JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

# Ascorbic Acid, Phenolic Acid, Flavonoid, and Carotenoid Profiles of Selected Extracts from *Ribes nigrum*

Jessica Tabart,*[,†] Claire Kevers,[†] Danièle Evers,[§] and Jacques Dommes[†]

[†]Plant Molecular Biology and Biotechnology Unit, University of Liège, B22, Sart-Tilman, B-4000 Liège, Belgium

[§]Public Research Center − Gabriel Lippmann, Rue du Brill 41, L-4422 Belvaux, Luxembourg

**ABSTRACT:** Small fruits such as berries have low energy contents, but high contents of vitamins, micronutrients, and dietary fibers and constitute a good source of natural antioxidant compounds that are important constituents of the human diet. This study identified a large number of compounds in an extract of black currant showing high antioxidant activity and compared their profile in various parts of the plants (leaves, buds, and berries). If it was known that berries contained very high levels of natural phenolic compounds, this study showed that leaves and buds could also be considered good sources of natural antioxidants. Indeed, they contained high amounts of phenolic acids, flavonoids, and carotenoids. An acetone mixture can extract several classes of phenolic compounds with a good yield of flavonols, flavan-3-ols, and anthocyanins.

**KEYWORDS:** antioxidant, flavonoids, flavonols, anthocyanins, flavanols, ascorbic acid, phenolic acids, carotenoids

## INTRODUCTION

Phenolic compounds are one of the most widely occurring groups of phytochemicals. In the plant kingdom, these compounds can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins.[1] Different classes of phenolic compounds can be distinguished. Flavonoids and phenolic acids are the most abundant. Phenolic acids are synthesized from hydroxybenzoic acid and hydroxycinnamic acid. The flavonoids are subdivided into different classes: flavonols, anthocyanins, flavones, flavan-3-ols, flavanones, and isoflavones.[2] In the plant, they play an important role in growth and reproduction, providing protection against pathogens and predators as well as against abiotic stresses.[1,3] In the human diet, fruits and vegetables have low energy content, but high contents of vitamins, essential micronutrients, and dietary fibers. They are also the predominant source of flavonoids and phenolic acids. Many health-related properties, including antiviral and anti-inflammatory activities, antioxidant properties, and the ability to inhibit human platelet aggregation, have been described.[4−6]

Carotenoids belong to another important group of natural pigments because of their wide distribution, structural diversity, and numerous functions for photosynthesis and for life in an oxygen-containing atmosphere. The carotenoids are subdivided into two groups: carotenes and xanthophylls (or oxycarotenoids).[7] Recently, these pigments have been described to be implicated in the prevention of human health disorders such as heart disease and photosensitivity disease as well as certain forms of cancer.[8,9]

Small fruits constitute a good source of natural antioxidant substances. Extracts of fruits from various blackberry, raspberry, and gooseberry cultivars act effectively as free radical inhibitors.[10,11] In addition, the flavonoid content of small fruits has been investigated.[12] Black currant berries (*Ribes nigrum*) contain very high amounts of phenolic compounds. Fresh black currants are particularly rich in anthocyanins, but other phenolic compounds such as flavonols are also present.[13] Only a very small proportion of these berries is consumed fresh; most are processed for juice concentrates. Leaves and buds can also be used;[14] leaf micronisates and glycerinate extracts of buds are especially commercialized as food supplements. Declume[15] and Chrubasik[16] also demonstrate that leaf extracts of black currant show significant anti-inflammatory activity.

The first objective of this study was to identify most of the compounds present in an extract of black currant with high antioxidant activity. We also compared the profile in ascorbic acid, phenolic acids, flavonoids, and carotenoids of extracts from various parts of the plants (leaves, buds, and berries). The second objective was to check whether an acetone extraction method optimized for high antioxidant capacity[17] provided good yields of various classes of antioxidant compounds.

## MATERIALS AND METHODS

**Materials.** The buds, berries, and leaves of 2-year-old black currant plants (Noir de Bourgogne) were harvested, respectively, in March, July, and August in the Belgian Ardennes (Bihain). The various explants were directly cut out in pieces, frozen, and stored at −20 °C.

**Sample Preparation.** *Acetone Extraction.* One gram of frozen sample (berries, buds, or leaves) was ground with 1 g of quartz and also 10 mL of extraction solution: acetone/water/acetic acid (70:28:2).[14] The mixture was shaken during 1 h at 4 °C and centrifuged at 17000*g* for 15 min. The supernatant was removed, and the sample was extracted again according to the same procedure but incubated for only 15 min. The supernatants were pooled and then diluted as appropriate for the analyses.

*Specific Extractions. (a) For Ascorbic Acid.* Four grams of frozen material (berries, buds, or leaves) was ground with 1 g of quartz and also 80 mL of extraction solution (20 g/L metaphosphoric acid). The mixture was shaken during 1 h at 4 °C and centrifuged at 15000*g* for 15 min. For HPLC analysis, 10 mL of a L-cysteine solution (40 g/L) was added to 20 mL of the supernatant, and the pH was

**Received:** November 22, 2010
**Revised:** March 15, 2011
**Accepted:** March 21, 2011
**Published:** March 21, 2011

adjusted between 7.0 and 7.2 with a solution of trisodium phosphate (200 g/L). After 5 min, the pH was adjusted between 2.5 and 2.8 with a solution of metaphosphoric acid (20 g/L).[18]

*(b) For Phenolic Acids.*[19] Samples of 3.4 g of frozen material (berries, buds, or leaves) were ground with 1 g of quartz and also 49 mL of extraction solution (methanol/water/acetic acid; 90:1.5:8.5; v/v/v) containing 2 g/L of butylated hydroxyanisole (BHA). The mixture was sonicated during 1 h at room temperature. Fifty milliliters of 20 g/L ascorbic acid and 25 mL of 10 M NaOH were added for the hydrolysis of esterified phenolic acids during 16 h at room temperature under nitrogen. Then 12.5 mL of 12 N HCl was added for the extraction of glycoside forms of phenolic acids, and the mixture was incubated for 3 h at 85 °C before cooling on ice. The mixture was extracted five times with a solution of diethyl ether/ethyl acetate (50:50; v/v) and centrifuged at 1200*g* for 2 min. The organic phases were pooled and evaporated. All of the residues were dissolved in 2 mL of 2% acetic acid and filtered for HPLC analysis.

