Antioxidant properties of domesticated and wild *Rubus* species

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Abstract: The antioxidative capacities of a number of *Rubus* species of varied pigmentation have been investigated. In addition, total phenol, anthocyanin and ascorbic acid contents have been determined. Two methods to assess the antioxidant potential of fruit juices have been used. The antioxidant capacities of the fruit ranged from 0 to 25.3 μmol Trolox equivalents g^{-1} (TEAC) or from 190 to 66 000 μmol l^{-1} ferric reducing antioxidant power (FRAP). Ascorbic acid contributes only minimally to the antioxidant potential of *Rubus* juices (<10%, TEAC). There are apparent linear relationships between antioxidant capacity (assessed as both TEAC and FRAP) and total phenols (r_{xy} = 0.6713 and 0.9646 respectively). Also, anthocyanin content has a minor influence on antioxidant capacity (r_{xy} = 0.3774, TEAC; r_{xy} = 0.5883, FRAP). The sample with the highest antioxidant capacity (*Rubus caucasicus*) had the highest phenol content, but only a low percentage was represented by anthocyanins. The present study demonstrates the potential of certain wild *Rubus* species, notably *R caucasicus*, for improvement of nutritional value through germplasm enhancement programmes.

Keywords: *Rubus*; antioxidants; phenolics; anthocyanins; free radicals

INTRODUCTION

The damaging reactions of free radicals are widely implicated in the aetiology of numerous disease processes. Reaction of these typically electrophilic, reactive moieties with lipids, proteins and nucleic acids (oxidative damage) is considered a mechanism whereby the toxicity of these species is expressed. The increasing interest in antioxidants (molecules that can donate single electrons or hydrogen atoms to reactive free radicals) thus reflects the ever-growing list of degenerative disorders in which free radicals have been implicated. Low plasma antioxidant status has been associated with increased risk of cancer mortality,[1] whilst antioxidants derived from fruit and vegetables are believed to maintain health and afford protection from coronary heart disease.[2]

Numerous studies have shown that fruit and vegetables are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant properties. In addition to vitamin C, a great number of other phenolics (especially the flavonoids) have strong antioxidant activity *in vitro*. In fact, the vast majority of the activity seen in various fruit juice samples is associated with molecules other than vitamin C.[3] The potential for these compounds to act as antioxidants *in vivo* is dependent upon their bioavailability, an area currently receiving much attention.[4]

Blueberries are one of the richest sources of antioxidant phytochemicals encountered, with antioxidant capacities as high as 45.9 μmol Trolox equivalents g^{-1}.[5] Blueberries are a particularly rich source of phenolics (up to 5 g kg^{-1}), further emphasising the link between *in vitro* antioxidant capacity and phenolic content. This relationship appears to hold true from blueberries to Scotch whisky.[6]

Structure–activity relationships[7,8] of phenolic antioxidants indicate that a loose relationship exists between the number of free, aromatic —OH groups and antioxidant potential, and that the anthocyan(i-d)ins are potent antioxidant molecules. More potent still are some of the catechins. For example, epicatechin gallate (with seven free —OH substituents) is 4.9 times more potent as an antioxidant than both vitamins C and E.[7]

Rubus represents one of the most diverse genera of plants, comprising 12 subgenera of which four have high value as fruiting species.[9] This diversity is reflected in the wide range of fruit types and pigmentation found within the genus. *Rubus* species are widely distributed globally as wild and cultivated species and genotypes, from Arctic regions to Australasia. Of the cultivated *Rubus* the most popular is the red raspberry of the subgenus Idaeobatus, which is distributed throughout the temperate regions of Europe, Asia and North America as two species, *R idaeus* L and *R strigosus* Michx. Another important member of the Idaeobatus is the black raspberry, *R occidentalis* L, which has greatly increased levels of

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Contract/grant sponsor: Scottish Executive Rural Affairs Department
(*Received 18 January 2000; accepted 7 February 2000*)

anthocyanins (up to 4 g kg^{-1} fwt) compared to the red raspberry (0.2–0.6 g kg^{-1} fwt[10]). As such, significant variation in the antioxidant capacities of juices from *Rubus* would be expected.

