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Phytochemical analysis of boysenberry products

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Executive summary

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The aim of this project was to obtain a framework of phytochemical/functional data for a range of commercial processed Boysenberry product forms and other berry fruit benchmark products for use in marketing presentations. Many berry fruit, particularly blueberries and blackcurrants, have been the focus of attention over the last decade or more, especially with regards to their phenolic content, including anthocyanins, and for their antioxidant capacity. Boysenberries have received much less attention and hence there is a lack of phenolic and antioxidant data, but what data exists indicates they have considerable potential for promotion of their health benefits. In addition to the phenolics, the data points to Boysenberries having a folate content that contributes significantly to the recommended daily intake.

The samples analysed for this project included Boysenberry fruit, processed Boysenberry products and random commercial samples of items perceived as market benchmarks against which Boysenberry product forms may be judged for specific attributes. The following products were tested:

- Boysenberry products:
  - IQF whole Boysenberry fruit
  - 65 Brix Boysenberry juice concentrate
  - Natural strength Boysenberry seedless puree (consisting of two sets of two with different process procedures, reference A and F)
  - Boysenberry freeze-dried seedless powders (consisting of one sample of each of two process variants, CON and SLP)
  - Boysenberry extract freeze-dried powder (Oxi-Fend).
- Benchmark products:
  - IQF blackberry fruit
  - North American cultivated IQF blueberries
  - 50 Brix cranberry juice concentrate
  - 65 Brix blackcurrant juice concentrate
  - Pomegranate juice concentrate.

There were considerable differences in phenolic concentration (as determined by the standard Folin-Ciocalteu method) across the berry fruit products, when compared on a fresh weight basis (Table 1). When expressed on a dry weight basis there was significantly less variation in phenolic content, with the exception of Boysenberry Oxi-Fend Extract, which had almost six times more phenolics than most of the other samples (Table 2). One downside is that the F−C method suffers from a number of interfering substances, particularly ascorbic acid (vitamin C). However, for this set of samples it would appear that the phenolics are so much higher than the vitamin C concentrations that there is no interference.

In all samples but the Oxi-Fend powder anthocyanins were the predominant phenolic. However, a variety of other compounds were present, including significant amounts of ellagitannins in some samples. There was no relationship between total anthocyanin content and total phenolics across all samples either on a fresh weight or dry weight basis. Unlike the anthocyanins where the profile of compounds was the same across the different processed forms the phenolic composition differed across the various processed products. Even between batches of the same product types there was variation. There is no clear explanation for the variation in profile among the different samples. The appearance of some compounds in processed samples compared with the fresh fruit samples may indicate the release of bound phenolics. Some free ellagic acid was present in Boysenberry samples (mainly the concentrates) but there were significant amounts of ellagic acid released by hydrolysis and ellagitannins were detectable by HPLC. These results certainly differ from those reported previously from overseas analysis (large amounts of free ellagic acid and minimal in bound forms).

Processing appears to have different effects on the various phenolic classes. There were significant differences in anthocyanin concentrations between the two types of puree, and this was even greater once converted to a dry weight basis. This would indicate that the aseptic process results in loss of anthocyanins. Total phenolic concentration did not differ between the two processes (on a dry weight basis) indicating that the anthocyanins may be modified to other phenolics. It is surprising that the powder samples had comparatively less anthocyanins than would be expected, again pointing to their sensitivity to processing. Although the Oxi-Fend Boysenberry extract powder had much higher total phenolic content than the other two powders the anthocyanin contents were similar across all three powders. This indicates that the process may be adversely affecting the anthocyanins, perhaps resulting in the formation of complexes (polymers).

The ORAC and FRAP antioxidant capacity assays gave similar results with all samples showing strong activity. The trends observed were very similar to that of the total phenolics, and in fact there was a strong correlation between the two.

Besides the phenolics and their antioxidant activity Boysenberries have attracted some attention for their relatively higher folate content than other fruit. However, until now robust data were very limited. The data gathered here support the existing food composition database value for folate. Boysenberry products are a source of folate and although some is lost on processing significant amounts are still present.

The accumulated data gathered here highlights a number of attributes of Boysenberries that will be useful for marketing. Boysenberry fruit out-performed the two benchmark fruit (blackberries and blueberries) across all assays. The Boysenberry concentrates also performed well, outranking the cranberry and pomegranate concentrates. However, the blackcurrant concentrate was higher in phenolics and hence antioxidant activity. Despite this, the Boysenberry concentrates contained free ellagic acid and ellagitannins, not present in the blackcurrant concentrate sample. Even when concentrates are adjusted to natural strength (since this frequently reflects levels of commercial inclusion in finished products) Boysenberry performs well with only blackcurrant outperforming it across the board, except for ellagitannins where Boysenberry excels (Table 1). Although when expressed this way pomegranate has a higher total phenolic content Boysenberry has much higher anthocyanin content and antioxidant activity in both FRAP and ORAC assays. Pomegranate is on a par with Boysenberry for ellagic

acid (present in free and bound forms). Boysenberry concentrate is superior to cranberry in all attributes measured here.

In conclusion, this report identifies a number of valuable attributes of Boysenberries and products made from them. In particular, Boysenberries contain high concentrations of phenolics, especially anthocyanins and ellagic acid (present in various forms) plus folate. Although processing has some impact on phytochemical composition, particularly the anthocyanins, Boysenberry products such as concentrate, puree and powders are still valuable sources of these compounds. Together with the nutritional composition of selected Boysenberry products being collected in another project, the accumulated data will have use for marketing and promotional material. This report also highlights some areas for further work. LC-MS analysis is required if conclusive identification of phenolic compounds, particularly the ellagitannins, is wanted. Investigation of the effects of processing on phenolic composition may enable improvements to be made to reduce losses of compounds, such as the anthocyanins.

Table 1. Composition of berry fruit products (all values per 100 g fresh weight)

Sample ID	Dry matter (%)	TP (mg GAE)	ANC (mg)	Free EA (mg)	Free ET as EA **(mg)**	Total ET as EA **(mg)**	ET (mg)	T-ORAC (µmol TE)	PH-ORAC **(µmol TE)**	FRAP (µmol)	Total folate (µg)
Fruit											
Boysenberries #1	15.5	583[a]	234[c]	2[c]	101[c+e]	132[f]	68[c]	7,614[g]	6,914[a]	7,193[a]	65
Boysenberries #2	15.3	498[a]	218[c]	1[c]	96[c+e]	138[f]	69[c]	6,889[g]	6,419[a]	5,817[a]	80
Blackberries	14.8	401[a]	75[c]	6[c]	150[c+e]	226[f]	86[c]	4,434[g]	3,346[a]	5,113[a]	Na
Cultivated blueberries	18.9	431[a]	174[c]	nd[c]	3[c+e]	nd[f]	nd[f]	5,470[g]	6,876[a]	4,694[a]	na
Purees											
Aseptic Boysenberry seedless #1	12.7	418[a]	101[c]	7[c]	65[c+e]	123[f]	19[c]	6,263[g]	6,272[a]	6,264[a]	32
Aseptic Boysenberry seedless #2	14.0	469[a]	122[c]	9[c]	90[c+e]	151[f]	46[c]	6,345[g]	6,617[a]	6,702[a]	42
Frozen Boysenberry seedless #1	9.9	330[a]	133[c]	6[c]	75[c+e]	121[f]	20[c]	5,301[g]	5,567[a]	5,586[a]	40
Frozen Boysenberry seedless #2	10.0	319[a]	143[c]	7[c]	72[c+e]	104[f]	30[c]	5,831[g]	5,927[a]	5,888[a]	43
Concentrates[h]											
Boysenberry #1	66.0	2,476	749	67	427	461	167	42,533	42,533	31,211	120
Boysenberry #2	68.3	2,393	555	59	382	352	109	38,754	38,754	29,574	150
Blackcurrant	66.7	3,158	1386	nd	9	nd	nd	62,178	62,178	41,322	100
Cranberry	56.0	1,144	159	nd	nd	nd	nd	24,264	24,264	10,802	8.0
Pomegranate	72.0	2,956	46	20	312	287	nd	19,226	19,226	16,722	na
Concentrates adjusted to natural strength											
Boysenberry #1	8	305	92	8	53	57	21	5,235	5,235	3,841	15
Boysenberry #2	8	295	68	7	47	43	13	4,770	4,770	3,640	18
Blackcurrant	11.6	564	247	nd	2	nd	nd	11,096	11,096	7,374	18

Sample ID	Dry matter (%)	TP (mg GAE)	ANC (mg)	Free EA (mg)	Free ET as EA **(mg)**	Total ET as EA **(mg)**	ET (mg)	T-ORAC (µmol TE)	PH-ORAC **(µmol TE)**	FRAP (µmol)	Total folate (µg)
Cranberry	7.5	172	24	nd	nd	nd	nd	3,640	3,640	1,620	1.2
Pomegranate	12	546	8	4	58	53	nd	3,549	3,549	3,087	na
Powders											
Boysenberry Oxi-Fend extract	100	18,615[b]	392[d]	129[d]	811[d+e]	3,086[f]	713[d]	200,013[g]	295,702[b]	116,080[b]	530
Boysenberry concentrate F-D	100	2,573[b]	487[d]	13[d]	48[d+e]	209[f]	13[d]	27,256[g]	35,933[b]	25,207[b]	110
Boysenberry puree F-D	100	3,316[b]	344[d]	50[d]	341[d+e]	810[f]	35[d]	49,392[g]	59,220[b]	31,211[b]	150

TP = total phenolics (expressed as gallic acid equivalents), ACN = anthocyanins (expressed as cyanidin 3-glucoside equivalents), EA = ellagic acid (true free form, not released from free or bound ellagitannins), ET = ellagitannins (sum of individual ellagitannins, expressed as epicatechin equivalents), Free ET as EA = Free ellagitannins expressed as ellagic acid equivalents, Total ET as EA = free plus bound ellagitannins expressed as ellagic acid equivalents, T-ORAC = total Oxygen Radical Absorbance Capacity (sum of lipophilic and hydrophilic extractions), PH-ORAC = Phytochemicals & Health modified ORAC, FRAP = Ferric Reducing Antioxidant Power. See Appendix 1 for further explanations of some terms.

Key to extractions: [a] = 50% acetone, [b] = water, [c] = ethanol/water/formic acid (80:20:1), [d] = methanol/water/formic acid (50:50:1), [e] = hydrolysed with concentrated HCl (post extraction as given), [f] = hydrolysed with ethanol/HCl, [g] = two step process – lipophilic antioxidants extracted with hexane then subsequent extraction with acetone/acetic acid/water (70:29.5:0.5) to obtain hydrophilic antioxidants; [h] concentrate samples did not require extraction and were simply diluted in water/solvent as applicable to assay

[i] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Table 2. Composition of berry fruit products (all values per 100 g dry weight)

Sample ID	TP (mg GAE)	ANC (mg)	Free EA (mg)	Free ET as EA **(mg)**	Total ET as EA **(mg)**	ET (mg)	T-ORAC (µmol TE)	PH-ORAC (µmol TE)	FRAP (µmol)	Total folate (µg)
Fruit										
Boysenberries #1	3,763[a]	1,510[c]	13[c]	652[c+e]	852[f]	439[c]	49,123[g]	44,606[a]	46,406[a]	419
Boysenberries #2	3,257[a]	1,425[c]	7[c]	627[c+e]	902[f]	451[c]	45,026[g]	41,954[a]	38,020[a]	523
Blackberries	2,708[a]	507[c]	41[c]	1,014[c+e]	1,527[f]	581[c]	29,959[g]	22,608[a]	34,547[a]	na
Cultivated blueberries	2,281[a]	921[c]	nd[c]	16[c+e]	nd[f]	nd[c]	28,942[g]	36,381[a]	24,836[a]	na

Sample ID	TP (mg GAE)	ANC (mg)	Free EA (mg)	Free ET as EA **(mg)**	Total ET as EA **(mg)**	ET (mg)	T-ORAC (µmol TE)	PH-ORAC (µmol TE)	FRAP (µmol)	Total folate (µg)
Purees										
Aseptic Boysenberry seedless #1	3,294[a]	795[c]	55[c]	512[c+e]	969[f]	150[c]	49,315[g]	49,386[a]	49,323[a]	252
Aseptic Boysenberry seedless #2	3,351[a]	871[c]	64[c]	643[c+e]	1,079[f]	329[c]	45,321[g]	47,264[a]	47,871[a]	300
Frozen Boysenberry seedless #1	3,336[a]	1,343[c]	61[c]	758[c+e]	1,222[f]	202[c]	53,545[g]	56,232[a]	56,424[a]	404
Frozen Boysenberry seedless #2	3,192[a]	1,430[c]	70[c]	720[c+e]	1,040[f]	300[c]	58,310[g]	59,270[a]	58,880[a]	430
Concentrates[h]										
Boysenberry #1	3,751	1,135	102	647	698	253	64,444	64,444	47,289	182
Boysenberry #2	3,503	813	86	559	515	160	56,741	56,741	43,300	220
Blackcurrant	4,735	2,078	nd	13	nd	nd	93,220	93,220	73,789	150
Cranberry	2,042	284	nd	nd	nd	nd	43,329	43,329	16,195	14
Pomegranate	4,105	64	28	433	399	nd	26,703	26,703	23,225	na
Powders										
Boysenberry Oxi-Fend extract	18,615[b]	392[d]	129[d]	811[d+e]	3,086[f]	713	200,013[g]	295,702[b]	116,080[b]	530
Boysenberry concentrate F-D	2,573[b]	487[d]	13[d]	48[d+e]	209[f]	13	27,256[g]	35,933[b]	25,207[b]	110
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Key to extractions: [a] = 50% acetone, [b] = water, [c] = ethanol/water/formic acid (80:20:1), [d] = methanol/water/formic acid (50:50:1), [e] = hydrolysed with concentrated HCl (post extraction as given), [f] = hydrolysed with ethanol/HCl, [g] = two step process – lipophilic antioxidants extracted with hexane then subsequent extraction with acetone/acetic acid/water (70:29.5:0.5) to obtain hydrophilic antioxidants; [h] concentrate samples did not require extraction and were simply diluted in water/solvent as applicable to assay

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1 Introduction

The aim of this project was to obtain a framework of phytochemical/functional data for a range of commercial processed Boysenberry product forms and other berry fruit benchmark products for use in marketing presentations. Many berry fruit, particularly blueberries and blackcurrants, have been the focus of attention over the last decade or more, especially with regards to their phenolic content, including anthocyanins, and for their antioxidant capacity. Boysenberries have received much less attention and hence there is a lack of data, but what data exists indicates they have considerable potential for promotion of their health benefits. In addition to the phenolics, limited data points to Boysenberries having a folate content that contributes significantly to the recommended daily intake.

One of the major challenges is what data to gather and how to present it, especially with antioxidants since they are not nutrients and therefore there are no official methods or recommended daily intakes. However, there are a number of approaches that can be taken when examining antioxidants and their activity.