*(c) For Anthocyanins.* One gram of frozen sample (berries, buds, or leaves) was ground with 1 g of quartz and also 15 mL of 1% HCl in methanol.[20] The mixture was shaken during 2 h at room temperature and incubated overnight in the dark at −20 °C before centrifugation at 4000*g* for 15 min. The supernatant was collected, and the sample was extracted again with 15 mL of 1% HCl in methanol. The supernatants were pooled and then diluted as appropriate for the analyses.

*(d) For Flavanols (Based on Parva-Uzunalic et al.*[21]*).* One gram of frozen sample (berries, buds, or leaves) was ground with 1 g of quartz and also 10 mL of acetone 100%. The mixture was shaken during 1 h at 70 °C and centrifuged at 17000*g* for 15 min. The supernatant was removed, and the sample was extracted again according to the same procedure but incubated for only 15 min. The supernatants were pooled and then diluted as appropriate for the analyses.

*(e) For Carotenoids.* One gram of frozen sample (berries, buds, or leaves) was ground with 1 g of quartz and also 15 mL of 1% BHT (2,6-di-*tert*-butyl-4-methylphenol) in acetone.[22] The mixture was shaken during 30 min at 4 °C in the dark and centrifuged at 17000*g* for 10 min. The supernatant was removed, and the sample was re-extracted until the sample was colorless. The supernatants were pooled.

**Colorimetric Assays.** The concentration of *reduced ascorbic acid* was measured by using the 2,6-dichloroindophenol (DCIP) method of the Association of Vitamin Chemists.[23] Briefly, each molecule of vitamin C converted a molecule of DCIP into a molecule of $DCIPH_2$, and the conversion was monitored as a decrease in the absorbance at 520 nm. A standard curve was prepared using a series of known ascorbic acid concentrations. One milliliter of diluted sample (in 5% metaphosphoric acid) or ascorbic acid calibration solution was mixed with 500 μL of 10% metaphosphoric acid. Three hundred microliters of citrate buffer (pH 4.15) and 300 μL of DCIP (0.1 mg/mL) were added to 600 μL of this mixture. Optical density blanching was used. For each sample, the blank value was determined after the addition of 60 μL of ascorbic acid (1 mg/mL) with the aim of evaluating the interference due to the sample color. The results were expressed as micrograms of ascorbic acid (AA) per gram of frozen weight.

*Total phenolic content* was determined according to the Folin−Ciocalteu method.[24] An amount of 3.6 mL of an appropriate dilution of acetone extract was mixed with 0.2 mL of Folin−Ciocalteu reagent and, after 3 min of incubation, 0.8 mL of sodium carbonate solution (20% w/v) was added. The mixture was heated at 100 °C during 1 min. The absorbance at 750 nm was measured after cooling. A standard curve was done with chlorogenic acid. The results were expressed in milligrams of chlorogenic acid equivalents (CAE) per gram of frozen weight.

*Total flavonol* content was measured following the method of total flavonoids described by Lamaison and Carmat.[25] In a previous paper, we had demonstrated that this technique appears to be adequate only for flavonols.[26] Appropriately diluted acetone extracts (1 mL) were mixed with 1 mL of 2% $AlCl_3 \cdot 6H_2O$ in methanol. The absorbance at 430 nm was measured 10 min later. Quercetin was used as standard, and results were expressed as milligrams of quercetin equivalents (QE) per gram of frozen weight.

*Total anthocyanin* quantification was performed according to the pH-differential method.[27] The extract was diluted in a pH 1.0 solution (0.1 M HCl, 25 mM KCl) and in a pH 4.5 solution (0.4 M $CH_3COONa$). The absorbance of the mixtures was then measured at 535 and 700 nm against distilled water. The value $(Abs_{535}-Abs_{700})_{pH1.0} - (Abs_{535} - Abs_{700})_{pH4.5}$ corresponds to the absorbance due to the anthocyanins. Calculation of the anthocyanin concentrations was based on a cyanidin-3-glucoside (kuromanin) standard curve. Results were expressed as micrograms of kuromanin equivalents (KuE) per gram of frozen weight.

*Total flavanol* content was evaluated according to the vanillin assay.[28] Each molecule of vanillin reacted with a molecule of flavanol to produce a red chromophore. The conversion was monitored as an increase in the absorbance at 500 nm. One volume of sample diluted in methanol (before acetone was removed by evaporation and replaced by the same volume of water) was mixed with 2.5 volumes of 1% vanillin in methanol and 2.5 volumes of 9 M HCl in methanol. The mixture was incubated for 20 min at 35 °C before analysis. For each sample, a blank value was measured where vanillin solution was replaced by methanol alone. Catechin (0−1 mg/mL) was used as standard, and results were expressed as milligrams of catechin equivalents (CaE) per gram of frozen weight.

*Total carotenoid* content was evaluated by a spectrophotometric method described by Rodriguez-Amaya[29] using the measurement of the absorbance at 450 nm. From this value we subtracted the turbidity of the sample (assayed through absorbance at 700 nm). We used an extinction coefficient recommended for the mixture of carotenoids ($\varepsilon$ = 2500). The results were expressed as μg of carotenoids per gram of frozen weight.

**Separation and Measurement of Compounds by HPLC.** Analyses were performed in a liquid Elite Lachrom Merck Hitachi chromatograph equipped with an L2450 photodiode array detector (sampling period = 400 ms, spectral bandwidth = 4 nm). Separation was carried out using a LiChroCART steel cartridge (Merck), 250 mm × 4.6 mm, filled with 5 μm particles RP 18 at 30 °C for flavonols and at 40 °C for anthocyanidins and flavan-3-ols. Other separations were carried out using a Grace Smart RP 18 (Grace Davison Discovery Sciences), 250 mm × 4.6 mm, filled with 5 μm particles RP 18 at 30 °C for ascorbic acid and phenolic acids.

