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Antioxidant Capacity of Black Currant Varies with Organ, Season, and Cultivar

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Small berries such as black currant constitute one of the important sources of potential health-promoting phytochemicals because these fruits are rich sources of compounds with high antioxidant properties. In this work, antioxidant capacities of different parts (buds, leaves, fruits) of various black currant cultivars were compared throughout the growing season with the aim to prepare extracts with high antioxidant capacity. Buds (opened, at the end of March) and leaves (in June) had a higher content in phenolics and antioxidants than fully ripened berries (in July) and the best yield (per branch) was obtained with the leaves collected in June due to their higher biomass. The differences observed among the eight cultivars tested were small. Concerning flavonols, quercetin was dominant in all organs and cultivars, myricetin varied widely among the cultivars, and kampferol was very low.

KEYWORDS: Antioxidant; black currant; flavonols; phenolics

INTRODUCTION

Small berries constitute one of the important sources of potential health-promoting phytochemicals. These fruits are rich sources of phenolic compounds (*1*) such as flavonoids (*2*) and other polyphenols (*3*), which display potential health-promoting effects (*4*−*7*) due to their antioxidant properties (*8*−*10*). Anthocyanins, which belong to the group of flavonoids, are responsible for the red, violet, and blue colors of most berries and fruits (*7*, *11*, *12*). Especially high levels of anthocyanins and high antioxidant capacity are found in bilberries (*13*) and black currants (*14*, *15*). Myricetin, quercetin, and kaempferol are the principal flavonols, another group of flavonoids. Their amounts change greatly during black currant fruit ripening (*16*, *17*).

The content of phenolics in berries is affected by the degree of maturity at harvest, genetic differences (cultivar), preharvest environmental conditions, postharvest storage conditions and processing (*1*, *18*, *19*), and growing season (*20*) but not by cultivation practices (organic or conventional ways) (*16*). The potential health benefits of these fruits may be greatly increased if berries from cultivars with high contents of antioxidant compounds are used as raw materials (*16*).

Domestic berries, both wild and cultivated species, are consumed in abundance. Although studies have been conducted on these berries in terms of their antioxidant capacity and phenolic profile (*15*), nothing was done on the other parts of these plants.

Declume (*21*) and Chrubasik (*22*) demonstrated that leaf extract of black currant showed significant anti-inflammatory activity. However, no study has compared the antioxidant capacities of various parts of black currant. In this work we wanted to compare the antioxidant capacities of different parts of various black currant cultivars with the aim to prepare an extract with high antioxidant capacity. It is clear that, except for the berries, other parts cannot be consumed just as they are but can be conditioned as food supplements or added to food preparations or juices. Leaves and buds of black currant are already used in the preparation of some food supplements.

MATERIALS AND METHODS

Materials. One-year branches of black currant 'Noir de Bourgogne' were collected on different dates during the year 2004, in the Belgian Ardennes (at Bihain). Approximately 30 branches were randomly selected among more than 100 plants. Branches of eight cultivars of black currant were also collected in the botanical garden of the University of Liège during the year 2005. The cultivars were Black Down (BD), Ben Nevis (BN), Goliath (G), Noir de Bourgogne (NB), Tenah (T), Titania (Ti), Silvergieter (S), and Wellington (W). To obtain comparable samples, buds, leaves, or berries were collected at the same stages of development as described later under Results.

Immediately, the various types of samples (buds, leaves, flowers, berries, or stem pieces) were cut, weighed, frozen in liquid nitrogen, lyophilized, and stored at −20 °C prior to analysis at the end of the sample collection. The stability of the compounds in frozen samples was previously tested.

Sample Preparation. One gram of lyophilized sample was ground with 1 g of quartz and 5 mL of glycine buffer (0.87 M), pH 3 (with HCl). 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 two more times with 5 mL of the same buffer, incubated for

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Table 1. Linear Gradient Used for the Separation of Flavonols from Black Currant Extracts

time (min)	solvent A[a] (%)	solvent B[b] (%)
0	85	15
2	80	20
10	80	20
23	70	30
30	60	40
42	0	100
47	0	100
49	85	15

[a] Solvent A: water/acetonitrile (95:5) adjusted to pH 1.8 with perchloric acid. [b] Solvent B: water/acetonitrile (50:50) adjusted to pH 1.8 with perchloric acid.

15 min, and centrifuged using the same procedure. The supernatants were pooled and then diluted as appropriate for the analyses. Each sample was independently extracted in triplicate or more, and analyses were performed the same day.