1.1 Background: approaches to quantifying antioxidants

The focus on antioxidants has led to a demand for information from very diverse groups. Scientists looking at epidemiological data require detailed information on the antioxidant composition and efficacy of foods when attempting to make correlations between particular foods, or food components, and protection from disease. Food manufacturers want antioxidant data on their products to promote their benefits and increase sales. In order to make meaningful comparisons between foods and to set standards for regulatory and health claims it is important that data collection is standardised. To be effective, information must be in a form, or forms, that is useful and useable to these diverse user groups. For example, there is no point requiring a level of detailed information for a food label that would be impossible to fit on that label in a readable form.

Analysis of antioxidants in foods is a complex issue, firstly because of the number of compounds involved but also because of the variety of ways in which they act and the different food matrices they are present in (Frankel & Meyer 2000). There are many times more antioxidant components than there are nutrients quantified for standard food composition databases. It is not possible to generalise about antioxidants in the same way as core nutrients. For example, most fruit and vegetables contain some carbohydrate, a small amount of protein and some of a range of about 20 vitamins and minerals. What differs most between different fruits and vegetables are not the nutrients themselves but the amounts of these nutrients. Antioxidants have a much larger possible spectrum of compounds and differing amounts, but there is also much less commonality: each fruit or vegetable has its own unique array of antioxidants.

It has been agreed that antioxidant methods must be standardised but there is no agreement on the best methods to use (Finley 2005). In an ideal world, scientists would assess all antioxidants *in vivo* but this is not practical as animal models and human studies are expensive and not suitable for screening purposes. Hence, *in vitro* assay tools are needed that also can be applied to foods. There are three possible general approaches to quantifying antioxidants, as follows.

1.1.1 Quantify antioxidants by class

The most widely found and quantitatively significant antioxidants are carotenoids and phenolics. Both of these classes of antioxidants can be quantified as a group, and hence this grouping may be a way to simplify quantification. There is consensus that the Folin-Ciocalteu (F-C) method is an appropriate assay for quantification of total phenolics (Prior et al. 2005) and simple spectrophotometric methods are available for total carotenoids (Goodwin 1955). There are limitations to these general assays and they do not always provide enough information to be meaningful, e.g. with regards to bioavailability. However, this approach has advantages over quantifying only a selected number of antioxidants, as shown when comparing data collected by the two methods. Many phenolics are unaccounted for when measuring only those compounds quantified in existing databases. This is largely because phenolic acids are not included in existing composition databases, yet they are present in significant amounts in fruits and vegetables. There is also a range of other flavonoids and phenolics. The measurement of total phenolics has the advantage of covering all the compounds, but it also generalises a group of chemicals very varied in terms of activity, bioavailability and effects in vitro.

1.1.2 Measure individual antioxidant compounds

Another approach to measuring antioxidants is to identify and quantify all the individual compounds present. Because of the complexity of the composition of foods, separating out each compound is a major undertaking. Although technical possible with modern instruments (Tsao & Deng 2004) this presents significant challenges and expense. There is a huge number of antioxidants and within this huge range there is diversity in their chemistry. Some antioxidants, such as vitamin C, vitamin E and selenium, are relatively simple to quantify and are already included in standard food composition databases (e.g. Plant & Food Research, FOODFiles 2010; U.S. Department of Agriculture, Agricultural Research Service 2010a). However, there are hundreds of carotenoids and thousands of flavonoids, and even though high performance liquid chromatography (HPLC) can easily be applied to quantify flavonoids (Lister et al. 1994), the task presents various challenges. One challenge is that standards are not always available, although LC-MS can also be applied to flavonoids (Marston & Hostettmann 2006; Fossen & Andersen 2006).

Databases for antioxidants have been developed over the past few years, although the focus is not on antioxidants *per se,* but the classes of compounds. The U.S. Department of Agriculture, Agricultural Research Service is building up food composition databases for carotenoids (initially a separate database but now included in the main nutrient database, U.S. Department of Agriculture, Agricultural Research Service 2010a), flavonoids (U.S. Department of Agriculture, Agricultural Research Service 2007), proanthocyanidins (U.S. Department of Agriculture, Agricultural Research Service 2004), and isoflavones (U.S. Department of Agriculture, Agricultural Research Service, 2008). With a huge number of potential compounds to be included it is obviously not practical to include everything, and the approach taken with these databases is just to quantify the most commonly occurring compounds. For example, the flavonoids are deglycosylated to quantify the aglycones and only a limited number of these are reported (Table 3). Although quantifying flavonoids as aglycones rather than glycosides simplifies their analysis, it removes a level of detail that may be important. There may be underestimation of the quantities of flavonoids/phenolics present in foods when the major flavonoids are not covered in those analysed. These methods also do not account for efficacy and the possible synergistic/antagonistic actions between antioxidants in a food mixture (Huang et al. 2005).

Table 3. The subclasses of flavonoids and selected compounds reported in the USDA flavonoid database.

Subclass	Compounds
Anthocyanidins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin
Flavan-3-ols	Catechins and gallic acid esters of catechins, epicatechins and gallic acid esters of epicatechins, theaflavins and gallic acid esters of theaflavins, thearubigins
Flavanones	Eriodictyol, hesperetin, naringenin
Flavones	Apigenin, luteolin
Flavonols	Isorhamnetin, kaempferol, myricetin, quercetin

1.1.3 Determine antioxidant capacity

Although quantifying individual or groups of antioxidants is important, it does not provide any indication of their effectiveness or the interactions that may occur between the different compounds. The solution is to measure activity, e.g. antioxidant capacity. There are currently between 25 and 100 different methods used to measure antioxidants. This makes it difficult to compare one plant extract with another, or the disease prevention potential of one functional beverage compared with another. In many cases there is no uniformity in the way antioxidants are evaluated. You don't know what you're getting, and that's not fair to consumers. In addition, there is always the controversy over what is being detected in total antioxidant capacity assays – only phenols, or phenols plus reducing agents plus possibly metal chelators. Several reviews of antioxidant capacity methodology have been published (Frankel & Meyer 2000; Huang et al. 2005; Prior et al. 2005) and some of the possible methods are summarised in Table 4. The obvious question at this point has to be “which of these antioxidant capacity assays is best?” Unfortunately there is no gold standard or “one-size-fits-all” method. In fact, there is a degree of controversy in this area, of sufficient interest and importance to have warranted two International Congresses on Antioxidant Methods. Results of the first congress have been summarised in a white paper (Finley 2005) but results of the second have not been published.

Table 4. Examples of some *in vitro* antioxidant capacity assays.

Assay	Mechanism	Reference
ABTS - TEAC = Trolox Equivalent Antioxidant Capacity	Radical scavenging, electron transfer	
	I – radical generated using metmyoglobin	Miller & Rice-Evans (1996)
	II – radical generated using manganese dioxide	Miller & Rice-Evans (1997)
	III– radical generated using potassium persulfate	Re et al. (1999)
DPPH	Radical scavenging, electron transfer	Brand-Williams et al. (1995)
FRAP Ferric Reducing Antioxidant Power	Radical scavenging, metal ion reduction, electron transfer	Benzie & Strain (1996)
LPIC Lipid Peroxidation Inhibition Capacity	Radical scavenging, electron transfer in lipid membrane setting	Zhang et al. (2006a)
ORAC Oxygen Radical Absorbance Capacity	Radical scavenging, electron transfer	Cao et al. (1993)
TRAP Total Radical-trapping Antioxidant Parameter	Radical scavenging, hydrogen atom transfer	Valkonen & Kuusi (1997)

It is not always possible to directly compare the results of different assays, and even within an assay results for standards can vary between different laboratories (Table 5). These differences may be due to slight differences in methodology (there are three commonly used versions of the TEAC assay) and to a host of other reasons (Frankel & Meyer 2000; Prior et al. 2005). Antioxidants can deactivate radicals by two major mechanisms, Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). The end result is the same, regardless of mechanism, but kinetics and potential for side reactions differ. HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation. Hence, many scientists feel these are most relevant to reactions where antioxidants typically act. SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals. SET and HAT mechanisms almost always occur together in all samples, with the balance determined by antioxidant structure and pH. The difficulty is that no single assay can capture the different modes of action of antioxidants and the different radical reactions involved. The total antioxidant capacity needs to reflect both lipophilic and hydrophilic capacity, and at least for physiological activity it needs to reflect and differentiate both hydrogen atom transfer (radical quenching) and electron transfer (radical reduction).

Table 5. Antioxidant capacity of various standards measured by different assays.

Antioxidant	ORAC	TEAC	DPPH	FRAP	LPIC
Vitamin C	0.95[a]	1.42[d]; 1.0[e]	3.7[h]	2.26[d]	
Vitamin E (α-tocopherol)	0.5[a]	1.0[e]			
Quercetin	6.47[a]; 3.56[b]; 2.7[c]	3.68[d]; 4.7[e]; 3.74[f]; 3.10[g]		4.0[c]; 7.39d; 3.73[f]	4.34[b]
Rutin	0.78[a]; 3.6[c]	2.4[e]; 1.45[f]		2.4[c]; 1.17[f]	3.62[b]
Kaempferol	2.1[c]	1.03[d]; 1.3[e]		1.8[c]; 1.95[d]	1.58[b]
Apigenin		2.01[d]; 1.5[e]		2.01[d]	0.36[b]
Catechin	2.35[b]; 3.9[c]	3.16[d]; 2.4[e]; 3.30[f]		1.8[c]; 2.47[d]; 1.26[f]	3.11[b]
Caffeic acid	2.1[c]	1.3[e]; 1.18[f]; 0.98[g]	9.1[h]	1.5[c]; 1.13[f]	3.92[b]
Chlorogenic acid	3.06[b]; 2.0[c]	1.45[d]; 1.3[e]; 1.00[f]		1.5[c]; 3.22[d]; 0.99[f]	2.57[b]
Ferulic acid	1.26[b]	0.98[d]; 1.9[e]; 3.51[f]; 1.90[g]	2.33[h]	1.33[d]; 1.40[f]	2.23[b]

[a]Aruoma 2003; [b]Zhang et al. 2006a & b; [c]Aaby et al. 2004; [d]Soobrattee et al. 2005; [e]Rice-Evans et al. 1997; [f]Nilsson et al. 2005; [g]Re et al. 1999; [h]Brand-Williams et al. 1995.

Prior et al. (2005) proposed that three methods (ORAC, F-C phenolics assay, and TEAC) should be standardised for use in the routine quality control and measurement of antioxidant capacity of dietary supplements and other botanicals. This choice of methods is based upon two methods with differing reaction mechanisms, with one utilising the peroxyl radical because of its predominance in biological systems and the other the SET mechanism utilising the ABTS radical (although another SET assay such as the FRAP could also be used). The F-C phenolics assay provides a third option for a simple, speedy, inexpensive, and robust assay that does not require specialised equipment, but can be automated for high-throughput assay. The ORAC assay represents a biologically relevant mechanism, one that can measure both lipophilic and hydrophilic antioxidant capacity and is adapted for high-throughput assay. Ultimately it is

desirable that a number of different assays or types of assay will be used to measure antioxidant capacity. However, this presents considerable challenges on how to interpret this information and present it in a meaningful way on food products.

1.1.4 Conclusions

So which of these approaches do we take to measure antioxidants? There is no simple answer, as each approach has its advantages and disadvantages (Table 6). No single approach provides the total picture and a combination of data is probably the best, especially when comparing foods. For example, a product could contain measures of total phenolics alongside any components from these groups of particular relevance (e.g. anthocyanins). In addition there could be a measure of efficacy (antioxidant capacity). To make real comparison across different foods this requires a large database of information to be built from which appropriate information can be selected depending on need. There is still some way to go before such comprehensive databases are established, especially containing Australasian data.

Table 6: Summary of the pros and cons of the possible approaches to quantifying antioxidants.

Method		
Measure individual antioxidant compounds	Pros	accurate quantification provides the detail that may be required for research purposes has significance for bioavailability, etc.
	Cons	so many compounds = huge databases not always practical to quantify all compounds analysis expensive & time consuming interpretation of data more complex level of detail not understood by general public
Quantify antioxidants by class (e.g. total phenolics, total carotenoids)	Pros	good coverage (don't miss individual compounds not analysed) makes comparison between foods easier cheap, less complex analysis simpler to present on products and easier for consumer to understand
	Cons	not always totally accurate (e.g. generalise about response of individual compounds) misses a level of detail that may be important
Determine antioxidant capacity	Pros	measure of efficacy not just chemical quantity if assay appropriate can demonstrate interactions/synergies captures activity of 'unknown' compounds
	Cons	need multiple assays to fully represent different modes of action and reactions with different reactive species doesn't portray the range of antioxidant compounds present relevance to *in vivo* efficacy uncertain

1.2 Experimental approach for this project

1.2.1 Phenolic composition

As discussed above phenolics can be quantified in two main ways: 1) in total as a class and 2) by specific compounds. For this project it is proposed to do both.

Total phenolics by Folin-Ciocalteu method

Straight F–C analyses of total phenolics are perceived as meaningful for customer response to comparative results. The F−C assay has for many years been used as a measure of total phenolics in natural products, but the basic mechanism is an oxidation/reduction reaction and, as such, can be considered another antioxidant method. The original F−C method developed in 1927 originated from chemical reagents used for tyrosine analysis in which oxidation of phenols by a molybdotungstate reagent yields a coloured product with λ_{max} at 745−750 nm. The method is simple, sensitive, and precise. However, the reaction is slow at acid pH, and it lacks specificity. Singleton and Rossi (1965) improved the method with a molybdotungstophosphoric heteropolyanion reagent that reduced phenols more specifically; the λ_{max} for the product is 765 nm. They also imposed mandatory steps and conditions to obtain reliable and predictable data: (1) proper volume ratio of alkali and F−C reagent; (2) optimal reaction time and temperature for colour development; (3) monitoring of optical density at 765 nm; and (4) use of gallic acid as the reference-standard phenol. The improved method outlined by Singleton and Rossi (1965) specified the conditions to minimise variability and eliminate erratic results.

The relationship between the F–C method and antioxidant capacity measurements by ORAC is usually good; however, differences in the way the antioxidant components in different foods react in this method differ from that of the HAT mechanism of ORAC.

One downside is that the F−C method suffers from a number of interfering substances, particularly sugars, aromatic amines, sulfur dioxide, ascorbic acid (vitamin C) and other enediols and reductones, organic acids, and Fe(II) (Everette et al. 2010). Correction for interfering substances should be made. In most plants, phenolics are the most abundant antioxidants present. Therefore, the F–C assay gives a good “ballpark” estimation of total phenolic content for most plants except in some cases, e.g. where the vitamin C content is high. Correction factors for vitamin C have been developed. These involve both the use of specific assays for vitamin C and extraction methods that remove most vitamin C (Everette et al. 2010). It is possible to remove vitamin C by pre-treating the samples with ascorbate oxidase. It is expected, based on NZ Concise Food Composition data, that the vitamin C content of the Boysenberry product samples will have little effect on F–C for the main range of Boysenberry processed samples but might contribute significantly to the F–C of blackcurrant juice concentrate.