*For ascorbic acid analysis*, the mobile phase was a gradient of water/metaphosphoric acid (199:1; v/v) (solution A) and 100% acetonitrile (solution B), at a flow rate of 1 mL/min. The elution gradient was performed as follows: isocratic elution 100% A, 0−3 min; linear gradient from 100% A to 100% B, 3−3.5 min; isocratic elution 100% B, 3.5−8.5 min; linear gradient from 100% B to 100% A, 8.5−9 min; isocratic elution 100% A, 9−20 min. Optical density was recorded at 254 nm.

*For phenolic acid analysis*, the elution gradient was performed using 2% ascorbic acid (solution A) and 100% acetonitrile (solution B) as follows: linear gradient from 100% A to 80% A/20% B, 0−1 min; isocratic elution 80% A/20% B, 1−4 min; linear gradient from 80% A/20% B to 100% A, 4−5 min; linear gradient from 100% A to 85% A/15% B, 5−30 min; linear gradient from 85% A/15% B to 32.5% A/67.5% B, 30−40 min; linear gradient from 32.5% A/67.5% B to 100% B, 40−42.5 min ; isocratic elution 100% B, 42.5−47.5 min; linear gradient from 100% B to 100% A, 47.5−50 min; isocratic elution 100% A, 50−60 min. Flow rate was 0.5 mL/min. Optical densities were recorded at 260, 275, and 325 nm.

*For flavonol aglycone analysis* of hydrolyzed (with a solution of 1.2 M HCl in methanol 50% and 3 mg/mL of L-ascorbic acid, v/v, at 80 °C during 1 h) extracts, the mobile phase[14] was a linear gradient of water/acetonitrile (50:50) adjusted to pH 1.8 with perchloric acid and

water/acetonitrile (95:5) adjusted to pH 1.8 with perchloric acid, at a flow rate of 1.2 mL/min. Optical density was recorded at 365 nm.

*For anthocyanidin analysis* of hydrolyzed (with a solution of 4 M HCl in methanol 50% and 3 mg/mL of L-ascorbic acid, v/v, at 80 °C during 2.5 h) extracts, the elution gradient[30] was performed using water/acetonitrile/formic acid (87:3:10) (solution A) and water/acetonitrile/formic acid (40:50:10) (solution B) as follows: linear gradient from 70% A/30% B to 50% A/50% B, 0−10 min; linear gradient from 50% A/50% B to 40% A/60% B, 10−13 min; linear gradient from 40% A/60% B to 70% A/30% B, 13−16 min; isocratic elution 70% A/30% B, 16−21 min. Flow rate was 0.8 mL/min. Optical density was recorded at 518 nm.

*For flavan-3-ol analysis* the mobile phase[31] was composed of 90% acetonitrile, 0.1% orthophosphoric acid, and 9.9% water. A gradient of flow was used: 0.4 mL/min to 3 min, a linear decrease to 0.3 mL/min at 10 min and to 0.2 mL/min at 13 min, and steady state to 25 min followed by a linear increase to 0.4 mL/min at 35 min. Optical density was recorded at 230 nm.

All of the samples were prepared in triplicates. Each sample analysis was performed in duplicate or triplicate. All of the results presented are the mean (±SE) of at least three independent experiments.

**Statistical Analysis.** The data were subjected to the statistical analysis of the variance (ANOVA-1) to evaluate significant differences between various explants of black currant. The difference was regarded as significant when $p < 0.05$.

## RESULTS AND DISCUSSION

The first aim of this study was to identify antioxidant compounds in the black currant acetone extracts (acetone/water/acetic acid, 70:28:2) from leaves, buds, and berries. This method of extraction of the antioxidant compounds was previously optimized[14] for the high antioxidant capacity of the extracts. We first determined the contents of total phenolics, phenolic acids, ascorbic acid, flavonols, anthocyanins, flavan-3-ols, and carotenoids. The second objective of this study was to check whether

