Antioxidants in soft fruit

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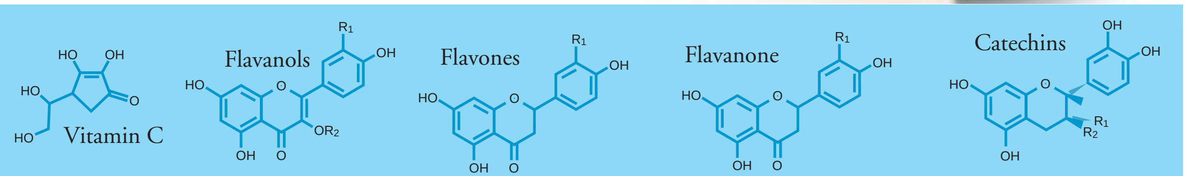
Introduction

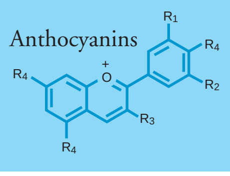
Now that the ability to produce sufficient quantities of food has largely been met in the developed world, the emphasis in agriculture and its associated research has shifted towards issues such as reduced production costs and more perceptible benefit to consumers through increased product quality and nutritive value. Within this latter area, a diverse group of plant compounds called 'antioxidants' has been the focus of intense research and associated economic and consumer interest. This is because it has been shown that oxidative stress, caused by an imbalance of pro-oxidants and antioxidants in the body, significantly increases the incidence of many degenerative and age-related diseases such as coronary heart disease (CHD), in particular atherosclerosis[1], and many cancers including those of the mouth, stomach and colon[2]. In addition, there is increasing evidence that changing one's diet to increase the intake of food relatively high in selected natural antioxidants, such as plant polyphenols, vitamin C, *etc.* (Fig. 1), can reduce the incidence of such diseases. Indeed, such a preventative, government-sponsored study[3], was initiated in 1972 in North Karelia, Finland, a region in which the diet was very similar to that which currently prevails in Scotland. In essence, the study aimed to reduce the consumption of saturated fat and double the fruit intake *per capita*. The researchers found that, over a period of 20 years, mortality rates caused by CHD and stroke were reduced by more than 50% in men and women. Those caused by cancer were reduced by 35% in men and 11% in women[3].

The underpinning mechanisms by which plant antioxidants exert their influences is best approached by bringing together the skills and knowledge of clinicians and nutritionists, with those of natural plant product chemists and plant breeders.

Soluble antioxidants

Antioxidant capacity Scottish soft fruit production is confined predominantly to genotypes of raspberry (*Rubus*), strawberry (*Fragaria*) and blackcurrant (*Ribes*). All of the cultivated varieties of these fruit are red or dark red coloured, to greater and lesser degrees, due to the presence of anthocyanins (Fig. 1). In addition, they also contain varying





Antioxidant	Anthocyanin	Flavonol/Flavone/Flavanone	Catechin
R1	H or OH	H or OH	H, OH, Glycosyl or Gallate
R2	H or OH	H or Glycosyl	H or OH
R3	H or Glycosyl	-	-
R4	OH or Glycosyl	-	-

Figure 1 The general chemical structures of vitamin C, anthocyanins, catechins, flavonols, flavone and flavanones.

amounts of catechins and flavonoids, compounds with structures similar to anthocyanins (Fig. 1). The intake of such compounds has previously been linked to reduced susceptibility to CHD[4]. At SCRI, extracts have been prepared from over 100 varieties of soft fruit and these have been analysed for their antioxidant capacity (ability to exert an antioxidant effect) using two independent *in vitro* methods (Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Ability of Plasma (FRAP)). Furthermore, their composition with regard to established antioxidants (phenols, anthocyanins, vitamin C, *etc.*) has been determined.

Overall, blackcurrant extracts have the greatest antioxidant activity, followed by raspberries, with strawberries marginally lower (Fig. 2). In general, and within species, varietal variation in antioxidant potential is minimal in strawberry but greater in raspberry. For example, a wild raspberry species (*R. caucasicus*, ex Russia) displayed an antioxidant potential almost double that of any of the cultivated varieties. Blackcurrant showed the greatest variability with regard to antioxidant potential. Some of the wild species, such as *Ribes sanguinium,* exhibit antioxidant levels more than double that found in the cultivated varieties.