Asia is a major centre of diversity for *Rubus*:[11] China alone has over 200 species, mainly in the Idaeobatus and Malachobatus subgenera.[12] Germplasm collected in 1992 on a plant exploration trip to Guizhou Province, China[11] has been grown at the USDA-ARS National Clonal Germplasm Repository (NCGR), Corvallis, Oregon, USA. The collected material as a whole shows great diversity of form and fruiting characters, with some accessions, eg *R coreanus* Miq, showing wide intraspecific variation in fruit colour. Such variability has been described previously for other Chinese *Rubus* species.[12,13]

The study reported here was carried out to examine the relationship between *in vitro* antioxidant capacity and phenolic content in a range of *Rubus* germplasm, including accessions from the Chinese collection held at the NCGR. Two independent assays for antioxidant activity were used throughout and the results from the *Rubus* species material are compared with those for the common red raspberry cultivar 'Glen Lyon'.

EXPERIMENTAL

Plant material

Seed of the *Rubus* species collected in Guizhou Province, China in 1992[11] on an expedition sponsored by the United States Department of Agriculture (USDA)-NCGR, Nanjing Botanical Garden, PRC and Guizhou Botanical garden, PRC was raised at the USDA-NCGR site in Corvallis, Oregon, USA (latitude 44.5 °N) and planted in the field in 1994 along with *R caucasicus* Focke, *R ulmifolius* Schott and *R ursinus* Cham and Schldl which are blackberry species from Eurasia and western North America respectively. Whole ripe berries of the following *Rubus* species from the Chinese collection and other accessions held at the USDA-NCGR were harvested and shipped frozen by express airfreight to the Scottish Crop Research Institute. *R caucasicus, R coreanus* (five accessions), *R hunanensis* Hand-Mazz, *R innominatus* S Moore (two accessions), *R lambertianus* Ser, *R niveus* Thunb, *R parvifolius* L, *R sumatranus* (Miq), *R tsangorum* Hand-Mazz, *R ulmifolius* and *R ursinus* (two accessions) have been analysed in the present study. For comparative purposes, fruit samples of the red raspberry cultivar 'Glen Lyon' (*R idaeus*) were collected at SCRI, Dundee, Scotland.

Preparation of juices

Berries were removed from the freezer and allowed to thaw overnight (20 °C), after which 500 g of fruit homogenised was in a blender (Waring) for 1 min with 10 units of pectinase. The resultant pulp was decanted into centrifuge bottles and allowed to stand at 4 °C overnight. Samples were then centrifuged for 20 min at $3500 \times g$ and the supernatant was filtered through Whatman No 1 paper.

Ascorbic acid determination

Ascorbic acid was determined by coupled liquid chromatography–mass spectrometry. Samples (10 µl) were loaded onto an anion exchange (Spherisorb SAX 5) column (250 mm × 4.6 mm, HPLC Technology, Macclesfield, UK) and eluted with a mobile phase of 0.25% TFA in water (0.7 ml min^{-1}). The flow was split (1:1) post-column, with 0.35 ml min^{-1} entering the APCI interface of a single-quadrupole API mass spectrometer (Finnigan MAT SSQ 710C, ThermoQuest, Hemel Hempstead, UK) in selected ion-monitoring mode. Ascorbic acid was estimated by the peak area (m/z=175) eluting at 4.1 min obtained by negative ion chemical ionisation (coronal discharge ~ −5 µA). Calibration was against freshly prepared standard solutions of ascorbic acid (0.1–10 mM, injection volume 5 µl, 0.5–50 ng per injection). Results were calculated as ascorbic acid kg^{-1} from determinations made in triplicate.