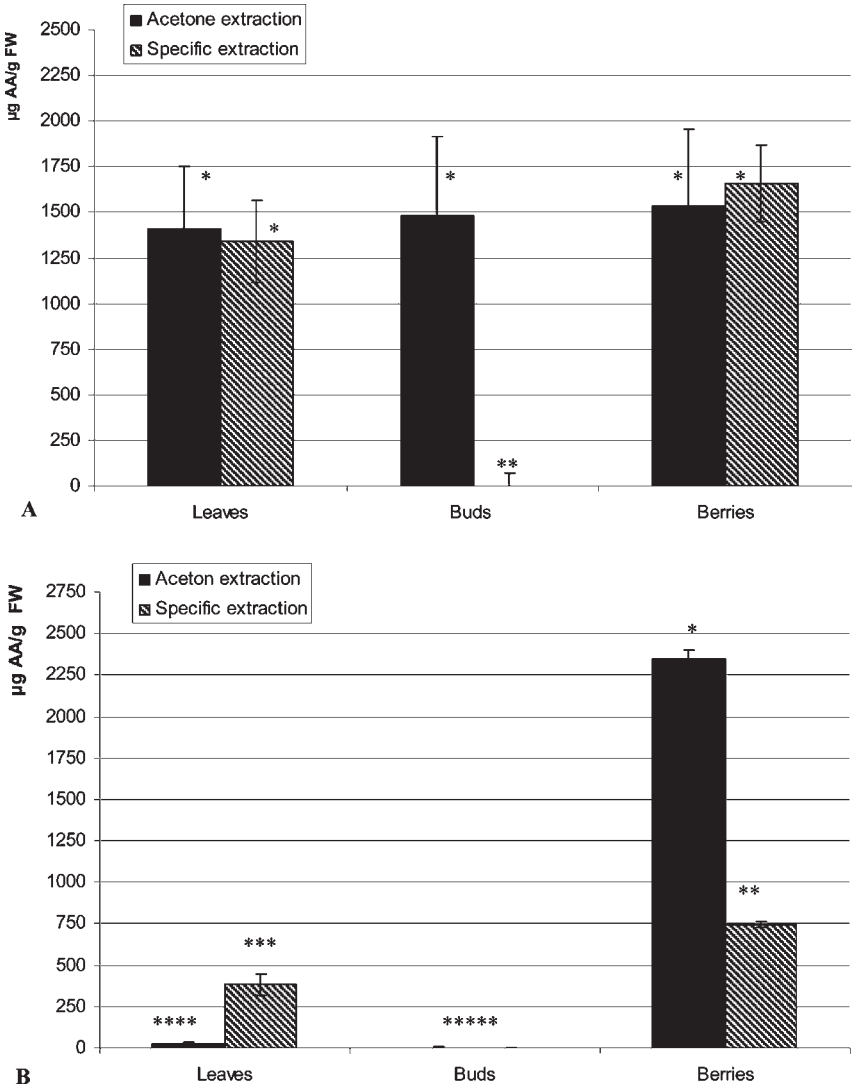


**Figure 1.** Determination of the contents in ascorbic acid of three black currant organs (berries, buds, and leaves) obtained by acetone and specific extractions: spectrophotometric (A) and HPLC (B) determinations. The concentration is expressed in micrograms of ascorbic acid per gram of frozen weight. No significant difference ($p > 0.05$) was observed for spectrophotometric assay between the three explants tested and between the two types of extraction used, except for specific extraction of buds ($n = 3$). For the three explants, analyzed by HPLC, a significant difference ($p < 0.05$) was observed ($n = 3$).


dx.doi.org/10.1021/jf104445c |J. Agric. Food Chem. 2011, 59, 4763–4770

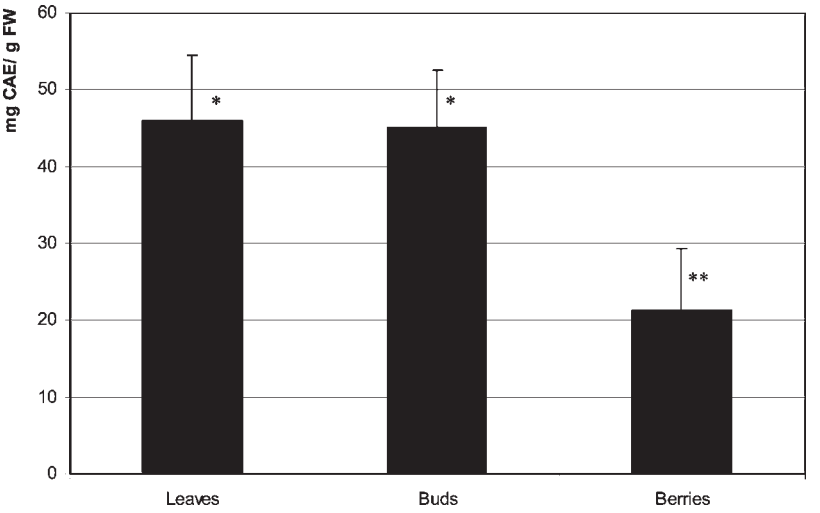


**Figure 2.** Determination of the total phenolic contents of three black currant plant organs (berries, buds, and leaves). The concentration is expressed in milligram equivalent chlorogenic acid per gram of frozen weight. For three analyzed explants, a significant difference ($p < 0.05$) was observed for the fruits compared with the leaves and buds ($n = 3$).

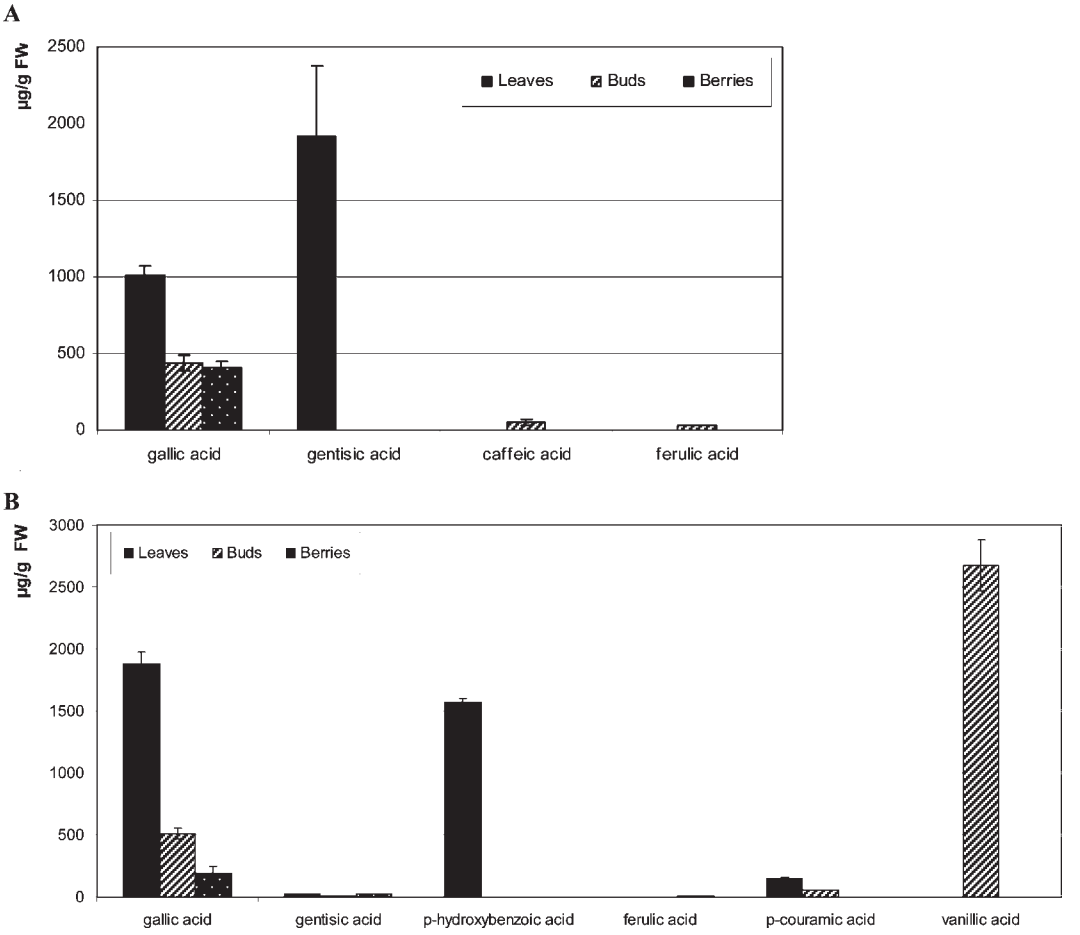


**Figure 3.** HPLC determination of the content in phenolic acids of three black currant extracts (leaves, buds, and berries) obtained by acetone extraction (A) and by specific extraction (B). The concentration is expressed in micrograms of phenolic acids per gram of frozen weight ($n = 3$).

the acetone mixture was an adequate solvent for high-yield extraction of different antioxidant compounds (by comparing the content of various antioxidant compounds in acetone extract and extracts obtained through compound-specific methods).