As part of a collaborative EU project termed 'NORTHBERRY'[5], the SCRI fruit antioxidant study has been widened to include a selection of soft fruit species native to, or popular in, Scandinavia. These included rowan (*Sorbus aucuparia*), sea buckthorn

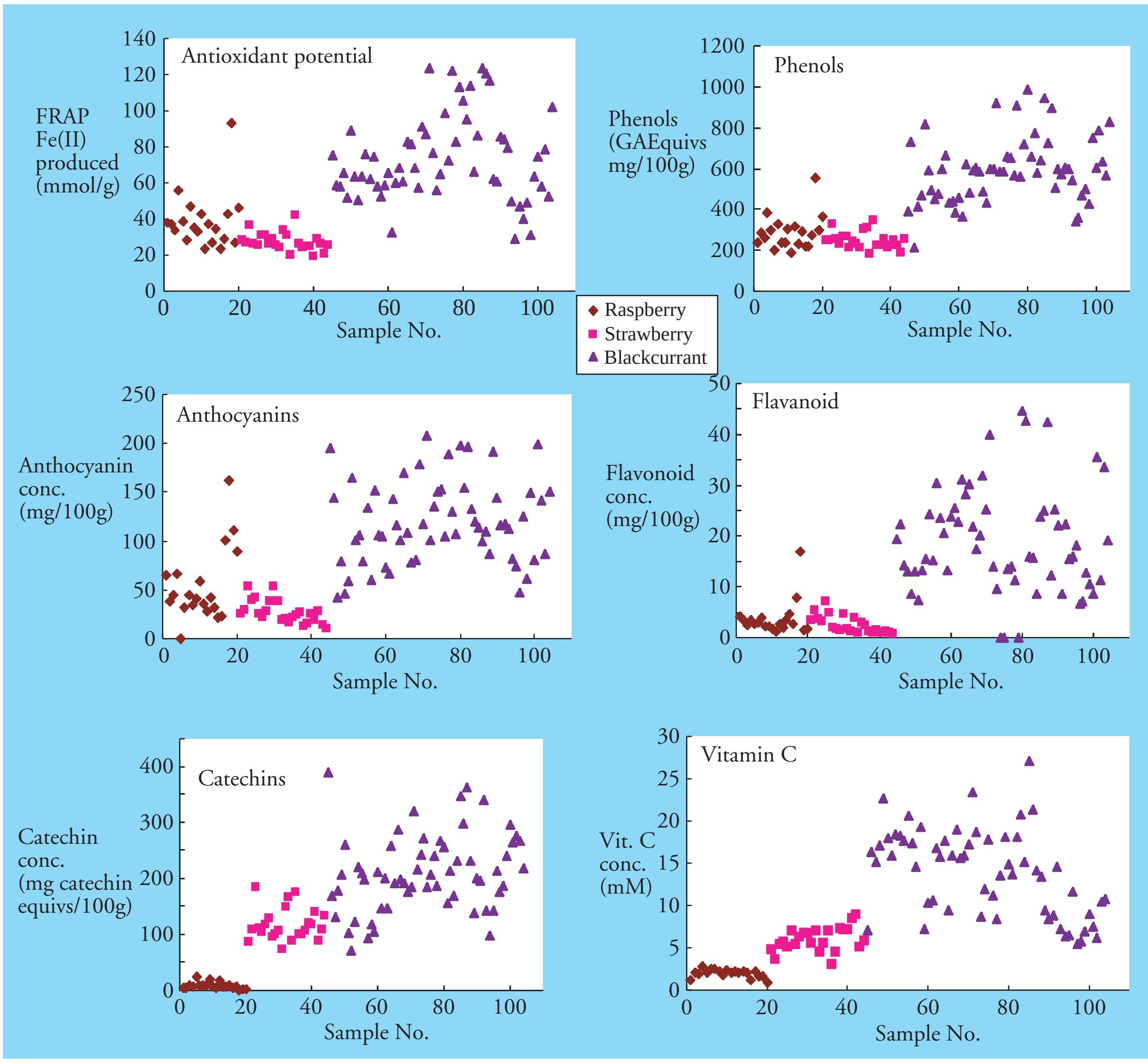


Figure 2 Species and varietal variation of antioxidant activity (FRAP), and anthocyanin, catechin, phenol, flavonoid and vitamin C contents of strawberry, raspberry and blackcurrant extracts.