Extractions concerning the comparison among varieties were performed in acetone mixture (acetone/water/acetic acid, 70:28:2). One gram of lyophilized sample was ground with quartz (1 g) and acetone mixture (10 mL). The mixture was shaken during 1 h at 4 °C and centrifuged at 17000*g* for 15 min. The supernatant was removed and the pellet washed with 5 mL of acetone mixture, shaken for 15 min, and centrifuged using the same procedure. The supernatants were pooled, and 70% of the volume was evaporated at 30 °C. The volume was then adjusted to 15 mL with water. Each sample was independently extracted in triplicate or more, and analyses were performed the same day.

This second type of extraction was used in the second part of the work because it allowed a better extraction of phenolics and antioxidants and the stability of the extracts was better (data not shown).

Total Phenolics. Total phenolics were determined according to the Folin−Ciocalteu method (*23*). If not very precise for phenolics, this protocol gave a good idea of the total phenolic content. Appropriately diluted extracts (3.6 mL) were mixed with 0.2 mL of Folin−Ciocalteu reagent, and 3 min later, 0.8 mL of sodium carbonate (20% w/v) was added. The mixture was heated at 100 °C during 1 min. After cooling, the absorbance at 750 nm was measured. Chlorogenic acid (Sigma) was used as standard, and results were expressed as milligrams of chlorogenic acid equivalents (CAE) per gram of dry weight of plant material. Analyses were performed in duplicate on each sample.

Antioxidant Capacity. Antioxidant capacity was determined by scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Tadolini et al. (*24*). Stock solution was prepared by stirring 75 mg of DPPH in 1 L of methanol overnight. Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Fluka Chemie GmbH, Buchs, Switzerland] was used as a standard and methanol as a blank.

In the assay, 0.75 mL of extract, standard (0−0.1 mM Trolox), or blank (methanol) and 1.5 mL of DPPH solution were mixed. The absorbance at 517 nm of samples, standards, and blanks was determined after 5 min. For each extract, a blank with 1.5 mL of methanol, instead of DPPH reagent, was included to correct for any sample absorbance at 517 nm. The percentage of the remaining DPPH was proportional to the antioxidant concentration, calculated relative to the antioxidant capacity of Trolox and expressed as milligrams of Trolox equivalent (TE) per gram of dry weight of plant material. Analyses were performed in duplicate.

Flavonol Analysis. Analyses were performed in a liquid Elite Lachrom Merck Hitachi chromatograph equipped with an L2450 photodiode array detector. Separation was carried out using a LiChroCART steel cartridge, 240 mm × 4 mm, filled with 5 μm particles of RP 18 and thermostated at 25 °C.

The mobile phase (*25*) was a linear gradient of water/acetonitrile (50:50) adjusted to pH 1.8 with perchloric acid (solvent B) in water/acetonitrile (95:5) adjusted to pH 1.8 with perchloric acid (solvent A), at a flow rate of 1.2 mL/min, as shown in **Table 1**. Spectra were recorded between 250 and 400 nm (sampling period, 400 ms; spectral bandwidth, 4 nm). Standards of flavonols were purchased from Extrasynthese (Genay, France).

All results presented are the means (± SE) of at least three independent experiments (extraction).

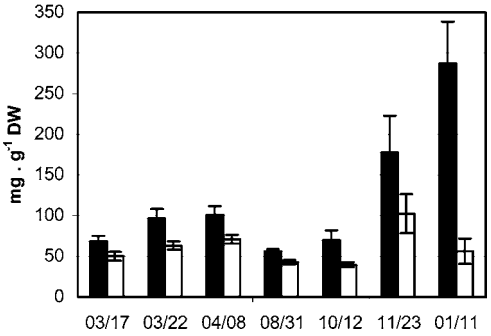

Figure 1. Total phenolics (black bars, mg of CAE g^{-1} of DW) and antioxidant capacities (white bars, mg of TE g^{-1} of DW) of buds of black currant 'Noir de Bourgogne' collected on different dates during the year ($n = 4$).

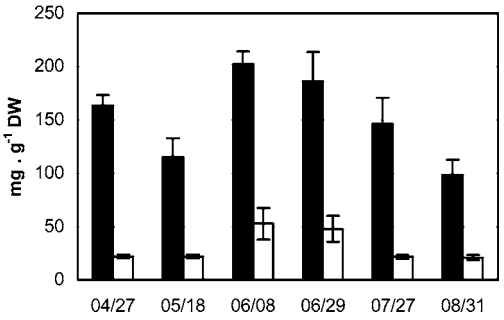

Figure 2. Total phenolics (black bars, mg of CAE g^{-1} of DW) and antioxidant capacities (white bars, mg of TE g^{-1} of DW) of leaves of black currant 'Noir de Bourgogne' collected on different dates during the year ($n = 4$).