Pigmentation, total anthocyanins and LC–MS of anthocyanins

Visible pigmentation was determined by diluting juices with distilled water to 1% (v/v) and recording the absorbance at 515 nm against a water blank (1 cm path length). Total anthocyanins were quantified according to the pH differential method of Cheng and Breen.[14] Briefly, anthocyanins were estimated through absorbance measurement at 510 and 700 nm in buffers at pH 1.0 and 4.5, where $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}] = \varepsilon cl$ and the extinction coefficient of cyanidin-3-*O*-*β*-glucopyranoside is 29 000 M^{-1}. All samples were analysed at 2% (v/v) with calibration against cyanidin-3-*O*-*β*-glucopyranoside (0–25 mg l^{-1}). Results are expressed as cyanidin-3-*O*-*β*-glucopyranoside equivalents kg^{-1} from determinations made in triplicate.

Juices were analysed for anthocyanidin composition by coupled liquid chromatography–mass spectrometry. Samples (7 µl) were loaded onto a column (Ultracarb C18, 150 mm × 2 mm, Phenomenex, Macclesfield, UK) equilibrated with 0.1% trifluoroacetic acid in water (solvent A). Components were eluted by a linear gradient to 85% solvent B (75:25 acetonitrile/water) over 80 min, with a flow rate of 0.25 ml min^{-1} throughout. A post-column flow splitter diverted ~70% of the eluant to a visible wavelength detector (515 nm), the remaining eluant being directed to a Finnigan electrospray interface. The mass spectrometer was configured for positive ion mode, and a CID (collision-induced fragmentation) voltage of 30 V was applied to bring about fragmentation of anthocyanins to the charge-bearing anthocyanidin base and neutral sugar moieties. Data were recorded in full-scan mode (270–332 m/z, scan time 1 s). The individual anthocyanidins were estimated according to peak areas (co-eluting with peaks from the visible

detector) obtained from the molecular ions: 271 (pelargonidin), 287 (cyanidin), 301 (peonidin), 303 (delphinidin), 317 (petunidin) and 331 (malvidin).

Total phenolics

Total soluble phenols were determined with half-strength Folin–Ciocalteu reagent by the method of Slinkard and Singleton.[15] Results are expressed as gallic acid equivalents kg^{-1} (analyses in triplicate).

TEAC assay

Analyses were performed by a slight modification of the method of Miller *et al.* [16] Samples (8.4 µl of 5 or 10% v/v juice (diluted with distilled water)) were mixed with buffer (25 mM phosphate, pH 7.4, 488.6 µl), met-myoglobin (70 µM stock in buffer, 36 µl) and 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS, 500 µM stock in buffer, 300 µl). Absorbance (734 nm) of the developing $ABTS^{\cdot+}$ chromophore was recorded 7.5 min after initiation following the addition of hydrogen peroxide solution (450 µM stock in water, 167 µl). In controls, distilled water replaced the hydrogen peroxide. All analyses were made in triplicate.

FRAP assay

A manual assay was used based upon the methodology of Benzie and Strain.[17] FRAP reagent was freshly prepared to comprise 1 mM 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mM ferric chloride in 0.25 M sodium acetate, pH 3.6. A 100 µl aliquot of *Rubus* juice (1% v/v in distilled water) was added to 900 µl of FRAP reagent and mixed. After standing at ambient temperature (~20 °C) for 4 min, absorbance at 593 nm was determined against a water blank. Calibration was against a standard curve (50–1000 µM ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulphate. FRAP values obtained from *Rubus* juices are presented as micromolar ferrous ion (ferric reducing power) of a 100% juice, from three determinations.

Statistical analysis

Data were analysed by Microcal Origin 4.10 (Microcal Software, Northampton, MA, USA). Linear regression analysis was performed, quoting the correlation coefficient r_{xy}.

RESULTS

Total antioxidant capacities of juices prepared from *Rubus* berries ranged from 0 to 25.3 µmol Trolox equivalent antioxidant capacity (TEAC) g^{-1} as assessed by the TEAC method (Table 1). The degree of pigmentation appears to be an important factor in determining the antioxidant capacity of berries, as evidenced by the higher TEAC values for the darker, red *R coreanus* accession E compared with the orange–coloured fruit of *R coreanus* accession A. The orange berries of *R coreanus* accession A had the lowest TEAC value (0.03 ± 0.03) of any of the berries studied, much lower than the yellow berries of *R hunanensis* (TEAC = 10.06 ± 1.16) and *R lambertianus* (TEAC = 9.81 ± 1.46). Data obtained using the FRAP assay