Quantification of individual phenolics by UPLC

Anthocyanins and other phenolics can be separated and quantified by HPLC/UPLC (further details on this analysis and in Appendix 2). There are limitations to accuracy depending on the availability of standards and peak identification. By using spectral data and retention time it is possible to roughly identify compounds but if conclusive identification is required them LC-MS must be carried out. For UPLC analysis, it is expected that the following compounds can be identified:

- Anthocyanins: expressed as cyanidin 3 glucoside equivalents (individual anthocyanins identified)

- Flavonol glycosides: expressed as rutin equivalents (myricetin, kaempferol and quercetin glycosides identified)
- Flavonols (aglycones): as quercetin equivalents (myricetin, kaempferol and quercetin identified if present)
- Flavanols (catechins): expressed as epicatechin equivalents (catechin and epicatechin identified if present)
- Cinnamic acid derivatives: expressed as chlorogenic acid equivalents (chlorogenic acid + isomers, coumaryl and quinic acid glycosides identified if present)
- Ellagitannins and ellagic acid: expressed as both epicatechin and ellagic acid equivalents for specific compounds, such as those described in Appendix 2 (note: composition may vary from this for non-Boysenberry samples).

There has been some published data on the phenolic content of Boysenberry fruit, although not other products. The most studied group of compounds has been the anthocyanins. Torre & Barritt (1977) reported that Boysenberries contained cyanidin 3-glucoside along with cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside. However, the methods they used are outdated and hence these identifications are questionable. Wada & Ou (2002) reported cyanidin 3-(6'-p-coumaryl)glucoside-5-glucoside and cyanidin 3-glucoside in relatively similar amounts. McGhie et al. (2006), on the other hand, reported three major peaks and four minor peaks. The major peaks were cyanidin 3-[2-(glucosyl)glucoside], cyanidin-3-[2-(glucosyl0-6-(rhamnosyl)glucoside] and cyanidin 3-glucoside. Scalzo et al. (2008) reported a similar composition but with slightly different proportions of the anthocanins. Cyanidin 3-rutinoside was present at a higher ratio and similar in concentration to the cyanidin 3-glucosylrutinoside, and cyanidin 3-xylosylrutinoside and cyanidin 3-sambubioside were also noted although in very low amounts. McGhie et al. (2006) use a different extraction solvent to that used by Wada & Ou (2002), which may be one explanation for the differences in the results or there may be differences between varieties.

Few other phenolics have been noted in the literature. Wada & Ou (2002) noted small amounts of gallic acid (9 mg/100 g) but rutin and isoquercetin were not detected. Two papers report on ellagic acid/ellagitannins. There has been a great deal of interest in ellagic acid as a potential anticarcinogen (Maas & Galletta 1991). However, published data on the ellagic acid content of various foods are limited. Strawberries, raspberries, and thornless blackberries (*Rubus eubatus*) have been reported to be good sources of ellagic acid (Daniel et al 1989; Wang et al. 1994; Rommel & Wrolstad 1993). Wada & Ou (2002) suggested that Boysenberry included relatively more free ellagic acid in addition to ellagitannins. Total ellagic acid content was around 70 mg/100 g (47–90 mg for other berry fruit including blackberries and raspberries). This result has been open to question and no published data exists for New Zealand Boysenberries in the product forms for commercial supply. However, Kool et al. (2010) note that acidic hydrolysates contain substantial amounts of ellagic acid and these may be derivated from ellagitannins. These authors reported four ellagitannin: galloyl-sanguiin H-6 (possible artefact of lambertianin C), sanguiin H-10 (an isomer), sanguiin H-6 and sanguiin H-2. As noted in above these compounds can be analysed by UPLC. Quantification of both free ellagic acid and total ellagic acid content following hydrolysis of the ellagitannins will be performed.

1.2.2 Antioxidant capacity

As noted above (Section 1.1.3) there are many different methods for measuring antioxidant capacity. Two methods were chosen for this project because they reflect different mechanisms discussed above – the ORAC assay utilizing HAT reaction mechanism and the FRAP assay utilizing the SET reaction mechanism.

ORAC assay

The ORAC assay is based upon the early work of Ghiselli et al. (1995) and Glazer (1990), as developed further by Cao et al. (1993). ORAC measures antioxidant inhibition of peroxyl radical induced oxidations and thus reflects classical radical chain breaking antioxidant activity by H atom transfer. In the basic assay, the peroxyl radical reacts with a fluorescent probe to form a non-fluorescent product, which can be quantitated easily by fluorescence. As originally configured, the ORAC assay is limited to measurement of hydrophilic chain breaking antioxidant capacity against only peroxyl radicals. This ignores lipophilic antioxidants that are particularly important against lipid oxidation in all systems as well as other radicals (HO•, HOO•, ONOO•, O2•-, etc.) that are very reactive physiologically. To be made more broadly applicable, the ORAC assay has been adapted to measure lipophilic as well as hydrophilic antioxidants using a solution of 50% acetone/50% water (v/v) containing 7% randomly methylated β-cyclodextrin (RMCD) to solubilise the antioxidants (Huang et al. 2002; Wu et al. 2004). The lipophilic and hydrophilic components are selectively extracted before assay (Prior et al. 2003). Researchers at the USDA have been using the ORAC assay to develop a database for total antioxidant capacity in foods (Wu et al. 2004).

The aim of this project is to obtain ORAC measurements that customers could compare with published databases such as USDA ORAC contents for other fruit and to see the pattern of ORAC retained after processing. Internal benchmarks will be included for comparison (blackcurrant and cranberry juice concentrates and a random sample of North American cultivated blueberry fruit) to allow internal comparison with Boysenberry in the analysis data set using same lab and methodology.

FRAP assay

The FRAP assay was originally developed by Benzie and co-workers (Benzie 1996; Benzie & Strain 1996) to measure reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals (Benzie & Szeto 1999; Ou et al. 2002; Gil 2000; Pellegrini et al. 2003; Proteggente et al. 2002). The FRAP assay is regarded by some as a useful assay because it directly measures antioxidants or reductants in a sample (Halvorsen et al. 2002). The TEAC and the ORAC assay, and many other antioxidant assays, are more indirect because they are based on the antioxidant's ability to react with or neutralise free radicals generated in the assay systems. The FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. Because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants. The results of many antioxidant assays depend strongly on the type of reactive species used. The FRAP assay, in contrast, uses antioxidants as reductants in a redox-linked colorimetric reaction. Furthermore, the other assays, but not the FRAP assay, use a lag phase type of measurement. One possible disadvantage with the FRAP assay is the fact that this assay does not react with thiols, because the reduction potential for thiols generally are below that of the Fe^{3+}/Fe^{2+} half-reaction. However, because only limited amounts of plant glutathione are absorbed by humans (Schafer & Buettner 2001), and almost no other antioxidant thiols are present in dietary plants (one exception is garlic, see below), the FRAP method may be suitable for assessment of total antioxidants in plants.

Because the redox potential of Fe(III)-TPTZ is comparable with that of ABTS•+, similar compounds react in both the TEAC and FRAP assays. Reaction conditions differ, though: TEAC is carried out at neutral pH, and the FRAP assay is conducted at acidic pH 3.6 to maintain iron solubility. Reaction at low pH decreases the ionisation potential that drives electron transfer and

increases the redox potential, causing a shift in the dominant reaction mechanism. Thus, TEAC and TRAP may give comparable relative values, but FRAP values are usually lower than TEAC values for a given series of antioxidant compounds (Pulido et al. 2000; Cao & Prior 2001; Erel 2004). Often, FRAP values have a poor relationship to other antioxidant measures. It has been argued that the ability to reduce iron has little relationship to the radical quenching processes (H transfer) mediated by most antioxidants. However, oxidation or reduction of radicals to ions still stops radical chains, and reducing power reflects the ability of compounds to modulate redox tone in plasma and tissues. The FRAP mechanism is totally electron transfer rather than mixed SET and HAT, so in combination with other methods can be very useful in distinguishing dominant mechanisms with different antioxidants.

Like the ORAC assay the FRAP assay is one of the few antioxidant assays with large pools of data for a range of foods. There are several papers providing extensive collections of FRAP data (e.g. Carlsen et al. 2010; Halvorsen et al. 2002, 2006). A database of FRAP values is to be available online at the University of Oslo's website.

The combination of ORAC and FRAP values should provide useful data because of the differences between the assays.

1.2.3 Folate

The terms folic acid and folate are often used interchangeably for this water-soluble B-complex vitamin. Folic acid, the more stable form, occurs rarely in foods or the human body but is the form most often used in vitamin supplements and fortified foods. Naturally occurring folates exist in many chemical forms. Folates are found in foods as well as in metabolically active forms in the human body (Institute of Medicine, Food and Nutrition Board 1998). Folate functions as a coenzyme in single-carbon transfers in the metabolism of nucleotides and amino acids. It is essential for the formation of thymidylate (TMP) for DNA synthesis, so that without folate, living cells cannot divide. The need for folate is higher when cell turnover is increased, such as in fetal development. It is also involved in purine synthesis, in the generation of formate and in amino acid interconversions. Homocysteine is methylated by methyl-THF (MTHF) to produce methionine, which is in turn used for the synthesis of S-adenosyl-methionine an important methylating agent *in vivo* (Wagner 1996).

Folate is difficult to measure in foods because it is present in different forms, so food databases can be inaccurate. However, the main sources of folate in Australia and New Zealand according to the National Nutrition Surveys undertaken in 1995 and 1997, respectively (Australian Bureau of Statistics 1998; Ministry of Health 1999), are cereals, cereal products and dishes based on cereals (about 27%) and vegetables and legumes (about 29%). Fruit provides about 8–10%. Orange juice is contributing a greater amount than in the past due to the recent introduction of fortification with folate. As the target compound is a core nutrient it is relatively easy to set recommended intake levels as there are published RDIs (recommended daily intakes). The RDI is "the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98 per cent) healthy individuals in a particular life stage and gender group" (NHMRC 2006). Note the following targets apply only to Australia and New Zealand as the RDIs will vary from country to country. Table 7 gives the EAR (Estimated Average Requirement), RDI and upper limit (UL; suggested upper intake levels) (as given in NHMRC 2006). Intakes of folate in the Australian and New Zealand populations are currently significantly below the RDI proposed here, with median intakes of about 300 µg/day for men and 230 µg/day for women. The current 90th percentile of intake of 416 µg/day in men is close to the new RDI and that of women (303 µg/day) close to the new EAR. The studies above indicate that an additional 100–

400 µg/day over current intakes may be required to optimise homocysteine levels and reduce overall chronic disease risk and DNA damage.

Table 7. Recommendations on intakes of folate.

Age group & gender		Folate as dietary folate equivalents		
		AI		UL
Infants	0–6 mo.	65		BM
	7–12 mo.	80		B/F
		EAR	RDI	UL
Children	1–3 yr	120	150	300
	4–8 yr	160	200	400
Boys	9–13 yr	250	300	600
	14–18 yr	330	400	800
Girls	9–13 yr	250	300	600
	14–18 yr	330	400	800
Men	19–30 yr	320	400	1,000
	31–50 yr	320	400	1,000
	51–70 yr	320	400	1,000
	>70 yr	320	400	1,000
Women	19–30 yr	320	400	1,000
	31–50 yr	320	400	1,000
	51–70 yr	320	400	1,000
	>70 yr	320	400	1,000
Pregnancy	14–18 yr	520	600	800
	19–30 yr	520	600	1,000
	31–50 yr	520	600	1,000
Lactation	14–18 yr	450	500	800
	19–30 yr	450	500	1,000
	31–50 yr	450	500	1,000

Abbreviations: AI adequate intake; BM, amount normally received from breast milk; B/F, amount in breast milk and food; EAR, estimated average requirement; RDI, recommended dietary intake; UL, Upper Level of Intake. See Appendix 1 for further explanation of some of these terms.

Note: For some of the nutrients the term 'equivalent' has been used to express the recommendations (e.g., Vitamin A is expressed in Retinol Equivalents, folate in Dietary Folate Equivalents; vitamin E in alpha-tocopherol equivalent).This reflects the fact that for some nutrients there is more than one chemical form in the food supply that provide a benefit. For example, for folate, there is naturally occurring food folate as well as folic acid used for food fortification. Folic acid is twice as active as food folate so not as much is needed to get the same biological benefit. The overall requirement may be met by a mixture of these so is expressed as dietary folate equivalents.

In addition the NHMRC (2006) has made recommendations on folate intake for prevention of chronic disease. An additional 100–400 µg DFE over current intakes (i.e. a total of about 300–600 µg DFE) may be required to optimise homocysteine content and reduce overall chronic disease risk and DNA damage. Current population intakes are well below the new recommended intakes. Increased consumption through replacement of nutrient-poor, energy-dense foods and drinks with folate-rich foods such as vegetables and fruits and wholegrain cereals is recommended as the primary strategy.

The folate content in Boysenberry fresh fruit has been highlighted in NZ Food composition tables and in market place as higher than in other fruits. Little data exists on folate in New Zealand Boysenberry fruit and the further processed variants. There are two objectives in

relation to folate analysis: 1) To compare contents in commercial samples of IQF fruit with those of other fruits for ingredient use, and 2) to obtain indication of the comparative level of folate following processing into other product forms.

2 Methods

2.1 Samples

The samples analysed were as follows (with the number of individual batch samples shown in brackets):

a) IQF whole Boysenberry fruit (2)

b) 65 Brix Boysenberry juice concentrate (2)

c) 50 Brix cranberry juice concentrate (1)

d) 65 Brix blackcurrant juice concentrate (1)

e) Natural strength Boysenberry seedless puree (4) (consisting of two sets of two with different process procedures reference A and F)

f) Boysenberry extract freeze-dried powder (1)

g) Boysenberry freeze dried seedless powders (2) (consisting of one sample of each of two process variants CON and SLP).

The total number of samples as above is 13. In addition, random commercial samples of items perceived as market benchmarks against which Boysenberry product forms may be judged for specific attributes were included:

h) North American cultivated IQF blueberries as benchmark for total anthocyanins, phenolics by F-C and antioxidant capacity

i) Pomegranate juice concentrate as benchmark for ellagitannins/ellagic acid, plus phenolics by F-C and antioxidant capacity

j) IQF blackberry fruit as benchmark for ellagitannins/ellagic acid plus phenolics by F-C and antioxidant capacity.

For specific sample descriptions and their unique identifiers see Table 8.

Table 8. Sample descriptions and their unique identifiers.

Sample ID	PFR#
Fruit	
IQF Boysenberries Sample 1	2246
IQF Boysenberries Sample 2	2247
IQF blackberries	2248
Cultivated blueberries CAN020	2249
Puree	
Aseptic Boysenberry seedless puree sample 1	2244
Aseptic Boysenberry seedless puree sample 2	2245
Frozen Boysenberry seedless puree sample 1	2284
Frozen Boysenberry seedless puree sample 2	2285

Sample ID	PFR#
Concentrate	
Boysenberry juice concentrate sample 1	2239
Boysenberry juice concentrate sample 2	2240
Blackcurrant juice concentrate	2241
Cranberry juice concentrate	2242
Pomegranate juice concentrate sample 0729-1309-1	2243
Powder	
Boysenberry Oxi-Fend Extract	2286
Boysenberry concentrate freeze-dried	2287
Boysenberry puree freeze-dried	2288

2.1.1 Sample handling/preparation

All products were held frozen until time of use for analysis, apart from the powder samples (items f and g above) which were held chilled in a refrigerator. The juice concentrate frozen samples, which over time can be subject to layering by freeze concentration within the container, were thoroughly mixed in order to obtain representative samples for analysis in duplicate. Solid samples were extracted for analysis and the particular extraction method was specific to the particular assay (these are detailed below).