**Ascorbic Acid (AA) Content.** Extraction of AA in the acetone mixture was compared to the specific extraction method. First, AA was assayed by using the colorimetric method (Figure 1). Both extraction methods gave similar results for leaves and berries, with approximately 1500 $\mu$g AA/g FW. Results were very different for buds: about 1500 $\mu$g AA/g FW was found when using the acetone mixture, whereas AA was barely detectable in the specific extract. HPLC determination confirmed the absence of AA in the bud specific extract but also showed contrasting results for leaves and berries (Figure 1). Compared to specific extraction, acetone extraction gave higher yields for berries (2347 vs 748 $\mu$g/g FW) and lower yields for leaves (28 vs 384 $\mu$g/g FW). The results obtained for berries by HPLC assay after acetone extraction were higher than (almost double) those obtained by Benvenuti et al.[13] on the black currant 'Noir de Bourgogne'. For the determination of the ascorbic acid, we could show great differences between the two assays used. These observations were due to the characteristics of the quantification methods: In the DCIP assay, only reduced L-(+)-ascorbic acid was measured, but DCIP could also react with other reducing substances contained in the extracts such as myricetin.[26] In the HPLC assay, vitamin C was quantified in its two forms: reduced L-(+)-ascorbic acid and D-(−)-dehydroascorbic acid. The HPLC method had greater sensitivity and specificity than colorimetric assays. We could show also that bud extracts (by the two extracting methods) did not contain ascorbic acid. Information about AA content in buds is scarce. In soybean, the buds contain ascorbic acid, and its concentration increases at flower induction.[32]

**Total Phenolic Compounds Content.** Total phenolic content was assayed in acetone extracts of leaves, buds, and berries (Figure 2). Leaves and buds showed significantly higher content (46.0 ± 8.4 and 45.1 ± 7.5 mg CAE/g FW, respectively) than berries (21.2 ± 8.0 mg CAE/g FW). Benvenuti et al.[13] obtained contents in berries of 5.5 ± 0.1 mg gallic acid equiv/g of FW (extracted in methanol/HCl 2% (95:5, v/v), whereas Cacace and Mazza[33] obtained 88.9 ± 2.4 mg CAE/g FW in an aqueous sulfur dioxide extraction solution at pH 3.8 on milled berries.

**Phenolic Acid Content.** For phenolic acids (Figure 3), the extraction by specific techniques yielded higher results compared to extraction in acetone mixture. In acetone extraction, only the free forms were detected. Phenolic acids are rarely free but generally linked or esterified. The acetone mixture was able to liberate a lot of gallic acid (from the three explants) and gentisic acid (only from leaves) and small amounts of caffeic and ferulic acids (only from buds). With the specific extraction and hydrolysis (Figure 3B), other compounds were found. Gallic acid was present in the extracts from the three explants with higher content in leaves. Leaf specific extracts contained about twice the amount of gallic acid as acetone extracts (1883 ± 90 vs 1015 ± 54 $\mu$g/g FW). Two other phenolic acids were also found in high quantity: *p*-hydroxybenzoic acid in leaves (1572 ± 32 $\mu$g/g FW) and vanillic acid in buds (2677 ± 206 $\mu$g/g FW). Zadernowski et al.[34] used a similar extraction method to the specific extraction used here on berries and generally found the same phenolic acids but in lower amounts. They also found that *m*-coumaric acid was predominant (1872 ± 145 $\mu$g/g FW); this phenolic acid was not found in our specific berry extract. These contrasting results could be due to varietal differences or difference in ripening stage of the berries.

**Flavonoid Content.** Flavonoids are important plant secondary metabolites accumulating in stressing conditions.[35] They are largely studied for their benefit on human health.

First, we evaluated the content of total *flavonols* in the three extracts (leaves, buds, and berries) (Table 1). The colorimetric technique used was described in the literature as able to quantify the content in total flavonoids, but it is based on the formation of a complex between the flavonoids and $AlCl_3$. We showed

**Table 1. Contents of Various Flavonol Aglycons in the Three Extracts (Leaves, Buds, and Berries) and Quantification of the Total Flavonol Content**

| | flavonols ($\mu$g/g FW) | | | |
|---|---|---|---|---|
| | myricetin | quercetin | kaempferol | total flavonols[a] (mg QE/g FW) |
| leaves | 139 ± 47 | 778 ± 203 | 322 ± 151 | 2.05 ± 0.34 a |
| buds | 241 ± 57 | 480 ± 74 | 87 ± 22 | 2.15 ± 0.09 a |
| berries | 35 ± 3 | 77 ± 19 | 10 ± 5 | 0.50 ± 0.09 b |

[a] Letters (a and b) indicate a significant difference determined by ANOVA-1 ($p < 0.05$) between the berries and the buds and leaves ($n = 3$).

**Table 2. Contents of Various Anthocyanidins in the Three Extracts (Leaves, Buds, and Berries) Obtained by Acetone Extraction (A) and Specific Extraction (B) and Quantification of the Total Anthocyanin Content**

| | (A) Acetone Extraction anthocyanidins ($\mu$g/g FW) | | | | | | |
|---|---|---|---|---|---|---|---|
| | delphinidin | petunidin | cyanidin | peonidin | pelargonidin | malvidin | total anthocyanins[a] ($\mu$g KuE/g FW) |
| leaves | 85 ± 14 | 514 ± 152 | 65 ± 19 | 35 ± 7 | 0 | 0 | 381 ± 72 a |
| buds | 70 ± 5 | 493 ± 140 | 14 ± 7 | 0 | 0 | 64 ± 18 | 260 ± 72 a |
| berries | 155 ± 56 | 641 ± 61 | 123 ± 41 | 21 ± 13 | 0 | 503 ± 74 | 1468 ± 222 b |
| | (B) Specific Extraction anthocyanidins ($\mu$g/g FW) | | | | | | |
| | delphinidin | petunidin | cyanidin | peonidin | pelargonidin | malvidin | total anthocyanins[a] ($\mu$g KuE/g FW) |
| leaves | 88 ± 18 | 1181 ± 115 | 363 ± 138 | 133 ± 75 | 258 ± 196 | 178 ± 119 | 429 ± 87 a |
| buds | 66 ± 7 | 114 ± 13 | 0 | 0 | 0 | 0 | 665 ± 92 b |
| berries | 92 ± 14 | 941 ± 167 | 3 ± 2 | 19 ± 10 | 0 | 10 ± 8 | 3180 ± 996 c |

[a] Letters (a–c) indicate a significant difference determined by ANOVA-1 ($p < 0.05$) between the three explants ($n = 3$).