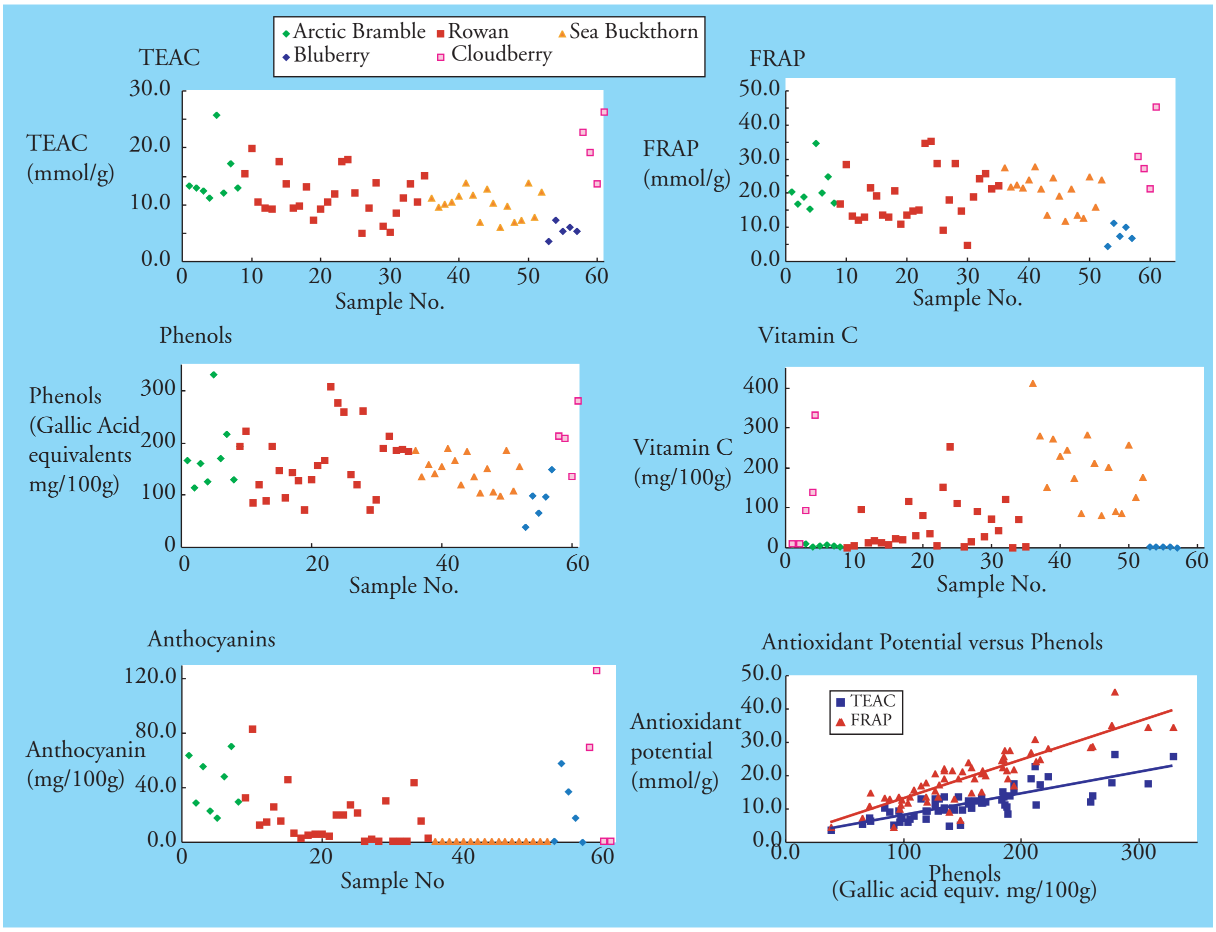


Figure 3 Species and varietal variation of antioxidant activity (TEAC and FRAP), and anthocyanin, phenol and vitamin C contents of extracts from fruit derived from the NORTHBERRY project.