2.1.2 Dry matter

In order to make full comparisons between samples the dry matter of all samples was determined. This was done by an oven dry method until samples had reached a stable weight.

2.2 Phenolic composition

2.2.1 Total phenolics

These analyses were carried out by the Phytochemicals and Health Laboratory at Lincoln. Fruit and puree samples were extracted with 50% acetone while powders were solubilised in water (complete solution was achieved with this whereas they were not with 50% acetone). Concentrates were diluted with water as appropriate to bring into range. Total phenolics in the extracts or the liquid samples were measured using Folin-Ciocalteu reagent, adapted from the method of Spanos & Wrolstad (1990) and based on original method of Singleton and Rossi (1965) with the modifications for improved accuracy. This assay is based on the colour reaction of phenolics with a phosphomolybdic-phosphotungstic acid reagent (Folin-Ciocalteu reagent). Absorbance of the samples at 765 nm was compared with a control and a gallic acid standard curve, with results expressed as gallic acid equivalents (mg GAE/100 g).

In addition to the standard method a paired aliquot of sample/extract was treated with ascorbate oxidase to remove any vitamin C present and hence eliminate possible interference.

2.2.2 HPLC analysis

The following analyses were conducted by Tony McGhie's lab, Palmerston North.

Sample extraction

A weighed portion of each sample was extracted or diluted with ethanol/water/formic acid and then diluted for analysis by UHPLC. Concentrate samples were made to an exact volume. After the initial run it was apparent that the three powder samples (PFR#2286-2288) did not fully extract in this solvent system. Hence an experiment was conducted to optimise extraction. Note that in the meantime the UHPLC system was changed and the repeats of the powders samples were actually run by LC-MS. The data from the solvent trial is given in Table 9. To explain the data, the top block is the original data. The second block is those same extracts analysed and quantified by LC-MS. The numbers are a little different but quite good considering there is a different basis for quantitation. The 3rd, 4th, and 5th blocks are variations of the extraction procedure. The 1 g sample extracted with methanol give the best results and this was confirmed visually in that in these extract there was only a clear/white residue left, that is all the anthocyanin is transferred into the liquid phase. The concentrate powder seemed to contain a filler/drying aid and it was suspected that it was maltodextrin and may have explained why ethanol was not a good solvent. However, the only additive in the sample of FD powder from concentrate was 5% sipernat anti-caking agent added at time of milling of dry product and maltadextrin is not therefore apparent cause of behaviour.

The comparison between DAD and MS data is reasonably good, with the exception of the ellagitannin values which show considerable differences between the DAD and MS results. This will be because the results are calculated as epicatechin equivalents. Ellagitannins concentrations calculated as 'epicatechin equivalents' should be treated as estimates only as epicatechin is quite a different compound to the ellagitannin. Quantitation of ellagitannins is a work in progress. The 'total ellagitannin' value calculated as ellagic acid equivalents would be the best data for comparison between sample and for comparison to previously published data.

Table 9. Comparison of different solvents for the extraction of anthocyanins from the three powder samples.

		Anthocyanin Concentration (mg/100 g FW)			
	Solvent	Cy-sop	Cy-glurut	Cy-glu	Cy-rut
Original DAD Data (48 hrs, 5 g sample)					
Oxi-Fend Extract	EtOH/H_2O/FA 80:20:1	42	17	3	6
Concentrate FD	EtOH/ H_2O /FA 80:20:1	33	17	22	3
Puree FD	EtOH/ H_2O /FA 80:20:1	118	64	79	8
LCMS Original Extracts					
Oxi-Fend Extract	EtOH/ H_2O /FA 80:20:1	51	18	nd	4
Concentrate FD	EtOH/ H_2O /FA 80:20:1	35	11	14	nd
Puree FD	EtOH/ H_2O /FA 80:20:1	135	60	47	6
LCMS Repeat (72 hrs, 5 g sample)					
Oxi-Fend Extract	EtOH/ H_2O /FA 80:20:1	266	88	6	23
Concentrate FD	EtOH/ H_2O /FA 80:20:1	143	61	46	8
Puree FD	EtOH/ H_2O /FA 80:20:1	140	66	53	7
LCMS Repeat (72 hrs, sonication, 1 g sample)					
Oxi-Fend Extract	EtOH/ H_2O /FA 80:20:1	157	58	nd	16
Concentrate FD	EtOH/ H_2O /FA 80:20:1	139	63	50	nd
Puree FD	EtOH/ H_2O /FA 80:20:1	138	61	53	nd

	Solvent	Anthocyanin Concentration (mg/100 g FW) Cy-sop	Cy-glurut	Cy-glu	Cy-rut
LCMS Methanol (72 hrs, sonication, 1 g sample)					
Oxi-Fend Extract	MeOH/ H_2O /FA 50:50:1	270	97	nd	25
Concentrate FD	MeOH/ H_2O /FA 50:50:1	253	125	95	14
Puree FD	MeOH/ H_2O /FA 50:50:1	185	87	72	nd

nd = not detected; Cy-sop = cyanidin 3-sophoroside; Cy-glurut = cyanidin 3-glucosylrutinoside; Cy-glu = cyanidin 3-glucoside; Cy-rut = cyanidin 3-rutinoside

Polyphenol quantification

The concentrations of polyphenolic components were determined by UHPLC. Compounds were separated using a standard reversed phase column with a binary solvent gradient elution (A = 0.5% H_3PO_4, B = acetonitrile). The different classes of phenolic compounds were detected and quantified as follows:

- Anthocyanins: detected at 530 nm and quantified as cyanidin 3-glucoside equivalents
- Flavonols: detected at 370 nm and quantified as quercetin 3-glucoside equivalents
- Flavanols: detected at 210 nm and quantified using authentic standards
- Cinnamic acids: 325 nm and quantified as 3-caffeoyl quinic acid (chlorogenic) equivlants.
- Ellagitannins: 210 nm and quantified as epicatechin equivalents.

Ellagic acid analysis

True free ellagic acid was quantified directly in the extracts as used above. Ellagitannins are often measured as ellagic acid equivalents following acid-hydrolysis. Two measures of ellagitannin were carried out on the berry fruit samples. Firstly, a portion of the extract prepared for polyphenol (fruit and puree samples were extracted with ethanol/water/formic acid, powder samples with methanol/water/formic acid and concentrates diluted as required with ethanol/water/formic acid) was hydrolysed with concentrated HCl at 80ºC and the ellagic acid that was generated was measured. This is referred to as ‘free ellagitannin’. Secondly, the original samples (fruit, powder, or concentrate) were hydrolysed with an ethanol/conc HCl mixture and the ellagic acid that was generated was measured. This is referred to as bound ellagitannins. Ellagic acid was detected at 370 nm and quantified using an authentic standard of ellagic acid.

2.3 Antioxidant capacity

These analyses were carried out by the Phytochemicals and Health Laboratory at Lincoln.

2.3.1 ORAC

The Oxygen Radical Absorbance Capacity (ORAC) assay is one of the most popular tests being used today to rank the antioxidant potential of foods. The ORAC assay measures antioxidant inhibition of peroxyl radical-induced oxidation (Cao et al. 1993). The procedure used was based on a previous report by Ou and co-workers (2001). Trolox, a water-soluble analogue of vitamin E, was used as a control standard.

Samples were prepared using the hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC) method developed by Prior et al. (2003). Samples were extracted with hexane, followed by centrifugation and removal of the hexane layer to give the L-ORAC fraction. Residual hexane was evaporated, and then the residue was extracted with 10 mL of acetone/water/acetic acid, (70:29.5:0.5, v/v/v) to give the H-ORAC fraction. The total antioxidant capacity ORAC (T-ORAC) score is the sum of these two numbers.

In addition we ran our standard total ORAC method was run using the samples as for total phenolics, i.e. fruit and puree samples were extracted with 50% acetone while powders were solubilised in water (complete solution was achieved with this whereas they were not with 50% acetone). Concentrate samples do not require extraction and were simply diluted as appropriate.

2.3.2 FRAP

This assay measures the ability of a substance to reduce Fe^{3+} to Fe^{2+} using TPTZ at pH = 3.6 (Benzie & Strain 1996).The formation of a blue-coloured TPTZ-ferrous ion complex is measured spectrophotometrically at 593 nm.

Extracts were prepared as for the total phenolics assay, i.e. fruit and puree samples were extracted with 50% acetone while powders were solubilised in water (complete solution was achieved with this whereas they were not with 50% acetone). Concentrate samples do not require extraction and were simply diluted as appropriate.

2.4 Folate

Folate (total folates) was analysed by the National Measurement Institute in Australia using the standard microbiological method used for food composition databases.

3 Results and discussion

3.1 Dry matter

In order to make full comparisons in was important to take into account the varying water content of the samples. The dry matters of all berry fruit samples are given in Table 10 (note powders are already dry). In most cases the pairs of the same types of products were similar in dry matter but there was quite a difference in the dry matter content of the two different puree processes.

Table 10. Dry matter of samples analysed

Sample ID	PFR#	Dry matter (%)
Fruit		
Boysenberries #1	2246	15.5
Boysenberries #2	2247	15.3
Blackberries	2248	14.8
Cultivated blueberries	2249	18.9
Purees		
Aseptic Boysenberry seedless #1	2244	12.7
Aseptic Boysenberry seedless #2	2245	14.0
Frozen Boysenberry seedless #1	2284	9.9
Frozen Boysenberry seedless #2	2285	10.0
Concentrates		
Boysenberry #1	2239	66.0
Boysenberry #2	2240	68.3
Blackcurrant	2241	66.7
Cranberry	2242	56.0
Pomegranate	2243	72.0

3.2 Phenolic composition

3.2.1 Total phenolics (F–C method)

There were considerable differences in phenolic concentration (as determined by the standard Folin-Ciocalteu method) across the berry fruit products, when compared on a fresh weight basis (Table 11). The highest phenolic concentration was in the Boysenberry Oxi-Fend Extract and the lowest in the Boysenberry frozen seedless puree samples. When expressed on a dry weight basis there was significantly less variation in phenolic content, with the exception of Boysenberry Oxi-Fend Extract which had almost six times more than most of the other samples. When concentrates were adjusted to natural strength (since this frequently reflects levels of commercial inclusion in finished products) Boysenberry had a lower phenolic content than the blackcurrant and pomegranate but higher than cranberry.

Table 11. Total phenolic content (expressed as gallic acid equivalents, GAE, per 100 g on both a fresh and dry weight basis) in berry fruit samples. Fruit and puree samples were extracted with 50% acetone, powder samples solubilised with water and concentrates simply diluted as required.

Sample ID	Total phenolics (mg GAE/100 g FW)	Total phenolics (mg GAE/100 g DW)
Fruit		
Boysenberries #1	583	3,763
Boysenberries #2	498	3,257
Blackberries	401	2,708
Cultivated blueberries	431	2,281
Purees		
Aseptic Boysenberry seedless #1	418	3,294
Aseptic Boysenberry seedless #2	469	3,351
Frozen Boysenberry seedless #1	330	3,336
Frozen Boysenberry seedless #2	319	3,192
Concentrates		
Boysenberry #1	2,476	3,751
Boysenberry #2	2,393	3,503
Blackcurrant	3,158	4,735
Cranberry	1,144	2,042
Pomegranate	2,956	4,105
Concentrates adjusted to natural strength[a]		
Boysenberry #1	305	-
Boysenberry #2	295	-
Blackcurrant	564	-
Cranberry	172	-
Pomegranate	546	-
Powders		
Boysenberry Oxi-Fend extract	18,615	18,615
Boysenberry concentrate F-D	2,573	2,573
Boysenberry puree F-D	3,316	3,316

[a] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Comparing the Boysenberry samples alone, on a fresh weigh basis total phenolic content was higher in the powders (particularly the Oxi-Fend Extract) than in the concentrates, followed by the fruit, with the purees being lowest. However, on a dry weight basis all were relatively similar with the exception of Boysenberry Oxi-Fend Extract. The Boysenberry fruit samples differed significantly between the two batches but both were higher than the benchmark fruit (blackberries and blueberries), both on the basis of fresh and dry weights. There were also significant differences in the total phenolic contents of the four Boysenberry purees when expressed on a fresh weight basis. The two asceptic samples were significantly higher than the two frozen seedless purees. However, when converted to a dry weight basis all four samples had relatively similar total phenolic contents. On the other hand, the two Boysenberry concentrate samples had very similar phenolic contents. The Boysenberry concentrates were lower in phenolics than the blackcurrant concentrate but much higher than the cranberry concentrate and slightly lower than the pomegranate concentrate.

Whilst the seedless puree are not made from the same lines as the IQF Boysenberries analysed the F–C analyses on the seedless puree samples are in the same ball park as the IQF whole fruits expressed on a dry weight basis. If there is little transfer of phenolics from the seed to the puree during processing and the pulp contributes only 77% to the dry weight of whole fruit the contribution from phenolics extraction from the seed might be a significant contributor to the whole fruit total phenolics content by F–C. The only way to determine the degree to which the seeds contribute would be to analyse them separately. There are some reports in the literature of the phenolic content of Rubus seeds. Boysenberry seeds had a total phenolic content of 4,910 mg per 100 g and blackberries were similar (Bushman et al. 2004).

In addition to the standard phenolic assay a modification of the assay was carried out to eliminate any potential interference from vitamin C. None of the samples showed a significant drop in measured phenolics (Table 12), in fact some samples showed a slight increase, although virtually all samples were within the normal assay variability. It is apparent from these results for this set of samples that the phenolics are so much higher than the vitamin C concentrations that there is no interference. Even the blackcurrant concentrate did not show a drop in phenolic content with the removal of vitamin C. We do know that in other berry fruit products, e.g. Barkers blackcurrant concentrate and commercial blackcurrant and apple juices, the vitamin C does cause interference and that addition of ascorbate oxidase significantly reduces the measured phenolic content.

Table 12. Total phenolic content (expressed as gallic acid equivalents, GAE, per 100 g fresh weight) in berry fruit samples with the removal of interference from vitamin C by addition of ascorbate oxidase (asc ox). Fruit and puree samples were extracted with 50% acetone, powder samples solubilised with water and concentrates simply diluted as required.