RESULTS

Antioxidants in Various Parts of Black Currant Collected during Growth over the Year. *Buds*. The variations of total phenolics and antioxidant capacity were measured during the year 2004 on buds of black currant 'Noir de Bourgogne' (**Figure 1**). On March 17, the buds were closed (± 6 mm long), on March 22, they were "splinted" (± 8 mm long), and on April 8, the buds were opened (± 15 mm long). At the end of the summer, new buds were present, but they remained very small (2−3 mm long) until the end of the winter.

The level of phenolics did not change significantly from March to October but increased later with a maximum in January. The changes of the antioxidant capacity were similar except in January. Maxima were observed in April and November. Whereas the antioxidant capacity was 1.5−2 times higher in November, the weight of buds by branch was very low (5−10 times less). Thus, the best yield per branch was obtained in the spring, when the buds were opened.

Leaves. The leaves were collected from April to August (**Figure 2**). There was no difference between the measurements done in April (10−25 mm width) and in May (15−35 mm width) on the young leaves, but in June, when the leaves were well developed (± 70 mm width), the level of phenolics and the antioxidant capacity were higher. During the summer, the leaves necrosed except in the apex. The higher antioxidant capacity observed in June corresponded to the best yield because, at this time, the amount of leaves per branch was also the highest.

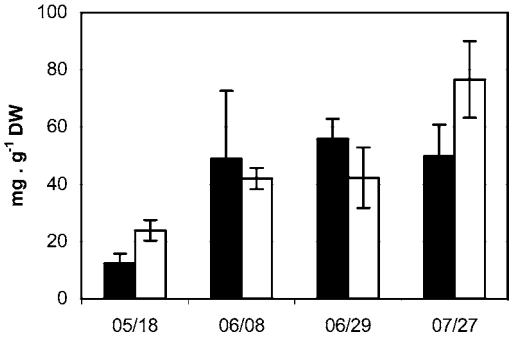

Figure 3. Total phenolics (black bars, mg of CAE g^{-1} of DW) and antioxidant capacities (white bars, mg of TE g^{-1} of DW) of berries of black currant 'Noir de Bourgogne' collected on different dates during the year ($n = 3$).

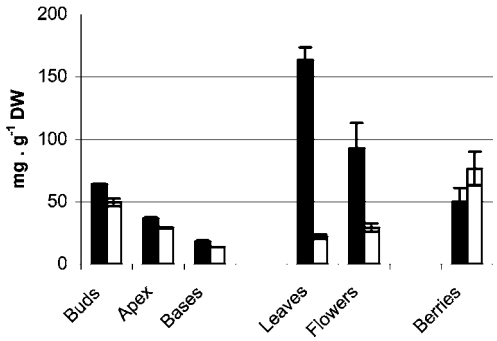

Figure 4. Total phenolics (black bars, mg of CAE g^{-1} of DW) and antioxidant capacities (white bars, mg of TE g^{-1} of DW) of various parts of black currant plants 'Noir de Bourgogne'. Buds, apex (2 cm long), and bases (2 cm long) of stems (without buds) were collected on March 17, leaves and flowers on April 22, and berries on July 27 ($n = 3$).

Berries. The berries were collected from May to July (**Figure 3**). The phenolic content increased during the growth of the berries and then stabilized during ripening. An increase of the antioxidant capacity was observed throughout the sampling period. The antioxidant capacity was maximal when the berries were ripe. At that moment, the fruit weight was highest. Thus, the yield of the antioxidant capacity was also maximal at this time.

Comparison with Other Parts. The phenolic level in leaves and flowers was higher than that in the other parts of the plant (**Figure 4**), whereas antioxidant capacity was superior in buds. The phenolic level and antioxidant capacity decreased from the apex to the basis of the stem.

Antioxidants in Buds, Leaves, and Berries of Various Black Currant Cultivars. Total phenolics and antioxidant capacities were measured in four types of explants (splinted and opened buds, well-developed leaves, and ripe berries) from eight commercial cultivars of black currant. These explants were collected at the time when the yield was previously shown to be maximal (**Figure 5**).