**Table 3. Identification and Quantification of Monomers of Flavan-3-ols in Black Currant Extracts (Leaves, Buds, and Berries) Obtained by Acetone Extraction (A) and Specific Extraction (B) and Quantification of the Total Content in Flavan-3-ols**

| | (A) Acetone Extraction | | | | | |
|---|---|---|---|---|---|---|
| | flavan-3-ols ($\mu$g/g FW) | | | | | |
| | epigallocatechin | gallocatechin | catechin | epicatechin | epigallocatechin gallate | total flavan-3-ols[a] (mg CaE/g FW) |
| leaves | 150 ± 86 | 382 ± 132 | 19 ± 9 | 2,5 ± 1,6 | 0 | 1.47 ± 0.87 a |
| buds | 599 ± 113 | 667 ± 45 | 91 ± 9 | 48 ± 21 | 37 ± 35 | 15.30 ± 0.93 b |
| berries | 114 ± 20 | 226 ± 4.5 | 29 ± 10 | 8 ± 0.5 | 0 | 3.42 ± 0.15 c |
| | (B) Specific Extraction | | | | | |
| | flavan-3-ols ($\mu$g/g FW) | | | | | |
| | epigallocatechin | gallocatechin | catechin | epicatechin | epigallocatechin gallate | total flavan-3-ols[a] (mg CaE/g FW) |
| leaves | 0 | 0 | 0 | 0 | 0 | 9.0 ± 4.4 a |
| buds | 110 ± 4 | 241 ± 21 | 36 ± 3 | 69 ± 22 | 44 ± 7 | 32.0 ± 3.4 b |
| berries | 79 ± 33 | 108 ± 35 | 78 ± 1 | 35 ± 4 | 25 ± 16 | 9.7 ± 1.4 a |

[a] Letters (a−c) indicate a significant difference determined by ANOVA-1 ($p < 0.05$) between the three explants ($n = 3$).

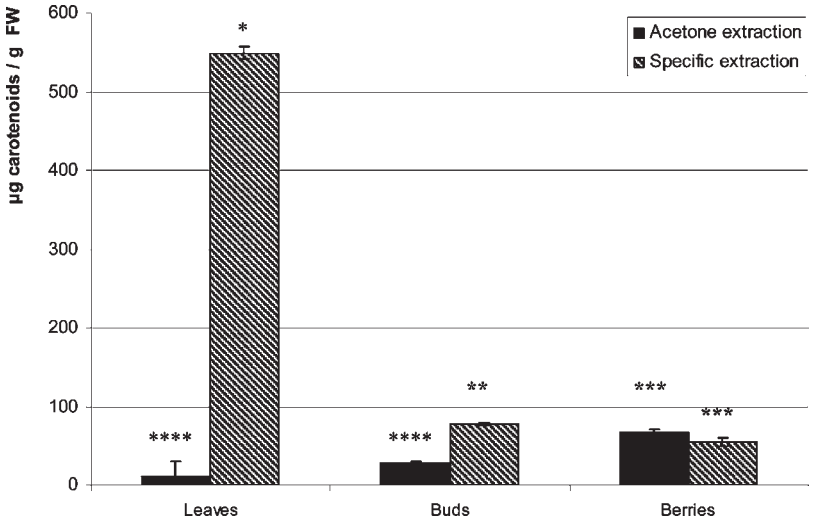


**Figure 4.** Total carotenoid content in extracts of black currant plant organs (leaves, buds, and berries) after acetone and specific extractions. The results are expressed in micrograms of carotenoids per gram of frozen weight ($n = 3$). A significant difference in the total carotenoid content ($p < 0.05$) was observed between the three explants analyzed ($n = 3$).

previously that only the flavonols were measured[26] by this technique. The contents in total flavonols of the berries (0.50 ± 0.09 mg QE/g FW) were significantly lower than in leaves and buds. The leaves and the buds had similar contents (2.05 ± 0.34 and 2.15 ± 0.09 mg QE/g FW, respectively). Cacace and Mazza[33] assayed flavonols in extracts from frozen berries by HPLC-MS analysis and reported slightly higher contents (1.90 ± 0.14 mg QE/g of FW). After separation and HPLC analysis, we showed that quercetin was more abundant than the two other aglycons (myricetin and kaempferol) in the three explants with a higher amount in leaf extracts (1.6 and 10 times more than in buds and berries, respectively). Similar results on the abundance of aglycons were obtained by Borges et al.[36] This group determined eight flavonols by HPLC-MS analysis with a better yield than in the present study. Häkkinen et al.[37] also reported similar contents in berries for the three aglycons (quercetin, between 33 and 68 $\mu$g/g FW; kaempferol, ≤10 $\mu$g/g FW; myricetin, 55 $\mu$g/g FW). The same conclusions were obtained by the group of Jakobek:[38] 44 $\mu$g myricetin/g FW, 21 $\mu$g quercetin/g FW, and 8 $\mu$g kaempferol/g FW. For the majority of cultivars, quercetin is prevalent, followed by myricetin and, finally, kaempferol. However, the contents of quercetin and myricetin considerably varied with the cultivar.[37] For the variety 'Silvergieter', no myricetin was present, whereas for the 'Rosenthals langtraubige Schwarze' variety, myricetin was prevalent, followed by quercetin.