Sample ID	Total phenolics (mg GAE/100 g FW)	Total phenolics + asc ox (mg GAE/100 g FW)
Fruit		
Boysenberries #1	583	559
Boysenberries #2	498	514
Blackberries	401	420
Cultivated blueberries	431	455
Purees		
Aseptic Boysenberry seedless #1	418	415
Aseptic Boysenberry seedless #2	469	462
Frozen Boysenberry seedless #1	330	328
Frozen Boysenberry seedless #2	319	314
Concentrates		
Boysenberry #1	2,476	2,301
Boysenberry #2	2,393	2,232
Blackcurrant	3,158	3,215
Cranberry	1,114	1,436
Pomegranate	2,956	2,975
Powders		
Boysenberry Oxi-Fend extract	18,615	19,124
Boysenberry concentrate F-D	2,573	2,765
Boysenberry puree F-D	3,316	3,303

Comparative published data for total phenolics is shown in Table 13. However, there is a lack of published data for many processed products and so values for the relevant fruits are given. One of the Boysenberry fruit samples had a phenolic content in the range of what we have previously measured but one was slightly higher (although on a par with a single value reported by Wada & Ou 2002). Both the blackberry and blueberry total phenolic contents measured in this study are in the range of the published data. One paper (Muller et al. 2010) reported the phenolic content of Boysenberry puree (from a German supplier); their value of 330 mg/100 g is almost identical to the frozen purees measured in this study. The phenolic content of the Boysenberry concentrates measured here was similar to what we have measured previously, as was the blackcurrant concentrate. We found no published data for cranberry concentrate (only juices) but based on the fruit values it would be expected to be lower than the Boysenberry or blackcurrant concentrates as found here. Only one published phenolic value for pomegranate concentrate was found (Muller et al. 2010) and this is much lower than that measured here.

Table 13. Comparative and published data for total phenolic content in berry fruit (expressed as gallic acid equivalents, GAE, per 100 g fresh weight). Note sample preparation varied.

Sample ID	Total phenolics (mg GAE/100 g FW)
Fruit	
Boysenberries	400–550[a]; 599[b]
Blackberries	300–640[a]; 477[c]; 412[d]; 173–305[e]; 174–197[g]; 417–555[h]
Cultivated Blueberries	225–740[a]; 311[c]; 285[d]; 44–362[f]; 261–585[h]
Blackcurrants	650–1,150[a], 1,202[c]; 498–1,342[i]
Cranberry	220[a]; 503[c]; 287[d]; 120–177[j]; 315[k]
Pomegranate	338[c,d]
Puree	
Boysenberry	330[l]
Concentrates	
Boysenberry	2,500–3,200[a]
Blackcurrant	3,000–4,700[a]; 2,340[m]
Pomegranate	848[l]

[a] Data from Phytochemicals & Health Group; [b] Wada & Ou 2002; [c] U.S. Department of Agriculture, Agricultural Research Service 2010a; [d] Wolfe et al. 2008; [e] Koca & Karadeniz 2009; [f] You et al. 2011; [g] Milivojevic et al. 2011; [h] Sellappan et al. 2002; [i] Moyer et al. 2002; [j] Wang & Stretch 2001; [k] Zheng & Wang 2003; [l] Muller et al. 2010 (note for the pomegranate samples values were adjusted using the dilution factor provided in the paper); [m] Bermudez-Soto & Tomas-Barberan 2004 (note value per 100 ml rather than gram)

3.2.2 Anthocyanins by HPLC

The total anthocyanin contents (sum of individual peaks measured by HPLC) in the berry fruit samples are given in Table 14. The blackcurrant concentrate had the highest anthocyanin concentration followed by the Boysenberry concentrates. The powder samples were next highest followed by the fruit samples (with the exception of blackberries which had the lowest anthocyanin concentration of all the products measured), with the lowest anthocyanin concentrations in the puree samples. The anthocyanin concentrations in Boysenberry fruits were higher than both blueberries and blackberries. When concentrates are adjusted to natural strength (since this frequently reflects levels of commercial inclusion in finished products) Boysenberry performs well with only blackcurrant outperforming it.

There were significant differences in anthocyanin concentrations between the two types of puree, and this was even greater once converted to a dry weight basis. This would indicate that

the aseptic process results in loss of anthocyanins. Total phenolic concentration did not differ between the two processes (on a dry weight basis) indicating that the anthocyanins may be modified to other phenolics. It is surprising that the powder samples were comparatively lower than would be expected in anthocyanins despite a modified extraction procedure. Although the Oxi-Fend Boysenberry extract powder had much higher total phenolic content than the other two powders the anthocyanin contents were similar across all three powders. This indicates that the process may be adversely affecting the anthocyanins. It was noted that once on solution the powder had a brownish tint rather than pure purple. This may indicate complexes have formed.

Table 14. Total anthocyanin content (expressed as cyanidin 3-glucoside equivalents, CGE, per 100 g on both a fresh and dry weight basis) in berry fruit samples. Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1).

Sample description	Total Anthocyanins (mg CGE/100 g FW)	Total Anthocyanins (mg CGE/100 g DW)
Fruit		
Boysenberries #1	234	1,510
Boysenberries #2	218	1,425
Blackberries	75	507
Cultivated blueberries	174	921
Purees		
Aseptic Boysenberry seedless #1	101	795
Aseptic Boysenberry seedless #2	122	871
Frozen Boysenberry seedless #1	133	1,343
Frozen Boysenberry seedless #2	143	1,430
Concentrates		
Boysenberry #1	749	1,135
Boysenberry #2	555	813
Blackcurrant	1,386	2,078
Cranberry	159	284
Pomegranate	46	64
Concentrates adjusted to natural strength[a]		
Boysenberry #1	92	-
Boysenberry #2	68	-
Blackcurrant	247	-
Cranberry	24	-
Pomegranate	8	-
Powders		
Boysenberry Oxi-Fend extract	392	392
Boysenberry concentrate F-D	487	487
Boysenberry puree F-D	344	344

[a] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

There was no relationship between total anthocyanin content and total phenolics across all samples either on a fresh weight or dry weight basis (even with the outlier Boysenberry Oxi-Fend sample excluded). The relationship was strengthened when only Boysenberry samples were examined, but only with the outlier Oxi-Fend extract data removed. See Appendix 3 for details.

Comparative published data for total anthocyanins is shown in Table 15. Like the total phenolics, there is a lack of published data for many processed products and so values for the relevant fruits are given. In most cases the values obtained in this study are in line with what has been reported. The exception is the blackberry fruit sample where total anthocyanins were lower than what has been published previously (although total phenolics were in line with published data). The two Boysenberry fruit samples are at the higher end of the published data, and notably higher than overseas data.

Table 15. Comparative and published data for total anthocyanin content in berry fruit (generally expressed as cyanidin 3-glucoside equivalents, CGE, per 100 g fresh weight). Extraction solvents varied between studies.

Sample ID	Total anthocyanins (mg CGE/100 g FW)
Fruit	
Boysenberries	190–210[a]; 131[b]; >160[c]; 3–366 (avg. 151)[d]
Blackberries	151–360[a]; 91[b]; 83–244[c]; 90[e]; 95–158[f]; 125–152[g]
Cultivated Blueberries	117–180[a]; 25–495[c]; 49–423[d]; 18-29[f]; 116–224[h]; 89–331[i]
Blackcurrant	350–850[a]; 250[c] ; 96–720[d]; 272[e]; 128–411[j]
Cranberry	50[a]; 78[c]; 92[e]; 20-66[k]
Pomegranate	5–27[l]; 138[m]
Concentrates	
Boysenberry	500–650[a]
Blackcurrant	500–1,200[a]; 78[n]

[a] Data from Phytochemicals & Health Group; [b] Wada & Ou 2002; [c] Mazza & Miniati 1993; [d] Scalzo et al. 2008; [e] USDA Flavonoid Database release 2.1 2007; [f] Koca & Karadeniz 2009; [g] Pantelidis et al. 2007; [h] You et al. 2011; [i] Ehlenfeldt & Prior 2001; [j] Moyer et al. 2002; [k] Wang & Stretch 2001; [l] Tehranifar et al. 2010; [m] Kulkarni & Aradhya 2005; [n] Bermudez-Soto & Tomas-Barberan 2004 (note value per 100 ml rather than gram).

Examining the profile of individual anthocyanins there are major differences between the berry fruit types but within the Boysenberries the profile was basically the same (Table 16). In addition, Tables 18–23 show quantification of the individual anthocyanins in each of the berry fruit types. Boysenberries showed four main peaks although they do also contain cyanidin 3-xylrutinoside as a minor component, but this could not be separated from cyanidin 3-glucoside. For the two Boysenberry fruit samples the anthocyanin profiles were very similar, although sample #1 had a slightly higher proportion of cyanidin 3-sophoroside and lower cyanidin 3-glucoside (Table 24). Looking at the processed products compared to the frozen fruit it would appear that cyanidin 3-glucoside is more sensitive to loss as it proportionally decreases while the sophoroside is more stable and forms a greater percentage of total anthocyanins in the processed products. However, overall the changes are not that dramatic.

Blackberries had the simplest profile with only two anthocyanins present while blueberries contained at least 13 anthocyanins. Blueberries contain Dp-ara, Cy-gal, Pn-gal, Pn-glu, and Pn-ara but individual measures could not be obtained due to coelution with other anthocyanins (for full names of anthocyanins see Table 17). Anthocyanin components of cranberry and pomegranate were identified by reference to published scientific reports.

Table 16. Presence of the different anthocyanins in the berry fruit samples (also see Appendix 4 for chromatograms). Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1).

	Anthocyanin[a]																			
Sample ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Fruit																				
Boysenberries #1					●				●	●		●								
Boysenberries #2					●				●	●		●								
Blackberries										●		●								
Cultivated blueberries[b]			●	●			●	●		●	●		●	●	●	●	●		●	●
Purees																				
Aseptic Boysenberry seedless #1					●				●	●		●								
Aseptic Boysenberry seedless #2					●				●	●		●								
Frozen Boysenberry seedless #1					●				●	●		●								
Frozen Boysenberry seedless #2					●				●	●		●								
Concentrates																				
Boysenberry #1					●				●	●		●								
Boysenberry #2					●				●	●		●								
Blackcurrant				●		●				●		●								
Cranberry								●		●			●		●			●		
Pomegranate	●	●		●						●										
Powders																				
Boysenberry Oxi-Fend extract					●				●	?[c]		●								
Boysenberry concentrate F-D					●				●	●		●								
Boysenberry puree F-D					●				●	●		?[c]								

[a] 1 = delphinidin-diglucoside, 2 = cyanidin-diglucoside, 3 = delphinidin-galactoside; 4= delphinidin-glucoside, 5 = cyanidin-sophoroside, 6 = delphinidin-rutinoside, 7 = delphinidin-arabinoside, 8 = cyanidin-galactoside, 9 = cyanidin-glucosylrutinoside, 10 = cyanidin-glucoside, 11 = petunidin-galactoside, 12 = cyanidin-rutinoside, 13 = cyanidin-arabinoside, 14 = petunidin-glucoside, 15 = peonidin-galactoside, 16 = petunidin-arabinoside, 17 = malvidin-galactoside, 18 = peonidin-arabinoside, 19 = malvidin-glucoside, 20 = malvidin-arabinoside

[b] Note for blueberries Dp-ara and Cy-gal coelute, but Dp-ara is by far the major component this peak was quantified and labelled as Dp-ara. Pn-gal, Pn-glu and Pn-ara are minor components in most blueberries and probably coelute with Pt-ara, Mv-gal and Mv-glu respectively. This cannot be confirmed as standards are not commercially available

[c] Small amounts of these compounds were detected in original analysis but not in the samples re-run with improved extraction (see Table 9)

Table 17. Abbreviations and full names of the anthocyanins cited in this report.

Abbreviation	Full name
Cy-ara	Cyanidin 3-arabinoside
Cy-diglu	Cyanidin 3,5-diglucoside
Cy-gal	Cyanidin 3-galactoside
Cy-glu	Cyanidin 3-glucoside
Cy-glurut	Cyanidin 3-glucosylrutinoside
Cy-rut	Cyanidin 3-rutinoside
Cy-sop	Cyanidin 3-sophoroside = glyucosyl-glucoside
Dp-ara	Delphinidin 3-arabinoside
Dp-gal	Delphinidin 3-galactoside
Dp-glu	Delphinidin 3-glucoside
Dp-rut	Delphinidin 3-arabinoside
Mv-ara	Malvidin 3-arabinoside
Mv-gal	Malvidin 3-galactoside
Mv-glu	Malvidin 3-glucoside
Pn-ara	Peonidin 3-arabinoside
Pn-gal	Peonidin 3-galactoside
Pt-ara	Petunidin 3-arabinoside
Pt-gal	Petunidin 3-galactoside
Pt-glu	Petunidin 3-glucoside

Table 18. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE) in the Boysenberry samples expressed on a fresh weight basis (mg/100 g FW) with figures in brackets on a per 100 g dry weight basis (note powder samples already dry). See Table 17 for full names of anthocyanins. Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1).

Sample ID	Anthocyanin Concentrations (mg CGE)				
	Total	Cy-sop	Cy-glurut	Cy-glu	Cy-rut
Fruit					
Sample #1	234	104	44	78	7
	(1,510)	(671)	(284)	(503)	(45)
Sample #2	218	86	39	86	7
	(1,425)	(562)	(255)	(562)	(46)
Purees					
Aseptic seedless #1	101	45	25	28	3
	(795)	(354)	(197)	(220)	(25)
Aseptic seedless #2	122	57	24	37	4
	(871)	(407)	(171)	(264)	(27)
Frozen seedless #1	133	68	25	38	3
	(1,343)	(687)	(253)	(384)	(29)
Frozen seedless #2	143	65	33	41	5
	(1,430)	(650)	(330)	(410)	(48)
Concentrates					
Sample #1	749	338	165	220	26
	(1,135)	(512)	(250)	(333)	(39)

Sample ID	Anthocyanin Concentrations (mg CGE)				
	Total	Cy-sop	Cy-glurut	Cy-glu	Cy-rut
Sample #2	555 (813)	247 (362)	133 (195)	153 (224)	24 (35)
Powders					
Oxi-fend Extract	392	270	97	nd	25
Boysenberry concentrate	487	253	125	95	14
Boysenberry puree	344	185	87	72	nd

nd = not detected

Table 19. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE) in the blackcurrant sample (see Table 17 for full names of anthocyanins).

	Anthocyanin concentrations (mg CGE/100 g FW)				
	Total	Dp-glu	Dp-rut	Cy-glu	Cy-rut
Blackcurrant juice concentrate	1386	133.1	569.7	71.5	611.7

Table 20. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE, per 100g fresh weight) in the cranberry sample (see Table 17 for full names of anthocyanins).

	Anthocyanin concentrations (mg CGE/100 g FW)					
	Total	Cy-gal	Cy-glu	Cy-ara	Pn-gal	Pn-ara
Cranberry juice concentrate	159	31.4	2.0	47.5	43.2	34.4

Table 21. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE, per 100g fresh weight) in the pomegranate sample (see Table 17 for full names of anthocyanins).

	Anthocyanin concentrations (mg CGE/100 g FW)			
	Total	Cy-diglu	Dp-glu	Cy-glu
Pomegranate juice concentrate	46	11.6	8.2	19.7

Table 22. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE, per 100g fresh weight) in the blackberry sample (see Table 17 for full names of anthocyanins).

