Contribution of Cell Wall-Bound Compounds to the Antioxidant Capacity of Soft Fruit

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Abstract

Global studies have shown that increased consumption of fruit and vegetables is a major contributory factor in reducing the incidence of degenerative diseases. Current theory holds that diseases of ageing result from cumulative damage to cells by free radicals. This has provided an impetus to look for natural antioxidants with a view to increasing their dietary intake by humans. Soft fruit species such as *Rubus* (raspberry), *Ribes* (blackcurrant) and *Fragaria* (strawberry) contain many types of antioxidant compounds including ascorbic acid, flavonoids, phenols, anthocyanins etc. To date the contributions made by these individual classes to the overall antioxidative status have not been fully determined, nor has the influence of genetic variation both within and between species. In addition to this virtually all previous studies have concentrated on fruit extracts, or juices, whilst the cell wall material (pulp/pomace) has been disregarded. This is particularly important when assessing the total antioxidant capacity (TAC) of fruit since, more often than not, the whole fruit is consumed rather than just the juice. We have endeavoured to correct this oversight by carrying out some preliminary experiments with assays modified to assess solid samples. The contribution to TAC from associated and covalently bound species in *Fragaria* and *Ribes* is discussed

Materials and methods

Cell wall associated antioxidants
Blackcurrant fruit were puréed using a Waring blender and the purée centrifuged at 10,000 rpm for 45 mins. The pellet was washed twice in d.H_2O and re-centrifuged. The pellets were soxhlet extracted with d.H_2O, methanol or acetone for 4 hrs and the extracts subject to total phenols[1], TEAC[2] and FRAP[3] analyses.

Cell wall bound antioxidants
Blackcurrant (Ben Alder & Ben Lomond) and strawberry (Rhapsody & El Santa) fruit were puréed, washed, pelleted, methanol-soxhlet extracted and milled as above. The residues (0.5g) were extracted with 0.1 or 1.0 M NaOH for 16 hrs at 20°C. After centrifugation the supernatants were acidified with 1M TFA and extracted with ethyl acetate. The organic layer was concentrated, dissolved in methanol and subject to modified (antioxidant) TEAC and FRAP analyses. Briefly, cell wall material was pre-wetted in an appropriate buffer and the methods followed as described by Benzie and Strain[2] (FRAP) and Re *et al*[3] (TEAC) except that immediately prior to reading the absorbance the sample was quickly centrifuged to pellet the solid.

Results

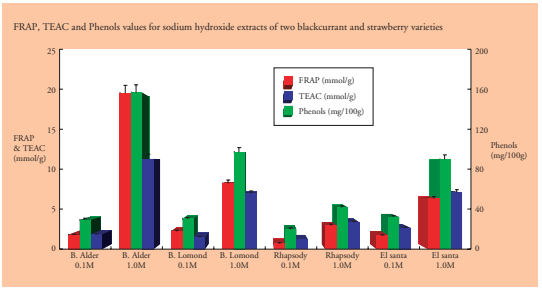
Cell wall associated Antioxidants
Soxhlet extraction with solvents of extreme polarity (acetone and water) did not result in the extraction of significant amounts of non-covalently bound anthocyanins. However extraction with medium polarity solvents, such as methanol and ethanol, did extract significant amounts of anthocyanins. Extraction of phenols was directly related to solvent polarity with 2-3 times more phenols extracted by water than by acetone. This suggests the majority of the non-covalently bound phenolics are present as glycosides. The extractions also reflect the varietal differences with Ben Alder containing a greater proportion of extractable anthocyanins and phenols. This is reflected in the TEAC and FRAP values with Ben Alder consistently the greater.

The importance of the wall associated anthocyanins, and hence whole fruit consumption, can be demonstrated by estimating their relative proportions in fresh fruit. By assuming that the cell wall accounts for ~30% of the fresh fruit weight (R. Brennan, Pers. Comm,) and that the remainder is juice with a specific gravity of 1.2, then 100g of blackcurrant fruit provides 355 mg anthocyanin in the juice and 487 mg anthocyanin associated with the cell wall.

Variety	Ben Alder					Ben Lomond				
Extraction solvent	Water	Methanol	Ethanol	Acetone	Juice*	Water	Methanol	Ethanol	Acetone	Juice*
Total Anthocyanins (mg 100g-1)*	0.0	1624.0	1330.0	93.0	608.6	15.0	733.0	610.0	117.0	539.9
Total phenols (mg 100g-1)*	2224.0	1906.0	919.0	924.0	673.8	1327.0	1051.0	452.0	572.0	533.9
Ascorbic acid (mg 100g-1)*	nd	nd	nd	nd	63.9	nd	nd	nd	nd	55.5
Ascorbic acid (millimolar)	nd	nd	nd	nd	3.6	nd	nd	nd	nd	3.1
TEAC (mmol/g)*	115.1	122.2	80.1	64.3	66.4	64.8	59.8	32.9	24.4	61.1
FRAP (mmol/g)*	203.1	212.0	140.5	107.3	118.6	113.6	101.4	56.1	43.1	110.8
% of TEAC contributed by ascorbic acid	nd	nd	nd	nd	5.4	nd	nd	nd	nd	5.1

* The values for the corresponding juices are expressed as (mg 100^{-1}) nd - not determined

Cell wall bound antioxidants
The value of alkali-extractable phenols and TEAC and FRAP activities were uniformly less in the 0.1M NaOH extracts although the distinction is less significant for the alkaline extracts of strawberry cell walls. As before the levels of phenols were greatest in the Ben Alder extracts although the levels are only 10% of those extracted by water alone (see table). The significant feature of the alkali extract values is the shift in relative FRAP and TEAC values on going from blackcurrant to strawberry. This suggests that the major species contributing to the TEAC and FRAP values are different. Although both TEAC and FRAP measure antioxidant activity their specificities are not the same, that is they are indicative of reducing species with very different one electron redox reduction potentials[4,5]. Generally the FRAP values are greater than the corresponding TEAC values but for the strawberry alkali extracts this is not the case suggesting that significantly different species are covalently bound to strawberry cell walls.



Conclusion

We have shown that a significant proportion of the total antioxidant capacity of fruit is associated with, and/or covalently bound to, the cell wall, especially for blackcurrant. Initial studies have shown that a large varietal variation in TAC exists and this may be exploitable in breeding strategies which encompass this parameter as an additional quality trait[6].

References
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