Concerning the buds, there was no significant difference between the different cultivars (**Figure 5**) even though the splinted buds of the Wellington cultivar did show lower phenolic content and antioxidant capacities than all of the other cultivars.

The phenolic contents and the antioxidant capacities of the leaves and the berries were very similar whatever the cultivars except for Black Down and Wellington, in which phenolic contents were lower.

If both phenolic contents and antioxidant capacities, measured per gram of dry weight, were similar for buds and leaves, the results obtained for the berries were lower, 3 times for phenolic contents and 6 times for antioxidant capacities.

The yield by branch was calculated for each explant of each cultivar (**Figure 6**). The results were very different from those obtained by unit of dry weight. There were important differences in the amount of each type of explant between the cultivars.

Yield in total phenolic and antioxidant capacity per branch was >20 times higher for the leaves than for the other explants.

Flavonol Contents in Buds, Leaves, and Berries of Black Currant Cultivars. Flavonols were present as aglycons or as glycosides. The ratios between the three main flavonol aglycon

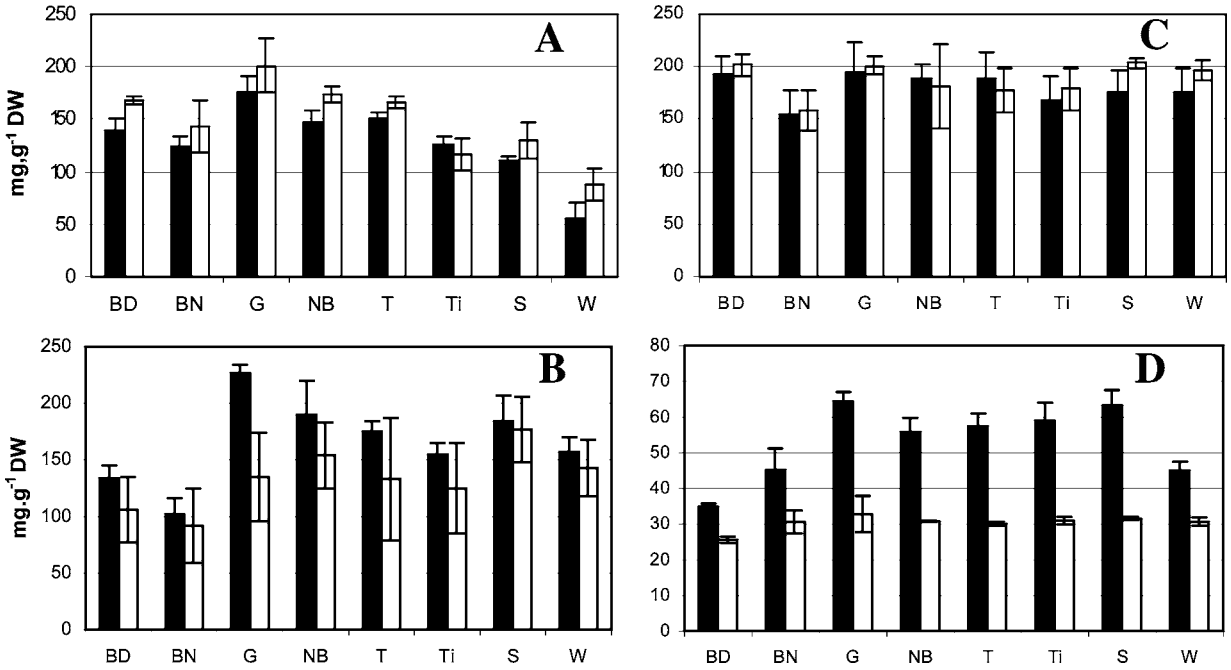

Figure 5. Total phenolics (black bars, mg of CAE g^{-1} of DW) and antioxidant capacities (white bars, mg of TE g^{-1} of DW) of buds (**A**, splinted; **B**, opened), leaves (**C**), and berries (**D**) of various cultivars of black currant: Black Down (BD), Ben Nevis (BN), Goliath (G), Noir de Bourgogne (NB), Tenah (T), Titania (Ti), Silvergieter (S), and Wellington (W) ($n = 3$).