	Anthocyanin Concentrations (mg CGE/100 g FW)		
	Total Acy	Cy-glu	Cy-rut
IQF blackberries	75	73.9	0.6

Table 23. Individual anthocyanin concentrations (expressed as cyanidin 3-glucoside equivalents, CGE, per 100g fresh weight) in the blueberry sample (see Table 17 for full names of anthocyanins).

	Anthocyanin concentrations (mg CGE/100 g FW)											
	Total Acy	Dp-gal	Dp-glu	Dp-ara[a]	Cy-glu	Pt-gal	Cy-ara	Pt-glu	Pt-ara	Mv-gal	Mv-glu	Mv-ara
Cultivated blueberries CAN020	174	28.4	7.4	26.7	1.5	16.6	3.4	6.9	11.9	36.7	13.1	21.8

[a]Dp-ara and Cy-gal coelute, but Dp-ara is by far the major component so this peak was just labelled and calculated as Dp-ara. Also note Pn-gal, Pn-glu and Pn-ara are minor components in most blueberries and probably coelute with Pt-ara, Mv-gal and Mv-glu respectively (although this could not be confirmed with HPLC alone).

Table 24. Percentage of individual anthocyanins in the Boysenberry samples.

	Anthocyanins			
Sample ID	Cy-sop	Cy-glurut	Cy-glu	Cy-rut
Fruit				
Sample #1	44	19	33	3
Sample #2	39	18	39	3
Purees				
Aseptic seedless #1	45	25	28	3
Aseptic seedless #2	47	20	30	3
Frozen seedless #1	51	19	29	2
Frozen seedless #2	45	23	29	3
Concentrates				
Sample #1	45	22	29	3
Sample #2	45	24	28	4
Powders				
Oxi-Fend Extract	69	25	0	6
Boysenberry concentrate	52	26	20	3
Boysenberry puree	54	25	21	0

3.2.3 Phenolics by HPLC

The phenolics quantified by HPLC are shown in Table 25, with the exception of ellagic acid and ellagitannins, which are covered in the subsequent section. Note that Boysenberry, or any fruit, contain a large number of compounds and therefore will present many ‘peaks’ in a chromatogram. The compounds measured in this study probably account for the major components of Boysenberry, further compounds or ‘peaks’ could be identified if there is a specific reason. Minor peaks may be of minimal significance as are present in such small amounts. Concentration of sample fractions will allow detection of a wider array of compounds than running samples directly as can allow elucidation of compounds otherwise “swamped” by the major compounds. It is important to remember that HPLC cannot conclusively identify all phenolics and LC-MS is required to confirm identity.

Note that quercetin 3-rutinoside could not be measured in the Boysenberry or blackberry samples because it coelutes with ellagic acid. In addition, there are some differences resulting from reanalysis of the powder samples with the modified extraction. The two essential differences between the original and the retest of the powders are the extraction solvent and the use of LCMS in the second analysis. The LCMS has high mass accuracy and is very specific, much more than UV/vis HPLC, and more sensitive. Therefore, since these compounds were not detected using the LCMS, they are not present in the samples. This of course means that the original detection of these compounds (based on retention time) by HPLC is incorrect and reflects the lack of specificity of UV/vis HPLC and is an example of the inherent inaccuracy of HPLC when components are at low concentrations.

The profiles are quite different for the various berry fruit with Boysenberry fruit having the simplest phenolic profile. Tables 27–32 show quantification of the individual phenolic compounds. Unlike the anthocyanins where the profile of compounds was the same across the different processed forms the phenolic composition differed across the various processed products. Even between batches of the same product types there was variation. There is no clear explanation for the variation in profile between the different samples. The appearance of some compounds in processed samples over the fruit samples may indicate the release of bound phenolics.

Table 25. Presence of the different phenolics in the berry fruit samples (also see Appendix 4 for chromatograms). Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1).

	Polyphenol															
	1	2	3	4	5	6	7[a]	8	9	10	11	12	13	14	15	16
Fruit																
Boysenberries #1	●		●				●		●							
Boysenberries #2			●				●		●							
Blackberries	●		●				●	●	●	●			●			●
Cultivated blueberries				●	●	●	●	●	●	●			●	●	●	
Purees																
Aseptic Boysenberry seedless #1			●				●		●		●					
Aseptic Boysenberry seedless #2	●		●				●		●		●					
Frozen Boysenberry seedless #1						●	●		●		●					
Frozen Boysenberry seedless #2	●		●			●	●		●		●					
Concentrates																
Boysenberry #1			●		●		●	●	●		●					
Boysenberry #2			●				●	●	●		●		●			●
Blackcurrant			●	●	●	●		●	●	●	●		●	●	●	
Cranberry	●	●	●	●	●	●			●	●	●	●	●	●	●	●
Pomegranate	●	●												●		
Powders																
Boysenberry Oxi-Fend extract	●	●	●		b		b		b		●		b	b	b	
Boysenberry concentrate F-D							b				●					b
Boysenberry puree F-D	b						b		b		●		b			

Key: 1 = catechin, 2 = procyanidin B2, 3 = epicatechin, 4 = myricetin-rutinoside, 5 = myricetin-glucoside, 6 = quercetin-rutinoside, 7 = quercetin-galactoside, 8 = quercetin-glucoside, 9 = kaempferol 3-rutinoside, 10 = kaempferol 3-glucoside, 11 = quercetin, 12 = kaempferol, 13 = 5CQA (coumaroylquinic acid = chlorogenic acid), 14 = 3CQA, 15 = 4CQA, 16 = pCouQA. For full names of compounds see Table 26.

[a] Although on the basis of diode array detection on the HPLC was quantified as quercetin 3-galactoside when the powders were run by LC-MS it was shown not to be. It is unclear which samples may actually contain this compound and LC_MS would be required to conclusively ascertain its presence in fruit, puree and concentrates.

[b] Small amounts of these compounds were detected in original analysis but not in the samples re-run with improved extraction

Table 26. Abbreviations and full names of the flavonoids cited in this report.

Abbreviation	Full name
Cat	catechin
ProCyB2	procyanidin B2
Epicat	epicatechin
My-rut	myricetin-rutinoside
My-glu	myricetin-glucoside
Q-rut	quercetin-rutinoside
Q-gal	quercetin-galactoside
Q-glu	quercetin-glucoside
K-rut	kaempferol 3-rutinoside
K-glu	kaempferol 3-glucoside
Quer	quercetin
Kmpf	kaempferol
5CQA	5-coumaroylquinic acid
3CQA	3-coumaroylquinic acid
4CQA	4-coumaroylquinic acid
pCouQA	*p*-coumaroylquinic acid

Table 27. Individual phenolic concentrations in the Boysenberry samples expressed on a fresh weight basis (mg/100 g FW) with figures in brackets on a per 100 g dry weight basis (note powder samples already dry). Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1). See Table 26 for full names of compounds.

	Polyphenol Concentration												
	Cat	ProCy B2	EpiCat	My-glu	Q-rut	Q-gal	Q-glu	K-3rut	Quer	5CQA	3CQA	4CQA	pCouQ A
Fruit													
IQF Sample 1	1.2	nd	2.5	nd	nd	8.2	nd	1.6	nd	nd	nd	nd	nd
	(7.7)		(16.1)			(52.9)		(10.3)					
IQF Sample 2	nd	nd	3.1	nd	nd	3.8	nd	0.8	nd	nd	nd	nd	nd
			(20.3)			(24.8)		(5.2)					
Purees													
Aseptic seedless sample 1	nd	nd	0.8	nd	nd	3.5	nd	0.8	0.7	nd	nd	nd	nd
			(6.3)			(27.6)		(6.3)	(5.5)				
Aseptic seedless sample 2	0.5	nd	0.9	nd	nd	5.2	nd	1.0	0.6	nd	nd	nd	nd
	(3.6)		(6.4)			(37.1)		(7.1)	(4.3)				
Frozen seedless sample 1	nd	nd	nd	nd	7.8	3.4	nd	0.7	0.5	nd	nd	nd	nd
					(78.8)	(34.3)		(7.1)	(5.1)				
Frozen seedless sample 2	0.6	nd	2.4	nd	8.1	4.6	nd	0.9	0.5	Nd	nd	nd	nd
	(6.0)		(24.0)		(81.0)	(46.0)		(9.0)	(5.0)				
Concentrates													
Sample 1	nd	nd	3.5	5.3	nd	15.4	1.7	2.5	6.8	Nd	nd	nd	nd
			(5.3)	(8.0)		(23.3)	(2.6)	(3.8)	(10.3)				
Sample 2	nd	nd	4.0	nd	nd	16.2	5.9	5.1	3.7	1.4	nd	nd	2.6
			(5.9)			(23.7)	(8.6)	(7.5)	(5.4)	(2.0)			(3.8)
Powders													
Oxi-Fend Extract F-D	37	21.1	46.7	nd	nd	nd	nd	nd	17.9	nd	nd	nd	nd
Boysenberry concentrate 1	nd	nd	nd	nd	nd	nd	nd	nd	3.8	nd	nd	nd	nd
Boysenberry puree 1	nd	nd	nd	nd	nd	nd	nd	nd	5.6	nd	nd	nd	nd

nd = not detected. My-rut, My-glu, Q-rut, Q-gal, Q-glu, K3rut, K3glu, and Kmpf concentrations are all expressed as Q-rut equivalents; cinnamic acids expressed as chlorogenic acid equivalents; catechin and epicatechin were quantified on the basis of authentic standards.

Table 28. Individual phenolic concentrations in the blackcurrant sample expressed per 100 g fresh weight. See Table 26 for full names of compounds.

	Polyphenol concentration (mg/100 g FW)										
	EpiCat	My-rut	My-glu	Q-rut	Q-glu	K3rut	K3glu	Quer	5CQA	3CQA	4CQA
Blackcurrant juice concentrate	5.0	53.7	38.4	25.5	23.1	1.8	4.6	3.7	7.8	12.6	3.1

Table 29. Individual phenolic concentrations in the cranberry sample expressed per 100 g fresh weight. See Table 26 for full names of compounds.

	Polyphenol concentration (mg/100 g FW)													
	Cat	ProCyB2	EpiCat	My-rut	My-glu	Q-rut	K3rut	K3glu	Quer	Kmpf	5CQA	3CQA	4CQA	pCouQA
Cranberry juice concentrate	3.6	77.1	34.4	27.1	3.0	38.9	8.9	14.3	27.6	1.4	1.0	37.7	3.9	1.1

Table 30. Individual phenolic concentrations in the pomegranate sample expressed per 100 g fresh weight. See Table 26 for full names of compounds.

	Polyphenol concentration (mg/100 g FW)		
	Cat	ProCyB2	3CQA
Pomegranate juice concentrate sample 0729-1309-1	2.8	21.6	1.9

Table 31. Individual phenolic concentrations in the blackberry sample expressed per 100 g fresh weight. See Table 26 for full names of compounds.

	Polyphenol concentration (mg/100 g FW)							
	Cat	EpiCat	Q-gal	Q-glu	K3rut	K3glu	5CQA	pCouQA
IQF Blackberries	0.6	2.8	0.8	2.1	1.2	0.7	2.2	0.8

Table 32. Individual phenolic concentrations in the blueberry sample expressed per 100 g fresh weight. See Table 26 for full names of compounds.

	Polyphenol concentration (mg/100 g FW)									
	My-rut	My-glu	Q-rut	Q-gal	Q-glu	K3rut	K3glu	5CQA	3CQA	4CQA
Cultivated blueberries	5.1	1.0	1.8	17.2	4.4	5.1	0.9	0.4	62.8	0.5

3.2.4 Ellagic acid & ellagitannins

It is important to note that even in the scientific literature there is considerable variation in terminology in this area. The term 'free' should always be used in conjunction with the solvent extraction conditions. Many papers due the term "free ellagic acid" even when samples are extracted in solvent at 100°C for 24 h.

True free ellagic acid (EA) is shown in Table 33 along with that released by hydrolysis. 'Total ET (EA equiv)' includes both the bound and the free ellagitannins converted to ellagic acid. This is the value that should be used for comparisons between Boysenberry and non-Boysenberry products. The value for 'Free ET (EA equiv)' refers to that portion of ET that can be extracted with solvent and is then converted to ellagic acid. This value should typically be lower than the 'total value' (and is for most samples). The third value is 'free EA' which is the actual amount of EA present in standard solvent extracts (no boiling, no acid hydrolysis). Generally in fruit this is low and insignificant as free ellagic acid is not normally present in whole, fresh fruit. The free ellagic concentrations were low in the Boysenberry fruit but significantly higher in the processed products, although not exceptionally high. This would indicate some of the ellagic acid is released from bound forms during processing, especially into concentrates and powders.

Table 33. Ellagic acid (free and from bound forms) concentrations in the berry fruit samples expressed on a fresh weight basis (mg/100 g FW) with figures in brackets on a per 100 g dry weight basis (note powder samples already dry). Free ellagic acid was determined in samples as prepared for flavonoids: fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1). A portion of each of these extracts was hydrolysed with concentrated HCl at 80ºC and the ellagic acid that was generated was measured ('free ellagitannin'). The original samples (fruit, puree, powder, or concentrate) were hydrolysed with an ethanol/conc HCl mixture and the ellagic acid that was generated was measured (free plus bound ellagitannins). Ellagic acid was quantified using an authentic standard.

Sample description		Hydrolysed samples	
	Free EA	Free ET (as EA equiv)	Total ET[a] (as EA equiv)
Fruit			
Boysenberries #1	2	101	132
	(13)	(652)	(852)
Boysenberries #2	1	96	138
	(7)	(627)	(902)
Blackberries	6	150	226
	(41)	(1,014)	(1,527)
Cultivated blueberries	nd	3	nd
		(16)	
Purees			
Aseptic Boysenberry seedless #1	7	65	123
	(55)	(512)	(969)
Aseptic Boysenberry seedless #2	9	90	151
	(64)	(643)	(1,079)
Frozen Boysenberry seedless #1	6	75	121
	(61)	(758)	(1,222)
Frozen Boysenberry seedless #2	7	72	104

Sample description	Hydrolysed samples		
	Free EA	Free ET (as EA equiv)	Total ET[a] (as EA equiv)
	(70)	(720)	(1,040)
Concentrates			
Boysenberry #1	67	427	461
	(102)	(647)	(698)
Boysenberry #2	59	382	352
	(86)	(559)	(515)
Blackcurrant	nd	9	nd
		(13)	
Cranberry	nd	nd	nd
Pomegranate	20	312	287
	(28)	(433)	(399)
Concentrates adjusted to natural strength[b]			
Boysenberry #1	8	53	57
Boysenberry #2	7	47	43
Blackcurrant	nd	2	nd
Cranberry	nd	nd	nd
Pomegranate	4	58	53
Powders			
Boysenberry Oxi-Fend extract	129	811	3,086
Boysenberry concentrate F-D	13	48	209
Boysenberry puree F-D	50	341	810

[a] released from both free and bound forms of ellagitannins

[b] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Note that the seed might be a contributor to the whole fruit ellagic acid. The only way to determine the degree to which the seeds contribute would be to analyse them separately. There are some reports in the literature of the ellagic acid content of Rubus seeds (determined after acid hydrolysis so that released from all ellagitannins). Boysenberry seeds had an ellagic acid content of 3,100 mg per 100 g and blackberries were similar (Bushman et al. 2004). Note that Bushman used the term “free ellagic acid” for samples extracted by methanol at 100°C for 24 h. Total ellagic acid was determined after drying these extracts and hydrolysing them in 2N trifluoroacetic acid in methanol at 100°C for 2 hours. Further work is required to conclusively determine what contribution seeds may make to the content of various forms of ellagic acid in processed products.