(*Hippophae rhamnoides*), blueberry (*Vaccinium spp)*, arctic bramble (*Rubus articus*) and cloudberry (*Rubus chamaemorus)*. This study has also allowed different Rubus species to be compared e.g. *R. idaeus* (raspberry), with *R. articus, R. chamaemorus and R. caucasicus.*

Significantly, blueberry, one of the current favourite sources of fruit antioxidants in the USA, proved, by FRAP assay at least, to be a less effective source of antioxidants than blackcurrants (Figs 2 & 3). Overall, blackcurrant displayed a three-fold greater antioxidant potential than blueberry.

Great care must be taken when comparing the antioxidant potentials within and between species, since the relative rankings can change depending on the method used to measure such potential. For example, the FRAP assay indicates that the antioxidant potential of raspberry (*Rubus idaeus*) is greater than that of arctic bamble (*R. articus*) and cloudberry (*R. chamaemorus*). However, using the TEAC assay, cloudberry has the greatest overall antioxidant potential. This highlights the fact that methods used to assess antioxidant capacity measure, or are sensitive to, different chemical moieties and their relative concentrations within plant extracts. Indeed, the variable rankings of antioxidant potential between raspberry (*R. idaeus*), arctic bamble (*R. articus*) and cloudberry (*R. chamaemorus*) with the use of a different method of measurement, may well reflect the different chemistries of the Rubus species. Both raspberry and arctic bramble have low levels of vitamin C, whilst cloudberry generally contains higher levels. The anthocyanin and total phenol contents also exhibit a range of values. Phenol content is notably higher in raspberry than in either cloudberry or arctic bramble. When the values for total phenols are compared against antioxidant capacities, be they derived from TEAC, FRAP or even spectroscopy-based (EPR) methods, a good relationship exists between the two parameters (Fig. 3). Total phenol content can be measured relatively easily and could form the basis of a screen for soluble antioxidant capacity in fruit.

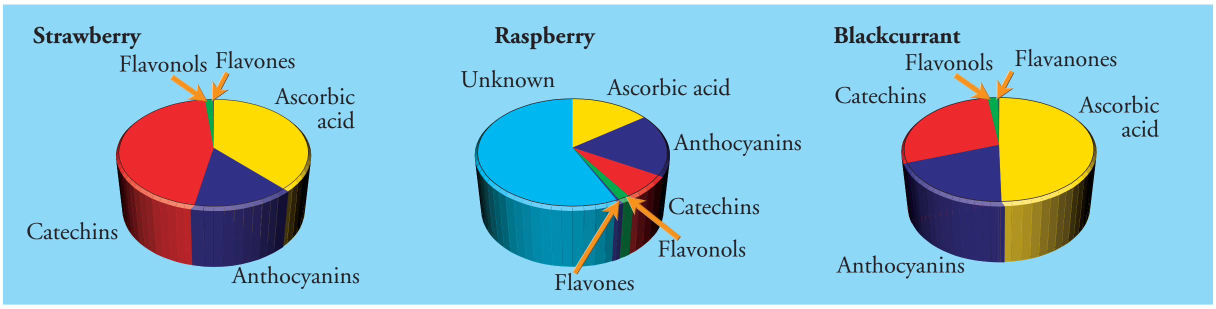


Figure 4 The relative contributions of components to the total antioxidant potential of strawberry, raspberry and blackcurrant extracts.

Composition of soluble antioxidant capacity in soft fruit The contribution made to total soluble antioxidant potential by vitamin C content is variable. For the various fruit(s) studied, the values are; blackcurrant 50%, strawberry 40% and raspberry 15%. Mass spectrometry indicates that the soft fruit analysed contained many polyphenolic compounds (Fig. 1). Anthocyanins accounted for about 20% of the antioxidant capacity of raspberry, blackcurrant and strawberry, whereas the contribution from flavonoids was minimal (<5%; Fig. 4). The contribution from catechins (Fig. 1) was least in raspberry (5-10%), greater in blackcurrants (25-20%) and greatest in strawberries (40-50% of the total antioxidant potential). Perhaps the most interesting feature is that more than half the soluble antioxidant capacity of raspberry remains unaccounted for. The direct relationship between total phenols and soluble antioxidant capacity suggest that the unknown fraction is probably phenolic and most likely glycosylated phenolics. Clarification of this is in progress.

Cell wall-associated antioxidants

The soft fruit studied are clearly excellent sources of antioxidants. However, virtually all published studies on plant antioxidants have confined themselves to soluble compounds and juices. Given that whole fruit is commonly eaten and that phenolics are ubiquitous in plant cell walls, it is possible that a significant proportion of the absolute antioxidant capacity of fruit is unaccounted for. Preliminary experiments have been undertaken to determine the presence of antioxidants associated with, or covalently bound to, the cell wall and the contribution they make to the total antioxidant capacity of the fruit.