Table 1. Antioxidant capacity and ascorbate, phenolic and anthocyanin contents of *Rubus* berries

Sample	*Colour*	*TEAC*[a]	*FRAP*[b]	*Ascorbic acid*[c]	A_{515}[d]	*Total phenols*[e]	*Anthocyanins*[f]	*A/P*[g]
R coreanus (A)	Orange	0.03 ± 0.03	191 ± 33	0.068 ± 0.004	0.034	0.267 ± 0.017	0.003 ± 0.001	0.011
R coreanus (B)	Orange	2.24 ± 0.08	3784 ± 50	0.081 ± 0.003	0.094	1.126 ± 0.070	0.085 ± 0.011	0.076
R coreanus (C)	Pale red	3.66 ± 0.59	7765 ± 96	0.081 ± 0.005	0.106	1.259 ± 0.047	0.086 ± 0.006	0.068
R coreanus (D)	Red	7.98 ± 1.61	5632 ± 55	0.092 ± 0.002	0.163	0.818 ± 0.069	0.113 ± 0.005	0.138
R coreanus (E)	Black	7.78 ± 0.62	13078 ± 241	0.102 ± 0.003	0.197	1.217 ± 0.031	0.343 ± 0.012	0.282
R ursinus (A)	Black	11.71 ± 0.75	55529 ± 895	0.123 ± 0.001	0.236	3.419 ± 0.158	1.050 ± 0.020	0.307
R ursinus (B)	Black	13.89 ± 0.94	27333 ± 494	0.164 ± 0.005	0.272	2.021 ± 0.130	0.619 ± 0.044	0.306
R innominatus (A)	Light red	5.74 ± 0.08	44260 ± 552	0.079 ± 0.007	0.105	3.264 ± 0.071	0.792 ± 0.011	0.243
R innominatus (B)	Dark red	11.53 ± 0.54	33436 ± 389	0.097 ± 0.003	0.237	2.502 ± 0.005	0.330 ± 0.007	0.132
R ulmifolius	Black	15.47 ± 1.34	34137 ± 443	0.106 ± 0.003	0.278	2.362 ± 0.006	0.590 ± 0.016	0.250
R parvifolius	Bright red	1.07 ± 1.00	17436 ± 205	0.069 ± 0.008	0.064	1.717 ± 0.041	0.116 ± 0.012	0.068
R caucasicus	Dark red	25.32 ± 1.28	65669 ± 997	0.141 ± 0.004	0.210	4.527 ± 0.007	0.325 ± 0.007	0.072
R niveus	Black	9.72 ± 0.38	40799 ± 780	0.099 ± 0.003	0.275	2.991 ± 0.028	1.186 ± 0.017	0.397
R sumatranus	Yellow	2.52 ± 1.07	13529 ± 245	0.114 ± 0.006	0.074	2.287 ± 0.133	0.034 ± 0.003	0.015
R tsangorum	Orange	3.47 ± 0.50	2681 ± 103	0.127 ± 0.001	0.081	0.840 ± 0.021	0.053 ± 0.003	0.063
R hunanensis	Orange	10.06 ± 1.16	20711 ± 432	0.164 ± 0.004	0.098	1.702 ± 0.016	0.067 ± 0.002	0.039
R lambertianus	Yellow	9.81 ± 1.46	51289 ± 635	0.079 ± 0.003	0.029	3.173 ± 0.003	0.001 ± 0.001	< 0.001
R idaeus (Glen Lyon)	Red	17.25 ± 1.03	25569 ± 344	0.242 ± 0.007	0.140	2.112 ± 0.004	0.387 ± 0.016	0.183

[a] µmol Trolox equivalents g^{-1} ± SEM ($n = 3$).
[b] µM FRAP ± SEM ($n = 3$).
[c] g kg^{-1} ± SEM ($n = 3$).
[d] Measured at 1% (v/v), 1 cm path length.
[e] g kg^{-1} ± SEM ($n = 3$).
[f] g kg^{-1} ± SEM ($n = 3$).
[g] Ratio anthocyanins/phenols (g g^{-1}).