The results reported here for Boysenberries differ from those reported by Wada & Ou (2002), who reported large amounts of free ellagic acid forms and minimal in bound forms. There are questions over the quality of the data from Wada and Ou. Firstly they appear to have misidentified the anthocyanins in Boysenberry, or they may have a very different phenolic composition than the Boysenberries grown in New Zealand. Secondly to measure ellagitannins in fruit they defined ‘free ellagitannins’ as those extract by methanol at 100°C. The extraction temperature used here is high and is probably why their ratio of ‘free’ to ‘total’ is high. In our studies ‘free’ ellagitannins are extraction in solvent at 4°C. The low temperature is used to avoid degradation of components.

Cranberry contained no free or bound ellagic acid while blueberries and blackcurrant concentrate contained no free ellagic acid and minimal amounts of free ellagitannins. As would be expected, blackberries had large amounts of ellagic acid. There are varying concentrations reported in the literature for blackberries. Vrhovsek et al. (2009) gave values between 58 and 240 mg per 100 g (determined as ellagic acid released after acid hydrolysis). Gasperotti et al. (2010) measured true free ellagic acid and found it to range between 4 and 8 mg per 100 g. They found total ellagic acid conjugates to range between 12 and 24 mg per 100 g while total ellagitannins ranged from 85 to 130 mg per 100 g.

In addition to the ellagic acid measures the parent ellagitannins were determined by HPLC (Table 34). As there are no standards available for ellagitannins, they were quantified as epicatechin equivalents. It should also be noted that it is hard to make direct comparisons between the powder samples and the other samples because of the way they were quantified (HPLC for the fruit samples and LCMS for the powders, so therefore the response and equivalence factors are different and the concentrations for these two sets of samples should not be compared). Ellagitannin identifications are based on previous research that used a Boysenberry powder (Kool et al. 2010). In the previous research gSanH6 and ET? coleuted. The ellagitannin labelled 'ET?' is more prevalent in fruit samples and may be the isomer of SanH6. Furuuchi et al. (2011) identified lambertianin C in Boysenberry juice along with sanguiin H-6 and/or lambertianin A and sanguiin isomers. There are substantial differences in the ellagitannin composition between fruit and processed Boysenberry. Pomegranate does not contain the same ellagitannins as Boysenberry, but they are present as indicated by the EA equivalent values. Note that characterisation and measurement of ellagitannins is a 'work in progress' and at the present stage analytical methods are not well developed. The recent move to LCMS has allowed us to better characterise ellagitannins and distinguish them for each other even when we do not get separation by LC, something we could not do with UV/vis.

Table 34. Ellagitannin concentrations in the berry fruit samples expressed on a fresh weight basis (mg/100 g FW) with figures in brackets on a per 100 g dry weight basis (note powder samples already dry). Fruit and puree samples were extracted with ethanol/water/formic acid (80:20:1), powder samples with methanol/water/formic acid (50:50:1) and concentrates diluted as required with ethanol/water/formic acid (80:20:1).

	Ellagitannin concentrations (mg epicatechin equiv/100 g FW)					
Sample description	Total ET	gSanH6	ET?	SanH10	SanH6	SanH2
Fruit						
Boysenberries #1	68	4	36	nd	26	1
	(439)	(26)	(232)		(168)	(6)
Boysenberries #2	69	2	37	nd	29	1
	(451)	(13)	(242)		(190)	(7)
Blackberries	86	2	26	3	50	6
	(581)	(14)	(176)	(20)	(338)	(41)
Cultivated blueberries	nd	nd	nd	nd	nd	nd
Purees						
Aseptic Boysenberry seedless #1	19	3	8	1	7	nd
	(150)	(24)	(63)	(8)	(55)	
Aseptic Boysenberry seedless #2	46	4	23	nd	19	1
	(329)	(29)	(164)		(136)	(7)
Frozen Boysenberry seedless #1	20	3	10	nd	7	nd
	(202)	(30)	(101)		(71)	

Sample description	Total ET	Ellagitannin concentrations (mg epicatechin equiv/100 g FW) gSanH6	ET?	SanH10	SanH6	SanH2
Frozen Boysenberry seedless #2	30	5	13	nd	11	1
	(300)	(50)	(130)		(110)	(10)
Concentrates						
Boysenberry #1	167	21	68	19	56	3
	(253)	(32)	(103)	(29)	(85)	(5)
Boysenberry #2	109	18	35	10	43	2
	(160)	(26)	(51)	(15)	(63)	(3)
Blackcurrant	nd	nd	nd	nd	nd	nd
Cranberry	nd	nd	nd	nd	nd	nd
Pomegranate	nd	nd	nd	nd	nd	nd
Powders						
Boysenberry Oxi-Fend extract	713	173	58	254	185	43
Boysenberry concentrate F-D	13	nd	nd	3	10	nd
Boysenberry puree F-D	35	nd	nd	nd	35	nd

nd = not detected

There is limited data in the literature for ellagitannins. Hager et al. (2010) analysed the individual ellagitannins by HPLC (although still expressed as ellagic acid equivalents) and reported the value for IQF fruit as 35 mg per 100 g. Gasperotti et al. (2010) also analysed individual ellagitannins in blackberry by HPLC but quantified them using purified standards. They reported values between 85 and 120 mg per 100 g. Thus, the values reported here seem to be in line with these.

Totals of all classes quantified above come out much lower than that from total phenolics as determined by the Folin method. The anthocyanins are the predominant class of phenolics in all samples except the Oxi-Fend extract where ellagitannins predominated. Interestingly, the other powder samples had very low concentrations of ellagitannins indicating the process used for the Oxi-Fend extract resulted in concentrating the ellagitannins.

3.3 Antioxidant capacity

3.3.1 ORAC

ORAC results are dependent on the extraction solvents and precise methods used. For the purposes of this study, to allow direct comparison with other published data, the hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC) method developed by Prior et al. (2003) was used. The Oxi-Fend extract had the highest ORAC capacity with the other two powders much lower (Table 35). Lipophilic activity was low in all samples, which is what would be expected. Concentrates also had high ORAC activity with fruit samples lower and puree samples lower again. Boysenberry fruit had higher ORAC values than both the benchmark fruit (blackberries and blueberries). The aseptic purees had higher ORAC values than the frozen purees on a fresh weight basis but the reverse was true on a dry weight basis (Table 36). When concentrates were adjusted to natural strength (since this frequently reflects levels of commercial inclusion in finished products) Boysenberry performs well with only blackcurrant outperforming it. As with the total phenolics the Boysenberry samples had more similar ORAC values when expressed on a dry weight basis with the exception of the Oxi-Fend sample that had higher activity and the concentrate powder than had lower activity. ORAC activity was

strongly correlated with total phenolic content (see Appendix 3). This had commonly been reported as phenolics are the primary antioxidants in most fruits and vegetables.

Table 35. Antioxidant capacity as determined by the ORAC assay (USDA method as per Prior et al. 2003), expressed on a per 100 g fresh weight basis. Samples first extracted with hexane to obtain lipophilic antioxidants then reside re-extracted with acetone/water/acetic acid (70:29.5:0.5, v/v/v) to obtain the hydrophilic antioxidants. Results are expressed as µmol Trolox Equivalents per 100 g.

	ORAC – USDA (µmol TE/100 g)		
Sample ID	H-ORAC	L-ORAC	Total-ORAC
Fruit			
Boysenberries #1	7,508	106	7,614
Boysenberries #2	6,777	113	6,889
Blackberries	4,289	144	4,434
Cultivated blueberries	5,412	58	5,470
Purees			
Aseptic Boysenberry seedless #1	6,143	120	6,263
Aseptic Boysenberry seedless #2	6,232	113	6,345
Frozen Boysenberry seedless #1	5,196	106	5,301
Frozen Boysenberry seedless #2	5,730	101	5,831
Concentrates[a]			
Boysenberry #1	42,533	-	42,533
Boysenberry #2	38,754	-	38,754
Blackcurrant	62,178	-	62,178
Cranberry	24,264	-	24,264
Pomegranate	19,226	-	19,226
Concentrates adjusted to natural strength[b]			
Boysenberry #1	5,235	-	5,235
Boysenberry #2	4,770	-	4,770
Blackcurrant	11,096	-	11,096
Cranberry	3,640	-	3,640
Pomegranate	3,549	-	3,549
Powders			
Boysenberry Oxi-Fend extract	199,471	542	200,013
Boysenberry concentrate F-D	26,862	394	27,256
Boysenberry puree F-D	48,802	591	49,392

[a] Because these are liquid samples no lipophilic extraction can be performed

[b] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Table 36. Antioxidant capacity as determined by the ORAC assay (USDA method as per Prior et al. 2003), expressed on a per 100 g dry weight basis. Samples first extracted with hexane to obtain lipophilic antioxidants then reside re-extracted with acetone/water/acetic acid (70:29.5:0.5, v/v/v) to obtain the hydrophilic antioxidants. Results are expressed as µmol Trolox Equivalents per 100 g.

	ORAC – USDA (µmol TE/100 g)		
Sample ID	H-ORAC	L-ORAC	Total-ORAC
Fruit			
Boysenberries #1	48,439	684	49,123
Boysenberries #2	44,294	739	45,026
Blackberries	28,980	973	29,959

Sample ID	ORAC – USDA (µmol TE/100 g) H-ORAC	L-ORAC	Total-ORAC
Cultivated blueberries	28,635	307	28,942
Purees			
Aseptic Boysenberry seedless #1	48,370	945	49,315
Aseptic Boysenberry seedless #2	44,514	807	45,321
Frozen Boysenberry seedless #1	52,485	1071	53,545
Frozen Boysenberry seedless #2	57,300	1010	58,310
Concentrates			
Boysenberry #1	64,444	-	64,444
Boysenberry #2	56,741	-	56,741
Blackcurrant	93,220	-	93,220
Cranberry	43,329	-	43,329
Pomegranate	26,703	-	26,703
Powders			
Boysenberry Oxi-Fend extract	199,471	542	200,013
Boysenberry concentrate F-D	26,862	394	27,256
Boysenberry puree F-D	48,802	591	49,392

In addition to the lipophilic and hydrophilic extractions the ORAC assay was performed on the same extracts as used for the total phenolics assay (i.e. fruit and puree samples were extracted with 50% acetone, powder samples solubilised with water and concentrates simply diluted as required). These results are shown in Table 37. Some of the results are very similar to the T-ORAC values although there are some differences, particularly for the powders and especially the Oxi-Fend (which was significantly higher when solubilised with water and analysed). These differences will relate to extractability (or solubility) of the samples by the different solvents and the lower values indicate incomplete extraction of the phenolics. It is clear that the extraction methods of Prior et al. (1993), although appropriate for material such as fruit, are not appropriate for extracts that are more water soluble. In using the results it may be more appropriate to use the higher values, noting the difference in sample preparation.

Table 37. Antioxidant capacity as determined by ORAC assay with samples extracted as per total phenolics (fruit and puree samples were extracted with 50% acetone, powder samples solubilised with water and concentrates simply diluted as required). Results are expressed as µmol Trolox Equivalents on both a fresh weight and a dry weight basis per 100 g.

Sample ID	PH-ORAC µmol TE/100 g FW	PH-ORAC µmol TE/100 g DW
Fruit		
Boysenberries #1	6,914	44,606
Boysenberries #2	6,419	41,954
Blackberries	3,346	22,608
Cultivated blueberries	6,876	36,381
Purees		
Aseptic Boysenberry seedless #1	6,272	49,386
Aseptic Boysenberry seedless #2	6,617	47,264
Frozen Boysenberry seedless #1	5,567	56,232
Frozen Boysenberry seedless #2	5,927	59,270

Sample ID	PH-ORAC µmol TE/100 g FW	PH-ORAC µmol TE/100 g DW
Concentrates		
Boysenberry #1	42,533	64,444
Boysenberry #2	38,754	56,741
Blackcurrant	62,178	93,220
Cranberry	24,264	43,329
Pomegranate	19,226	26,703
Concentrates adjusted to natural strength[a]		
Boysenberry #1	5,235	-
Boysenberry #2	4,770	-
Blackcurrant	11,096	-
Cranberry	3,640	-
Pomegranate	3,549	-
Powders		
Boysenberry Oxi-Fend extract	295,702	295,702
Boysenberry concentrate F-D	35,933	35,933
Boysenberry puree F-D	59,220	59,220

[a] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Comparative published data for antioxidant capacity as determined by the ORAC assay are shown in Table 38. Like the earlier data, there is a lack of published data for many processed products and so values for the relevant fruits are given. In most cases the values obtained in this study are in line with what has been reported.

Table 38. Comparative ORAC data (values expressed as µmol TE per 100 g fresh weight). Note sample extraction varied for the different reports and may explain some of the differences.

Sample ID	H-ORAC	L-ORAC	T-ORAC
Fruit			
Boysenberries	-	-	5,889-8,252[a]; 4,220[b]
Blackberries	5,802[c]	103[c]	5,905[c]; 6,221[d]; 1,480-2,260[e]
Cultivated blueberries	4,633[c]	36[c]	4,669[c]; 4,826[d]; 4,440-5,270[f]; 1,680-4,230[g]
Blackcurrants	7,880[c]	81[c]	7,957[c]; 3,690[h]
Cranberry	8,888[c]	202[c]	9,090[c]; 8,349[d]; 820-1,410[i]
Pomegranate	4,479[c]	-	4,479[c,d]
Puree			
Boysenberry	-	-	5,310[j]
Concentrates			
Boysenberry juice concentrate	-	-	30,993-40,114[a]
Pomegranate juice concentrate	-	-	13,600[j]

[a] Data from Phytochemicals & Health Group; b Wada & Ou 2002; c ORAC database 2010; d Wolfe et al. 2008; e Jiao & Wang 2000; f You et al. 2011; g Prior et al. 1998; h Moyer et al. 2002; i Wang & Stretch 2001; j Muller et al. 2010 (note for the pomegranate samples values were adjusted using the dilution factor provided in the paper)

3.3.2 FRAP

In many of the samples the FRAP values (Table 39) were similar to the ORAC values and there was a strong relationship between the two (see Appendix 3). However, the powder samples gave relatively lower activity in the FRAP assay. Like the ORAC assay values were closely related to total phenolic content (see Appendix 3). There has been limited published FRAP data on comparable products but what there is (Table 40) agrees with that reported here.