*Anthocyanins* are another group of pigments in plants showing health benefits. As listed in Table 2, total anthocyanins were significantly different among the explants studied and the extraction methods used. For acetone extraction, the berry extract had a very high content (1468 ± 222 $\mu$g KuE/g FW) compared with the two other explants (381 ± 72 and 260 ± 72 $\mu$g KuE/g FW, for leaf and bud extracts respectively). The specific extraction for anthocyanins described by Awika et al.[20] using 1% HCl in methanol gave significantly ($p < 0.05$) similar results for leaves

(429 ± 87 μg KuE/g FW). On the contrary, for berries and buds, the acetone mixture extracted significantly ($p < 0.05$) less anthocyanins than specific extraction (3180 ± 996 and 665 ± 92 μg KuE/g FW, respectively). The berries had an anthocyanin content 4−5 times higher than leaves and buds. The extracts obtained by the two extraction types were hydrolyzed before being analyzed by HPLC. With this technique, we were able to quantify the total content of the major anthocyanidins (aglycons of the anthocyanins) (Table 2): delphinidin, petunidin, cyanidin, peonidin, pelargonidin, and malvidin. Petunidin was the prevalent anthocyanidin found in the three types of explants with both extraction methods. Malvidin was also found in great quantity in the berries extracted with the acetone mixture. Extraction with 1% HCl led to higher or equivalent yields, except for petunidin in buds, cyanidin in berries, and malvidin in buds and berries. Anthocyanin content in berries was reported to be 2189 ± 20 mg KuE/kg FW by the pH-differential method used by the group of Jakobek,[38] 15.3 ± 0.4 mg KuE/g of frozen berries by Cacace and Mazza,[33] and 2.287 mg KuE/g FW by HPLC analysis by Benvenuti et al.[13] In this study we observed a great difference between the contents determined by colorimetric and HPLC assays. The colorimetric method gave lower contents than HPLC analysis. This was due to the specific reactivity of each anthocyanin in the assay[26] and the use of a glycosylated standard.

The total content in *flavan-3-ols* (Table 3) in acetone extracts was significantly higher in the buds (15.3 ± 0.9 mg CaE/g FW) compared to the two other explants. The same trend was observed in extract prepared by the specific method, although this extract always contained more flavan-3-ols than the acetone extract. However, the analyses by HPLC of the monomers present in the different extracts showed that the acetone mixture extracted a higher amount of monomers than the specific extraction. In our HPLC protocol, only the monomeric forms of flavan-3-ols present in the sample were quantified. Other polymeric forms were surely present in the extracts. To determine the contents in total monomers of flavan-3-ols, various techniques of acid hydrolysis and depolymerization described in the literature[39] were tested without success. On the level of the colorimetric assay, all forms of flava-3-ols (monomers and polymers) were detected. Moreover, some anthoycanins can also interfere, due to their color.

**Carotenoid Content.** For carotenoids, Morris et al.[40] and André et al.[22] used as extractive solvent 1% BHT in acetone. Compared to our acetone mixture, this specific extraction gave higher yields from leaves (549 ± 8 vs 10 ± 20 μg carotenoids/g FW) and to a lesser extent from buds (Figure 4). For berries, the yields for the two types of extraction were similar. An extractive mixture containing a high percentage of acetone is advised for the extraction of xanthophylls, but those were probably degraded in the acetone method because no precaution was taken here to allow their correct extraction and conservation.

In general, the black currant berries contained higher levels of natural phenolic compounds than other fruits such as *Rubus fruticosus*, *Rubus idaeus* L., *Ribes rubum*, and *Sambucus nigra*.[13,37,38] The black currant variety 'Noir de Bourgogne' has a lower level of phenolic compounds and ascorbic acid than other varieties of black currant but a higher level of anthocyanins.[13,14] In this work, we have shown that the acetone extracts of leaves and buds had total phenolic contents largely higher than the berry extracts, which was also confirmed after analysis of the content in total flavonoids. The most abundant flavonol aglycon in these extracts was quercetin. The content in ascorbic acid was similar in the three types of extracts if measured by spectrophotometric method, whereas HPLC analysis of berries showed a higher content in ascorbic acid than in the two other plant organs. The extracts also contained phenolic acids: gallic acid in extracts from the three explants, gentisic acid in leaves, and caffeic and ferulic acid in buds. The most abundant anthocyanidin, whatever the explant, was petunidin. With regard to the flavan-3-ols, the bud extracts presented a higher content compared to other explants. The predominant monomers of flavan-3-ols were gallocatechin followed by epigallocatechin.

In conclusion, the acetone mixture can extract several classes of phenolic compounds with a good yield for flavonols, flavan-3-ols, and anthocyanins. This method is not adequate for carotenoids. For phenolic acids, it extracts fewer compounds than the specific method. However, we have also shown that leaves and buds could be considered good sources of natural antioxidant compounds.

## AUTHOR INFORMATION

**Corresponding Author**
*E-mail: Jessica.Tabart@doct.ulg.ac.be.

## ACKNOWLEDGMENT

J.T. gratefully acknowledges the AFR Luxembourg and Mr. Andrianne from HerbalGem for providing the material. We greatly appreciate the skillful assistance of the APE staff (provided to CEDEVIT by the regional government of Wallonia).

## REFERENCES

(1) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317–333.

(2) Williamson, G.; Santos-Buelga, C. In *Methods in Polyphenol Analysis*; Royal Society of Chemistry: London, U.K., 2003.

(3) Teutter, D. Significance of flavonoïds in plant resistance: a review. *Environ. Chem. Lett.* **2006**, *4*, 147–157.

(4) Guardia, T.; Rotelli, A. E.; Juarez, A. O.; Pelzer, L. E. Anti-inflammatory properties of plant flavonoids; effects of rutin, quercetin and hesperetin on adjuvant arthiris in rat. *Farmaco* **2001**, *56*, 683–687.

(5) Ghedria, K. Les flavonoïdes: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. *Phytotherapie* **2005**, *4*, 162–169.

(6) Scalbert, A.; Claudine, C.; Morand, C.; Rémésy, C. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287–306.

(7) Olivier, J.; Palou, A. Chromatographic determination of carotenoids in foods. *J. Chromatogr., A* **2000**, *881*, 543–555.

(8) Mayne, S. T. β-Carotene, carotenoids, and disease prevention in humans. *FASEB J.* **1996**, *10*, 691–701.