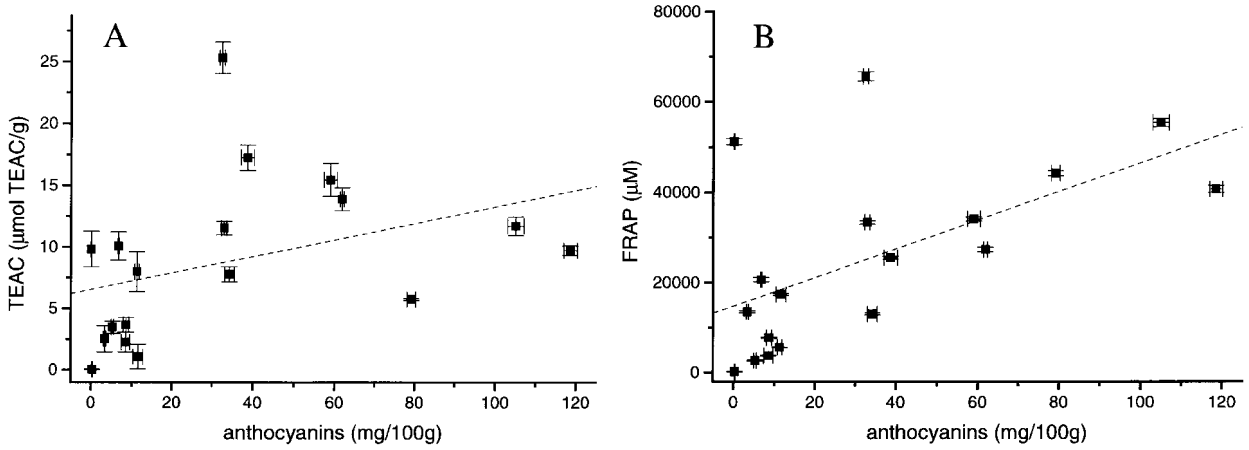

Figure 1. Influence of anthocyanin content on (A) TEAC and (B) FRAP antioxidant capacities of *Rubus* juices.

confirmed the patterns obtained using TEAC: *R coreanus* (accession A), 191 ± 33 μM FRAP; *R hunanensis*, 20711 ± 432 μM FRAP; *R lambertianus*, 51289 ± 635 μM FRAP. The juice with the highest antioxidative potential, as assessed by both methods, was that from *R caucasicus* (TEAC = 25.3 ± 1.28; FRAP = 65669 ± 997). 'Glen Lyon', a domesticated red raspberry cultivar studied as a comparison, had TEAC = 17.25 ± 1.03 and FRAP = 25569 ± 344.

The TEAC assay allows for calculation of the contribution that ascorbic acid makes to the total antioxidant capacity of complex mixtures of antioxidants, such as fruit juices.[18] The highest ascorbic acid content observed in the present study ('Glen Lyon', 0.242 g kg^{-1}) corresponds to a juice concentration of *ca* 1.4 mM. Ascorbic acid possesses a relative molar TEAC of 1.0,[18] and as such can be considered to contribute 1.4 μmol TEAC g^{-1} to the total observed TEAC of 17.25 μmol TEAC g^{-1}. The contribution of ascorbic acid to the total observed TEAC of the sample juice from 'Glen Lyon' is thus 8%. The contribution of ascorbic acid to the total observed TEAC is similarly low for all the *Rubus* species investigated: all ascorbic acid concentrations were determined to be less than 1 mM and hence contributed <10% to the observed TEAC (data from Table 1).

Total anthocyanins and total phenols were determined for all the juices. The highest anthocyanin content observed was from the juice of *R niveus* (1.186 g kg^{-1}), somewhat lower than the reported[10] anthocyanin content of *Rubus occidentalis* berries (2.14–4.28 g kg^{-1}). The lowest anthocyanin contents were 0.003 and 0.001 g kg^{-1} (*R coreanus* (A) and *R lambertianus* respectively). These two samples possessed very different antioxidant capacities: 0.03 and 9.81 (TEAC) and 191 and 51289 (FRAP) respectively. In contrast, the anthocyanin-rich *R niveus* displayed intermediate antioxidant capacity: 9.72 (TEAC) and 40799 (FRAP).