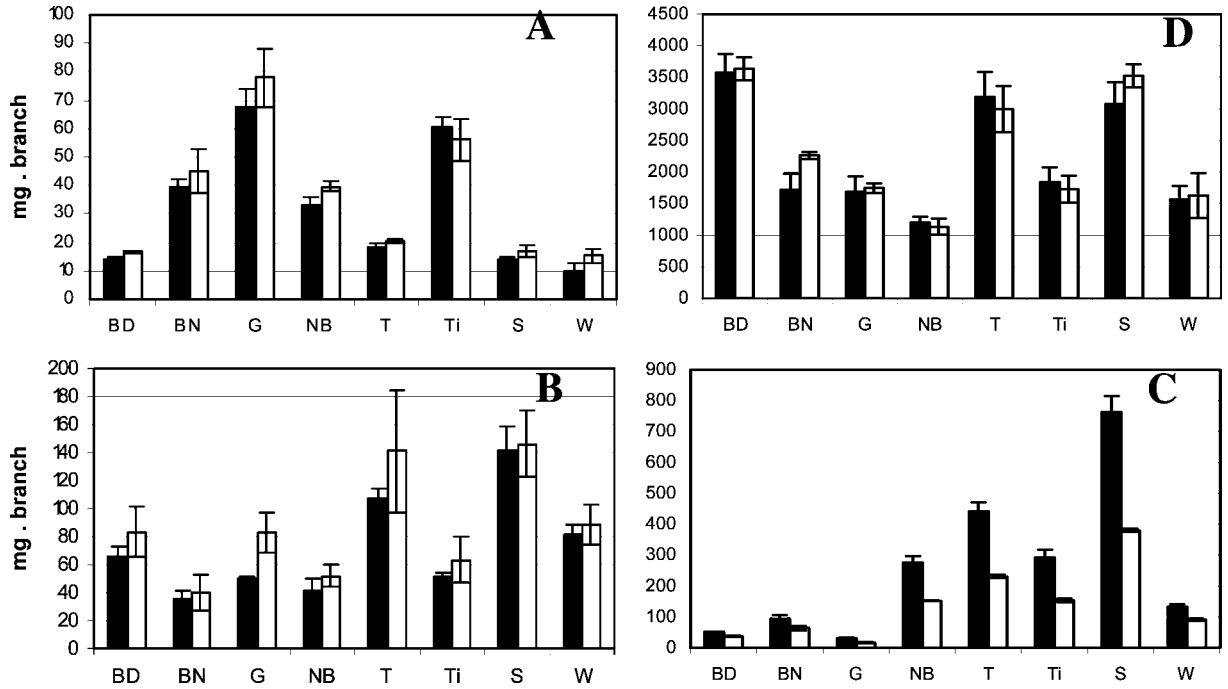

Figure 6. Yield by branch of total phenolics (black bars, mg of CAE branch^{-1}) and of antioxidant capacities (white bars, mg of TE branch^{-1}) of buds (**A**, splinted; **B**, opened), leaves (**C**), and berries (**D**) of various cultivars of black currant: Black Down (BD), Ben Nevis (BN), Goliath (G), Noir de Bourgogne (NB), Tenah (T), and Titania ($n = 3$).

Table 2. Flavonol Levels (Micrograms per Gram of Dry Weight) in Opened Buds of Various Cultivars of Black Currant: Black Down (BD), Ben Nevis (BN), Goliath (G), Noir de Bourgogne (NB), Tenah (T), Titania (Ti), Silvergieter (S), and Wellington (W) ($n = 3$)

flavonol	BD	BN	G	NB	T	Ti	S	W
myricitrin	197 ± 48	97 ± 52	35 ± 34	2 ± 0	169 ± 9	0	153 ± 20	0
rutin	407 ± 94	649 ± 149	433 ± 160	467 ± 80	753 ± 97	279 ± 44	589 ± 142	603 ± 63
isoquercitrin	165 ± 27	178 ± 19	122 ± 41	95 ± 1	178 ± 4	96 ± 5	140 ± 18	170 ± 11
astragalin	105 ± 21	140 ± 21	102 ± 34	70 ± 9	123 ± 9	82 ± 4	109 ± 8	110 ± 14
myricetin	109 ± 15	78 ± 31	26 ± 19	6 ± 1	102 ± 6	5 ± 1	80 ± 16	6 ± 1
quercetin	39 ± 5	27 ± 9	31 ± 4	13 ± 3	27 ± 1	20 ± 3	20 ± 7	16 ± 1
kampferol	4 ± 0	9 ± 1	8 ± 2	5 ± 1	10 ± 1	7 ± 1	9 ± 3	5 ± 1

Table 3. Flavonol Levels (Micrograms per Gram of Dry Weight) in Opened Buds, Leaves, and Berries of Black Currant Cultivars Goliath and Noir de Bourgogne ($n = 3$)