(9) Kanofsky, J. R.; Sima, P. D. Activity of a cationic carotenoid derivative in a mouse model of protoporphyria. *J. Photochem. Photobiol. B: Biol.* **2007**, *87*, 124–129.

(10) Heinonen, I. M.; Meyer, A. S.; Frankel, E. N. Antioxidant activity of berry phenolics on human low density lipoprotein and liposome oxidation. *J. Agric. Food Chem.* **1998**, *46*, 4107–4112.

(11) Su, M. S.; Silva, J. L. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinim ashei*) by-products as affected by fermentation. *Food Chem.* **2006**, *97*, 447–451.

(12) Pantelidis, G. E; Vasilakakis, M.; Manganaris, G. A.; Diamantidis, G. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem.* **2007**, *102*, 777–783.

(13) Benvenuti, S.; Pellati, F.; Melegari, M.; Bertelli, D. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. *Food Chem. Toxicol.* **2004**, *69*, 164–169.

(14) Tabart, J.; Kevers, C.; Pincemail, J.; Defraigne, J. O.; Dommes, J. Antioxidant capacity of black currant varies with organ, season, and cultivar. *J. Agric. Food Chem.* **2006**, *54*, 6271–6276.

(15) Declume, C. Anti-inflammatory evaluation of hydroalcoholic extract of black currant leaves (*Ribes nigrum*). *J. Ethnopharmacol.* **1989**, *27*, 91–98.

(16) Chrubasik, S. Pain therapy using herbal medicines. *Gynaekologe* **2000**, *33*, 59–64.

(17) Tabart, J.; Kevers, C.; Sipel, A.; Pincemail, J.; Defraigne, J. O.; Dommes, J. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and stability during storage. *Food Chem.* **2007**, *105*, 1268–1275.

(18) AFNOR. Foodstuffs — determination of vitamin C by high performance liquid chromatography. *NF EN 1430*, 2003.

(19) Barberousse, H.; Roiseux, O.; Robert, C.; Paquot, M.; Deroanne, C.; Blecker, C. Analytical methodologies for quantification of ferulic acid and its oligomers. *J. Sci. Food Agric.* **2008**, *88*, 1494–1511.

(20) Awika, J. M.; Rooney, L. W.; Waniska, R. D. Anthocyanins from black sorghum and their antioxidant properties. *Food Chem.* **2004**, *90*, 293–301.

(21) Parva-Uzunalic, A.; Skerget, M.; Knez, Z.; Einreich, B.; Otto, F.; Grüner, S. Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine. *Food Chem.* **2006**, *96*, 597–605.

(22) Andre, C. M.; Ghislain, M.; Bertin, P.; Oufir, M.; Del Rosario Herrera, M.; Hoffman, L.; Hausman, J. F.; Larondelle, Y.; Evers, D. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *J. Agric. Food Chem.* **2006**, *55*, 366–378.

(23) Association of Vitamin Chemists. *Methods of Vitamin Assay*, 3rd ed.; Interscience Publishers: New York, 1961.

(24) Caboni, E.; Tonelli, M. G.; Lauri, P.; Iacovacci, P.; Kevers, C.; Damiano, C.; Gaspar, T. Biochemical aspects of almond microcuttings related to *in vitro* rooting ability. *Biol. Planta.* **1997**, *39*, 91–97.

(25) Lamaison, J. L.; Carmat, A. Teneur en principaux flavonoïdes des fleurs et des feuilles de *Crataegus monogyna* Jacq. et de *Crataegus laevigata* (Poiret) DC en fonction de la végétation. *Plant. Med. Phytother.* **1990**, *25*, 12−16.

(26) Tabart, J.; Kevers, C.; Pincemail, J.; Defraigne, J. O.; Dommes, J. Evaluation of spectrophotometric methods for antioxidant compound measurement in relation to total antioxidant capacity in beverages. *Food Chem.* **2010**, *120*, 607–614.

(27) Nielsen, I. L.; Haren, G. R.; Magnussen, E. L.; Dragsted, L. O.; Rasmussen, S. E. Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography, Investigation of their pH stability and antioxidative potency. *J. Agric. Food Chem.* **2003**, *51*, 5861–5866.

(28) Nakamura, Y.; Tsuji, S.; Tonogai, Y. Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils. *J. Health Sci.* **2003**, *49*, 45–54.

(29) Rodriguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; Omni Research; ILSI Press: Washington, DC, 2001.

(30) Dutruc-Rosset, G. Détermination par CLHP de neuf anthocyanes principales dans le vin rouge et rosé. *Resolution OENO*.

(31) Sharma, V.; Gulati, A.; Ravindranath, S. D.; Kumar, V. A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC. *J. Food Compos. Anal.* **2005**, *18*, 583–594.

(32) Bharti, S.; Garg, P. P. Changes in the ascorbic acid content of the lateral buds of soybean in relation to flower induction. *Plant Cell Physiol.* **1970**, *11*, 723–727.

(33) Cacace, J. E.; Mazza, G. Extraction of anthocyanins and other phenolics from black currants with sulfured water. *J. Agric. Food Chem.* **2002**, *50*, 5939–5946.

(34) Zadernowski, R.; Naczk, M.; Nesterowicz, J. Phenolic acid profiles in some small berries. *J. Agric. Food Chem.* **2005**, *53*, 2118–2124.

(35) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481–504.

(36) Borges, G.; Degeneve, A.; Mullen, W.; Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, red currants, and cranberries. *J. Agric. Food Chem.* **2010**, *58*, 3901–3909.

(37) Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999**, *32*, 345–353.

(38) Jakobek, L.; Seruga, M.; Novak, I.; Medvidovic-Kosanovic, M. Flavonols, phenolic acids and antioxidant activity of some red fruits. *Dtsch. Lebensm. Rundsch.* **2007**, *103*, 369–378.

(39) Kelm, M. A.; Hammerstone, J. F.; Schmitz, H. H. Identification and quantitation of flavanols and proanthocyanidins in foods: how good are the data? *Clin. Dev. Immunol.* **2005**, *12*, 35–41.

(40) Morris, W. L.; Ducreux, L.; Griffiths, D.; Steward, D.; Davies, H. V.; Taylor, M. Carotenogenesis during tuber development and storage in potatoes. *J. Exp. Bot.* **2004**, *399*, 975–982.