The relationships between anthocyanin content and TEAC and FRAP are presented in Figs 1A and 1B respectively. An apparent linear relationship was observed between anthocyanin content and antioxidant capacity (r_{xy} = 0.3774, TEAC; r_{xy} = 0.5883, FRAP). Despite this, the juice of highest antioxidant capacity, from *R caucasicus*, is intermediate in anthocyanin content. In addition, the anthocyanin-devoid *R lambertianus* has a higher FRAP value than might be expected from the low anthocyanin content (Fig 1B).

Total phenolic content and its influence on antioxidant capacity are shown in Fig 2. Again there is a relationship between TEAC and total phenolics

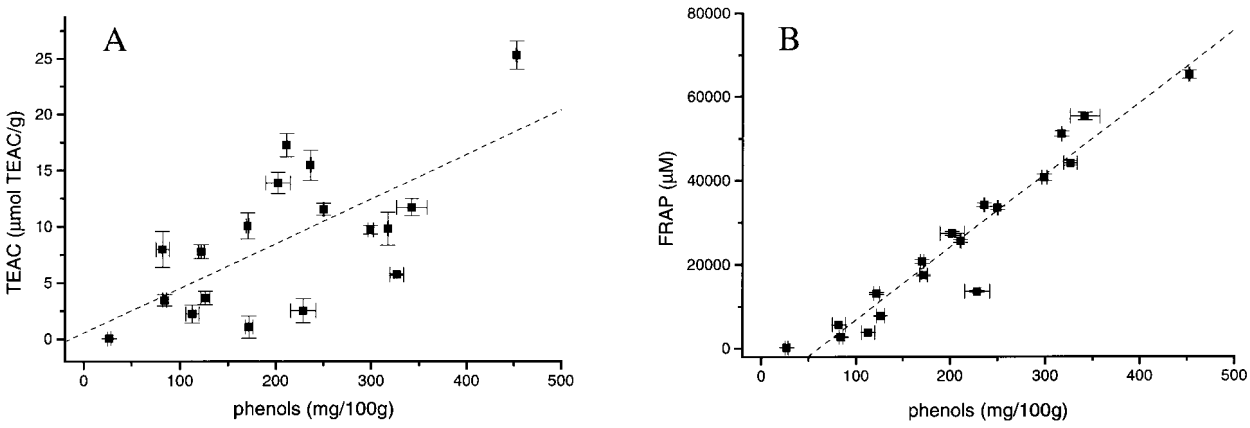

Figure 2. Influence of phenol content on (A) TEAC and (B) FRAP antioxidant capacities of *Rubus* juices.

(r_{xy} = 0.6713), the juice with the lowest phenolic content (*R coreanus* (A), 0.267 g kg^{-1}) having the lowest antioxidant capacity (TEAC = 0.03 μmol g^{-1}) and the juice with the highest phenolic content (*R caucasicus*, 4.527 g kg^{-1}) having the highest antioxidant capacity (25.3 μmol g^{-1}) (Fig 2A). The relationship between antioxidant capacity, as determined by the FRAP assay, and total phenols is presented in Fig 2B. Again the juices of *R coreanus* (accession A) and *R caucasicus* mark the extremes of a linear relationship (r_{xy} = 0.9646).

Of the phenolics within the juices, between 0 and 40% exist as anthocyanins (Table 1). The yellow berries of the anthocyanin-devoid species *R lambertianus* have an anthocyanin/phenol (A/P) ratio of < 0.001. For the intensely coloured *R niveus* this figure was 0.397, yet the two juices display similar antioxidant capacities as determined by both TEAC and FRAP. Juice of *R coreanus* (accession A), with poor antioxidant potential, had a low A/P of 0.012, as did the antioxidant-rich *R caucasicus* (A/P = 0.072). The representative red raspberry cultivar 'Glen Lyon' had an intermediate A/P of 0.183.