	Goliath			Noir de Bourgogne		
flavonol	buds	leaves	berries	buds	leaves	berries
myricitrin	35 ± 5	0	6 ± 1	2 ± 0	0	0
rutin	433 ± 160	2097 ± 427	260 ± 72	467 ± 80	2875 ± 503	277 ± 55
isoquercitrin	122 ± 41	1327 ± 282	75 ± 15	95 ± 1	747 ± 62	61 ± 6
astragalin	102 ± 34	752 ± 162	39 ± 6	70 ± 9	366 ± 18	38 ± 3
myricetin	26 ± 19	82 ± 15	8 ± 3	6 ± 1	41 ± 6	4 ± 1
quercetin	31 ± 4	53 ± 4	3 ± 1	13 ± 3	28 ± 4	2 ± 0
kampferol	8 ± 2	10 ± 2	0	5 ± 1	5 ± 2	0

forms (myricetin, quercetin, and kampferol) were different in the various cultivars tested. In buds, the myricetin level was higher in Black Down, Ben Nevis, Tenah, and Silvergieter, whereas in Wellington, Titania, and Noir de Bourgogne cultivars, quercetin was higher. The level of kampferol was always lower (**Table 2**). Among the glycosides, rutin was the most concentrated form. In all of the varieties, the level of rutin was many times higher than that of the other flavonols. The second form was isoquercitrin, also a glycoside of quercetin. Myricitrin (myricetin glycoside) was not present in some cultivars such as, for instance, Titania and Wellington.

In the leaves, the total level of flavonols, such as that of phenolics or antioxidants, was higher than in buds and berries (**Table 3**). In all of the different plant organs, rutin was always the most abundant among the flavonols and the ratio between the different flavonols measured was the same in the different plant organs of the same cultivar.

DISCUSSION

In all plant material tested, the total phenolic level was correlated with antioxidant activity as already shown in different berries (*15*) and other common foods (*26*, *27*). Due to differences in solubility, different extraction mixtures can affect the yield in total antioxidant capacity (*28*, *29*). This explained the difference between the phenolic amounts and antioxidant

capacities measured on the same type of explant extracted with glycine buffer (used for the variations during the year) or with acetone mixture (used for the cultivar comparison). The results were often higher with this last solvent, never lower.

Antioxidant capacity showed variations throughout the growing season in the different plant organs. Taking into account the biomass available for extraction, we defined an optimal period of harvest to ensure the highest yield in antioxidant activity. For the buds, the best yield was observed when the buds were just opened at the end of March or in the beginning of April. For the leaves, it was in June. For the berries, best yields were obtained in July, when they were fully ripened. Mikkonen et al. (*16*) and Wang and Lin (*19*) have already observed similar phenolic changes during black currant fruit ripening.

If bud extracts had the same content in phenolics and antioxidants as leaf extracts, the best yield was obtained from leaves due to their higher biomass. The antioxidant capacity of black currant leaves is not well studied (*30*), but interest in them as herbal medicinal products for the alleviation of osteoarthritis and rheumatic complaints (*22*) has been demonstrated. The antioxidant activities of the black currant berries have been studied more than for other berries (*2*, *3*), but we have shown that their antioxidant capacity was lower than that of the leaves and buds, leading to lower yield of antioxidant capacity. Ehlenfeldt and Prior found similar results in blueberry (*31*).

The differences observed among the eight cultivars tested were not important. Other research groups have observed some larger differences in black currant berries (*14*, *16*, *19*, *32*). Concerning flavonols, our results were in accordance with previous studies showing that black currant berries contained high amounts of quercetin (*2*); this aglycon (alone or as glycoside) was the dominant flavonol in all cultivars. Mikkonen et al. (*16*) have also shown that the amount of myricetin glycosides in black currant berries varied widely among black currant cultivars, whereas kampferol was known as a minor flavonol.

The increasing importance of functional ingredients in food provides new challenges for plant sciences to increase health-promoting phytochemicals in crop plants (*33*). Higher intakes of flavonoïds and other antioxidant compounds from foods are associated with reduced risks of cancer, heart disease, and stroke. Some experimental studies indicate that several plant flavonols, such as quercetin, myricetin, and rutin, are more powerful antioxidants than traditional vitamins (*34*, *35*) and have antitumor properties (*36*). The challenge is how to increase the levels of these beneficial phytochemicals in food. Black currant extract of leaves can be used as food supplements or incorporated in foods or beverages. The extract with the highest antioxidant capacity can be obtained with leaves collected in June and extracted with an acetone mixture.

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