Non-covalently bound cell antioxidants Anthocyanins were optimally extracted with medium polarity solvent (methanol and ethanol; Table 1). Extraction of phenols was directly related to solvent polarity with 2-3 times more phenols being extracted by water than by acetone. This suggests that the phenols are present as glycosides. Natural variation was evident, with levels of phenols greater in the blackcurrant cultivar Ben Alder than in cv. Ben Lomond. The importance of the non-covalently bound anthocyanins, and hence the importance of whole fruit consumption, can be demonstrated by comparing the relative proportions of soluble antioxidants in the juice and that present in whole fresh fruit. By assuming that the cell wall (pulp) 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 100 g fresh weight of blackcurrant fruit provides

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^{-1})*	115.1	122.2	80.1	64.3	66.4	64.8	59.8	32.9	24.4	61.1
FRAP (mmol g^{-1})*	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

Table 1 Composition and antioxidant capacities of solvent extracts of blackcurrant cell walls.

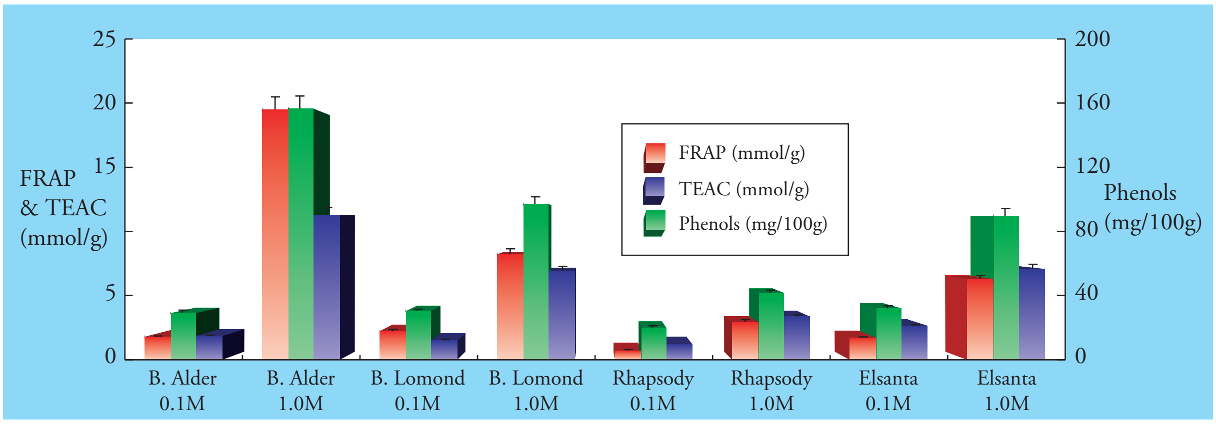


Figure 5 The antioxidant ability (TEAC and FRAP) and phenol contents for sodium hydroxide extracts of cell wall preparations of two blackcurrant and strawberry varieties.

355 mg anthocyanin in the juice and 487 mg anthocyanin associated with the cell wall!

Covalently bound cell wall antioxidants The levels of covalent, ester-bound, alkali-extractable phenols and TEAC and FRAP activities were uniformly less in 0.1 M than in 1.0 M NaOH extracts of both blackcurrant and strawberry varieties, although the distinction is less significant for strawberry (Fig. 5).

Although the levels of alkali-extractable phenols are only 10% of those extracted by water alone (Table 1), they follow a similar pattern to the non-covalently bound (methanol extractable) phenols and are greatest in the Ben Alder extracts.

Our studies indicate that soft fruit are undervalued as a source of natural antioxidants. Our research has shown that previous studies on fruit (and possibly vegetable) antioxidant capacity may have significantly underestimated the absolute antioxidant potential, since significant antioxidant activity remains associated and/or covalently bound to the cell wall. Also, given that covalently bound molecules contribute to a proportion of the overall antioxidant capacity, enzymes and metabolic pathways must exist which are responsible for their occurrence. These processes are targets for further study with a view to increasing the proportion of the covalently bounds antioxidants in fruit.

References

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