The anthocyanidin base composition of the juices is presented in Table 2. In most of the samples the dominant anthocyanidin was cyanidin, with lesser amounts of pelargonidin, peonidin, delphinidin and petunidin. Pelargonidin-containing anthocyanins were predominant in the four yellow and orange berries from *R sumatranus, R tsangorum, R hunanensis* and *R lambertianus*. Pelargonidin-containing anthocyanins also made a significant contribution to the anthocyanin pool of the antioxidant-rich berries of *R caucasicus* (30% of total).

Table 2. Anthocyanidin composition of *Rubus* berries

Sample	*Pg*	*Cy*	*Pn*	*Dp*	*Pt*
R coreanus (A)	0.090	0.874	0.000	0.036	0.000
R coreanus (B)	0.077	0.902	0.000	0.021	0.000
R coreanus (C)	0.038	0.924	0.000	0.038	0.000
R coreanus (D)	0.098	0.882	0.000	0.020	0.000
R coreanus (E)	0.030	0.932	0.000	0.038	0.000
R ursinus (A)	0.034	0.930	0.021	0.015	0.000
R ursinus (B)	0.018	0.952	0.012	0.018	0.000
R innominatus (A)	0.045	0.895	0.042	0.018	0.000
R innominatus (B)	0.022	0.944	0.014	0.020	0.000
R ulmifolius	0.020	0.964	0.002	0.014	0.000
R parvifolius	0.009	0.954	0.008	0.029	0.000
R caucasicus	0.302	0.677	0.003	0.018	0.000
R niveus	0.010	0.964	0.010	0.016	0.000
R sumatranus	0.605	0.395	0.000	0.000	0.000
R tsangorum	0.899	0.101	0.000	0.000	0.000
R hunanensis	0.893	0.089	0.000	0.000	0.018
R lambertianus	0.374	0.374	0.000	0.000	0.252
R idaeus (Glen Lyon)	0.028	0.798	0.007	0.167	0.000

Relative anthocyanidin profiles of black and red *Rubus* juices: Pg, pelargonidin; Cy, cyanidin; Pn, peonidin; Dp, delphinidin; Pt, petunidin; total 1.0. Malvidin derivatives were not detected in any sample.

DISCUSSION AND CONCLUSIONS

The involvement of free radicals, specifically their increased production, appears to be a feature of most, if not all human disease, including cardiovascular disease and cancer. As such, dietary antioxidants may be particularly important in fighting these diseases through affording protection against free radical damage to cellular DNA, lipids and proteins. The most intensely studied water-soluble antioxidant, ascorbic acid, has been shown in numerous supplementation studies to reduce the risk of developing cancer and heart disease. One of these studies shows that consumption of in excess of 750 mg day^{-1} reduces the risk of premature death by 60%.[19]

Ascorbic acid, however, is only one of many different antioxidant components found within animal and plant tissues. Because of the difficulty associated with the measurement of the individual antioxidant components of such complex mixtures, various methodologies have been developed for obtaining quantitative data from 'whole' samples. The two quantitative methods used in the present study, TEAC[16] and FRAP,[17] are just two of many such assays. These and other assays have been increasingly used to investigate fruit and vegetables to obtain antioxidant capacities of whole foodstuffs, amongst which red fruit appear to be particularly rich in antioxidant compounds.[3,5]

The present study has investigated the antioxidant capacities of novel germplasm of *Rubus* and analysed berry juices for compounds considered to contribute to this antioxidant potential, namely ascorbic acid, anthocyanins and phenolics. The total antioxidant potential of fruit extracts from *Rubus* species varied considerably. For example, as assessed by the TEAC method, the total antioxidant capacity of *R caucasicus* was approximately 800 times greater than that of the orange-coloured fruit from *R coreanus* (accession A). However, the antioxidative capacities of the darker, red accessions of *R coreanus* (accessions D and E) were only three times lower than that of *R caucasicus*. Similar results were obtained using the FRAP antioxidant assay. A representative red raspberry (*R idaeus*) cultivar ('Glen Lyon') yielded a high TEAC value (17.25) but only an intermediate FRAP value (25 569), indicating some discrepancy between the data obtained by the two assays.

An attempt has been made to rationalise the antioxidant potential in terms of the phenolic compounds present within the juices. In all the samples, ascorbic acid was found to make only a minor contribution to the total antioxidant capacity. This finding is in agreement with studies performed on grapes, grapefruit, tomato, orange and apple.[3] In the case of the antioxidant-rich *R caucasicus* we can estimate that the contribution by ascorbic acid to the total antioxidant capacity is around 3%. The majority of the antioxidant capacity thus appears to be associated with phenolics, of which the flavonoids are the dominant family. Flavonoids are low-molecular-weight polyphenolic compounds that are widely

distributed throughout fruit and vegetables,[20] many of which have been shown to possess antioxidant[21] and anticancer properties.[22] The *Rubus* species with highest antioxidant potential, *R caucasicus*, also had the highest phenolic content of 4.527 g kg^{-1}, close to that reported for blueberry samples analysed for antioxidant capacity.[5] Whilst analysed by another method (ORAC), that study reported antioxidant capacities of up to 45.9 μmol Trolox equivalents g^{-1}, considerably higher than those we now report for wild *Rubus*, although the use of a different assay and sample extraction with acetonitrile and acetic acid[5] hinders direct comparison. The same study demonstrated significant linear relationships between antioxidant capacity and both total anthocyanins and total phenolics. The present data lead to similar findings for the juices of *Rubus*.

Any association between the antioxidant potential of the juices and the proportion of phenolics present as anthocyanins (A/P) was not evident. This has also been demonstrated for blueberries.[5] It thus appears that the important criteria for high-antioxidant fruit are high total phenolics and high anthocyanin content, although the distribution between anthocyanins and non-anthocyanin flavonoids may be irrelevant. As indicated previously,[5] for a fruit such as blueberries where accumulation of flavonoids (and especially anthocyanins) occurs in the skin, an important factor associated with antioxidant capacity is the surface area/volume ratio of the berry. A small berry with a high ratio would be expected to possess a high anthocyanin and flavonoid content on a per weight basis and hence a high antioxidant potential. This would not be expected to apply to the same extent in *Rubus* where anthocyanins occur throughout the whole berry.

The breeding of *Rubus* cultivars has mainly focused on the fruit quality characters of berry size, shape, colour and firmness, pyrene size, soluble solids content, machine harvestability and, more recently, sensory characteristics.[23,24] In addition, a wide range of agronomic characteristics, such as pest and disease resistance, must be incorporated into a finished raspberry cultivar. However, the demands for particular fruiting characters from industry and end-users have, in some regards, resulted in a restriction of the genetic base. An estimate in 1970[25] suggested that 69% of all red raspberry cultivars released prior to this date had the cultivar 'Lloyd George' in their pedigrees, a figure that had risen to 90% for cultivars released between 1980 and 1989.[26] To remedy this, a number of measures were suggested, including the introduction of wild germplasm into the *Rubus* breeding programmes.

This investigation clearly shows the potential value of certain wild *Rubus* species, notably *R caucasicus*, within existing *Rubus* breeding programmes for the eventual improvement of nutritional value. At present, information on the absorption and metabolism of polyphenols, including the anthocyanins, is limited, although the study of bioavailability and metabolism of these phytomolecules is receiving much attention.[4,27] Clearly, the bioavailablity of these molecules would appear to be a prerequisite for potential health benefits associated through enhanced antioxidant capacity of berries.

R caucasicus, one of the non-Chinese types in this study, showed by far the highest antioxidant capacity. It is a native of temperate regions of Asia, mainly within the Russian Federation, producing small black fruits, sometimes in local cultivation,[28] and may therefore have some value as a specific donor of fruit quality generally. Studies on the heritability of antioxidant capacity are currently in progress at SCRI. Additionally, work on intraspecific hybridisation should produce an expansion of the genetic base of *Rubus* improvement programmes.

ACKNOWLEDGEMENTS

We thank Kirsten Wennstrom of the USDA-ARS for technical support and gratefully acknowledge the Scottish Executive Rural Affairs Department for funding this work.

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