Table 39. Antioxidant capacity as determined by the FRAP assay, expressed on both a fresh and dry weight basis per 100 g. Results are reported as absolute values in µmol of electrons/hydrogen atoms donated in the redox reaction per 100 g of sample. Samples were extracted as per total phenolics (fruit and puree samples were extracted with 50% acetone, powder samples solubilised with water and concentrates simply diluted as required).

Sample ID	FRAP µmol per 100 g FW	FRAP µmol per 100 g DW
Fruit		
Boysenberries #1	7,193	46,406
Boysenberries #2	5,817	38,020
Blackberries	5,113	34,547
Cultivated blueberries	4,694	24,836
Purees		
Aseptic Boysenberry seedless #1	6,264	49,323
Aseptic Boysenberry seedless #2	6,702	47,871
Frozen Boysenberry seedless #1	5,586	56,424
Frozen Boysenberry seedless #2	5,888	58,880
Concentrates		
Boysenberry #1	31,211	47,289
Boysenberry #2	29,574	43,300
Blackcurrant	41,322	73,789
Cranberry	10,802	16,195
Pomegranate	16,722	23,225
Concentrates adjusted to natural strength[a]		
Boysenberry #1		3,841
Boysenberry #2		3,640
Blackcurrant		7,374
Cranberry		1,620
Pomegranate		3,087
Powders		
Boysenberry Oxi-Fend extract	116,080	116,080
Boysenberry concentrate F-D	25,207	25,207
Boysenberry puree F-D	31,211	31,211

[a] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

Figure 40. Published FRAP data (values expressed as µmol TE per 100 g fresh weight). Note sample extraction varied for the different reports and may explain some of the differences.

Sample ID	FRAP µmol per 100 g FW
Fruit	
Blackberries	3,505–4,344[a]; 5,070[b]; 3,990[c]
Cultivated blueberries	741–1,369[a]; 3,640[b]; 2,154[c]; 3,000[d]
Blackcurrants	7,350[b]; 5,160[d]
Cranberry	5,030[b]; 3,289[c]; 1,860[d]
Pomegranate	1,133[b]; 1,800[e]
Puree	
Boysenberry	5,900[f]
Concentrates	
Pomegranate juice concentrate	9,050[f]

[a] Koca & Karadeniz 2009; [b] Halvorsen et al. 2002; [c] Halvorsen et al. 2006; [d] Borges et al. 2010; [e] Carlsen et al. 2010; [f] Muller et al. 2010 (note for the pomegranate samples values were adjusted using the dilution factor provided in the paper)

3.4 Folate

The folate concentrations in the selected berry fruit samples measured are given in Table 41. Note that in all these products natural folates are present (not folic acid) and hence the results expressed as micrograms/100 g are directly able to be taken as numerical contribution to RDI expressed as DFE (see Table 7). Examining the Boysenberry samples, on a fresh weigh basis folate contents were highest in the the Oxi-Fend Extract powder, followed by similar contents in the other two powders and the concentrates, followed by the fruit with the purees being lowest. On a dry weight basis folate contents were highest in the fruit samples as well as the Oxi-Fend Extract powder and frozen purees. Although the differently processed purees were similar on a fresh weight basis they were significantly different on a dry weight basis. Unlike many other components that are present in much higher concentrations in the concentrates (x times) than the fruit, folate was present at only double the concentration. In addition, when examined on a dry weigh basis folate was lower in the asceptic puree than the frozen puree. These results indicate that a significant proportion of folate is probably lost during processing. Folate can be significantly affected by processing and storage (Hawkes & Villota 1989). It is degraded during thermal processing, especially under more acidic conditions. No studies have been carried out with Boysenberries, but in beetroot processing resulted in considerable losses of folates, whereas losses during storage appeared to moderate (Jastrebova et al. 2003).

Table 41. Folate concentrations in selected berry fruit samples as determined by the microbiological method, expressed on both a fresh and dry weight basis per 100 g.

Sample ID	Total folate (µg/100 g FW)	Total folate (µg/100 g DW)
Fruit		
Boysenberries #1	65	419
Boysenberries #2	80	523
Blackberries	na	Na
Cultivated blueberries	na	Na
Purees		
Aseptic Boysenberry seedless #1	32	252

Sample ID	Total folate (µg/100 g FW)	Total folate (µg/100 g DW)
Aseptic Boysenberry seedless #2	42	300
Frozen Boysenberry seedless #1	40	404
Frozen Boysenberry seedless #2	43	430
Concentrates		
Boysenberry #1	120	182
Boysenberry #2	150	220
Blackcurrant	100	150
Cranberry	8	14
Pomegranate	na	na
Concentrates adjusted to natural strength[a]		
Boysenberry #1	15	-
Boysenberry #2	18	-
Blackcurrant	18	-
Cranberry	1.2	-
Pomegranate	na	-
Powders		
Boysenberry Oxi-Fend extract	530	530
Boysenberry concentrate F-D	110	110
Boysenberry puree F-D	150	150

na = not analysed

[a] Figures for concentrates were recalculated based on reconstitution by dilution to natural strength using the AIJN European standards for ss Brix.

According to the NZ Food Composition Database the folate content in Boysenberry fruits is 63 µg per 100 g FW (this is the same figure that is given in the USDA National Nutrient Database 2010). In our earlier study we found 37–44 µg/100 g in fruit and 40 µg/100 g in concentrates. The values obtained here are higher than those but in line with other data. The cranberry juice concentrate had low folate content, which is not surprising since NZ Food Composition Database gives value for fruit of 2 µg/100 g (1 µg/100 g in the USDA National Nutrient Database 2010). The blackcurrant concentrate had significant folate which is interesting as according to NZ Food Composition Database the folate content of blackcurrant fruit is only 3 µg/100 g (no figure provided in USDA National Nutrient Database 2010). In the literature a value of 17 µg/100 g has been reported for blackcurrant fruit as determined by a radioprotein-binding assay (Stralsjo et al. 2003). Subsequent nutritional analysis has shown New Zealand-grown blackcurrant to have significant folate content (report in preparation).

The specification for Oxi-Fend Boysenberry powder is reported to be >40 µg per 100 g as measured by an AOAC method (Indyk et al. 2000). This compares with a folate test result on the sample here of 530 µg per 100 g. The method used may be responsible for the difference. The AOAC method is a biosensor-based, non-labelled inhibition immunoassay so different to the microbiological assay used for this study. It was also developed for folate supplemented milk powders (so quite different to fruit). Folate naturally occurring in food is present in different forms and different to folic acid added to foods. It has been reported that the antibodies may underestimate folate vitamers in food present in conjugated forms. The microbiological method does have issues but at present is still the most widely used assay for foods and what most food composition databases use.

4 Conclusions

In conclusion, this report identifies a number of valuable attributes of Boysenberries and products made from them. In particular, Boysenberries contain high concentrations of phenolics, especially anthocyanins and ellagic acid (present in various forms) plus folate. Boysenberries have high antioxidant activity, in both ORAC and FRAP assays, due to the high phenolic content. Although processing has some impact on phytochemical composition, particularly the anthocyanins, Boysenberry products such as concentrate, puree and powders are still valuable sources of of these compounds.

The accumulated data gathered here highlights a number of attributes of Boysenberries that will be useful for marketing and promotion especially in comparison with other fruit currently used commercially. Boysenberry fruit out-performed the two benchmark fruit (blackberries and blueberries) across all assays. The Boysenberry concentrates also performed well, outranking the cranberry and pomegranate concentrates. However, the blackcurrant concentrate was higher in phenolics and hence antioxidant activity. Despite this, the Boysenberry concentrates contained free ellagic acid and ellagitannins, not present in the blackcurrant concentrate sample. Even when concentrates are adjusted to natural strength (since this frequently reflects levels of commercial inclusion in finished products) Boysenberry performs well with only blackcurrant outperforming it across the board, except for ellagitannins where Boysenberry excels. Although when expressed this way pomegranate has a higher total phenolic content Boysenberry has much higher anthocyanin content and antioxidant activity in both FRAP and ORAC assays. Pomegranate is on a par with Boysenberry for ellagic acid (present in free and bound forms). Boysenberry concentrate is superior to cranberry in all attributes measured here.

This report also highlights some areas for further work. LC-MS analysis is required if conclusive identification of phenolic compounds, particularly the ellagitannins, is wanted. Investigation of the effects of processing on phenolic composition may enable improvements to be made to reduce losses of compounds, such as the anthocyanins.

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Appendices

Appendix 1: Abbreviations, definitions and explanations of some terminology

AI = Adequate Intake (used when an RDI cannot be determined): The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

ANC =Anthocyanins

Dietary Folate Equivalents: For some nutrients the term 'equivalent' has been used to express the recommendations (e.g. Vitamin A is expressed in Retinol Equivalents, folate in Dietary Folate Equivalents; vitamin E in alpha-tocopherol equivalent).This reflects the fact that for some nutrients there is more than one chemical form in the food supply that provide a benefit. For example, for folate, there is naturally occurring food folate as well as folic acid used for food fortification. Folic acid is twice as active as food folate so not as much is needed to get the same biological benefit. The overall requirement may be met by a mixture of these so is expressed as dietary folate equivalents.

EA = Ellagic Acid

EAR = Estimated Average Requirement: A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

Ellagitannins: Ellagitannins (ETs) are polyphenols included within the so-called "hydrolyzable tannins" in which ellagic acid forms diesters with sugars (most often glucose). Present in plant cells in free and bound forms.

F-C = Folin-Ciocalteu: A mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants.

FRAP = Ferric Reducing Antioxidant Power: The FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants.

GAE = Gallic Acid Equivalents: Used to express total phenolic concentrations. Since the assay measures all phenolics, the choice of gallic acid as standard is based on the availability of a stable and pure substance, and gallic acid is both, and it is less expensive than other options. In addition, the response to gallic acid has been shown to be equivalent to many other phenolics in plants (with some exceptions).

ORAC = Oxygen Radical Absorbance Capacity: ORAC measures antioxidant inhibition of peroxyl radical induced oxidations and thus reflects classical radical chain breaking antioxidant activity by H atom transfer.

RDI = Recommended Dietary Intake: The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98 per cent) healthy individuals in a particular life stage and gender group.

TP = Total phenolics

UL = Upper Level of Intake: The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

Appendix 2: Further details of HPLC/UPLC analysis

A measurement of a compound in a sample is always an estimate of the true amount and the term accuracy refers to the closeness of the measurement to the true amount. Measurement accuracy is affected by sampling and analytical methods. The role of an analytical chemist is to generate the most accurate measurement possible within the constraints of the sampling and analytical methods employed.

HPLC (UHPLC) is a 'separate and measure' technique and generally the target compound must be physically separated before it can be measured. The only exception to this is where a spectral feature unique to the compound being measured is present. UV/vis has limited ability to provide unique and unambiguous spectral features, although anthocyanins are an example of compounds that can be measured in the presence of other phenolics; however, individual anthocyanins must still be separated from each other before measurement.

In HPLC the separation of components in a sample occurs as a function of time and the parameter known as 'retention time' is the key indicator used for the identification of a compound. Compound retention times are not absolute properties of compounds and vary with the particular setup of HPLC equipment. Retention time values are not transferable between HPLC equipment and so authentic standards compounds must always be included with batches of samples to ensure accuracy of the measurement.

Authentic standards of many phytochemicals are not available and in the absence of these, and when there is a lack of background knowledge of the composition of the sample, it is not possible to guarantee measurement accuracy when HPLC-UV/vis is used. This is the case with Boysenberry where we have limited knowledge of the composition and a number of components are only tentatively identified.

For Boysenberry samples the compounds below are the ones that can be measured with an acceptable degree of accuracy. This amounts to the quantitative analysis of 25 individual components. If there is a requirement to measure additional phenolic components, such as some of those identified by David Stevenson, detection by mass spectrometry is needed and the analysis will have to be done by LC-MS. If components (HPLC peaks) are detected in addition to the ones listed are detected, LC-MS will also have to be used for the analysis.

Compounds able to be quantified:

- Anthocyanins (as cyanidin 3-glucoside equivalents)
  - cyanidin 3-sophoroside
  - cyanidin 3-[2-(glucosyl)-6-(rhamnosyl)glucoside]
  - cyanidin 3-glucoside
  - cyanidin-3-[2-(xylosyl)-6-(rhamnosyl)glucoside]
  - cyanidin 3-rutinoside
- Flavonol glycosides (as quercetin 3-rutinoside equivalents)
  - quercetin 3-rutinoside
  - quercetin 3-glucoside
  - quercetin 3-galactoside
  - kaempferol 3-rutinoside
  - kaempferol 3-glucoside

- myricetin 3-rutinoside
- myricetin 3-glucoside

- Flavonol aglycones (as quercetin equivalents)
  - quercetin
  - kaempferol

- Flavanols (as epicatechin equivalents)
  - epicatechin
  - catechin
  - procyanidin B2

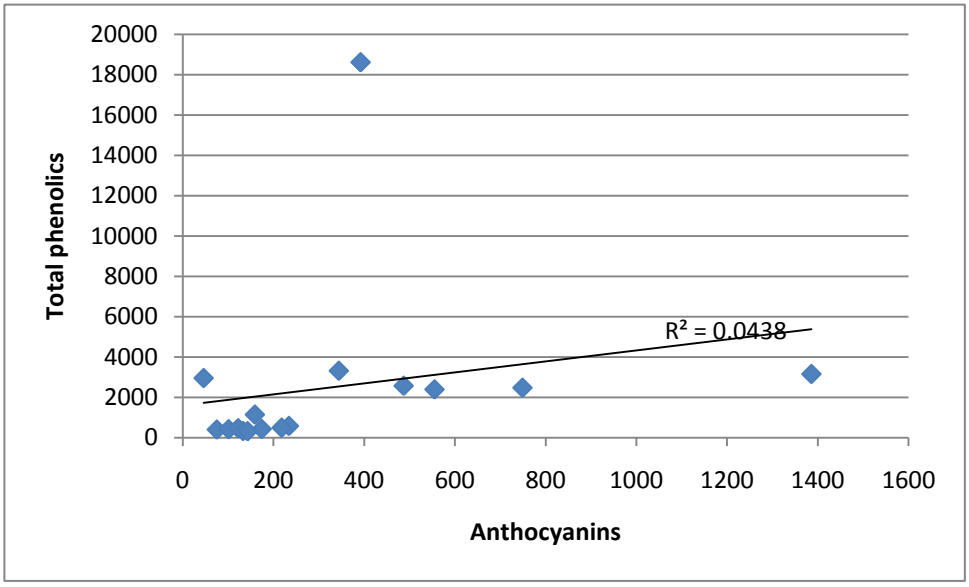
- Cinnamic acid (as 5-caffeoylquinic acid equivalents)
  - 5-caffeoylquinic acid (chlorogenic acid)
  - 4-caffeoylquinic acid
  - 3-caffeoylquinic acid
  - p-coumarylquinic acid

- Ellagitannins (as eipcatechin and ellagic acid equivalent)
  - sanguiin H-2
  - sanguiin H-6
  - sanguiin H-10 (isomer)
  - galloyl sanguiin H-6
  - total ellagitannin as ellagic acid
  - free ellagitannin as ellagic acid.

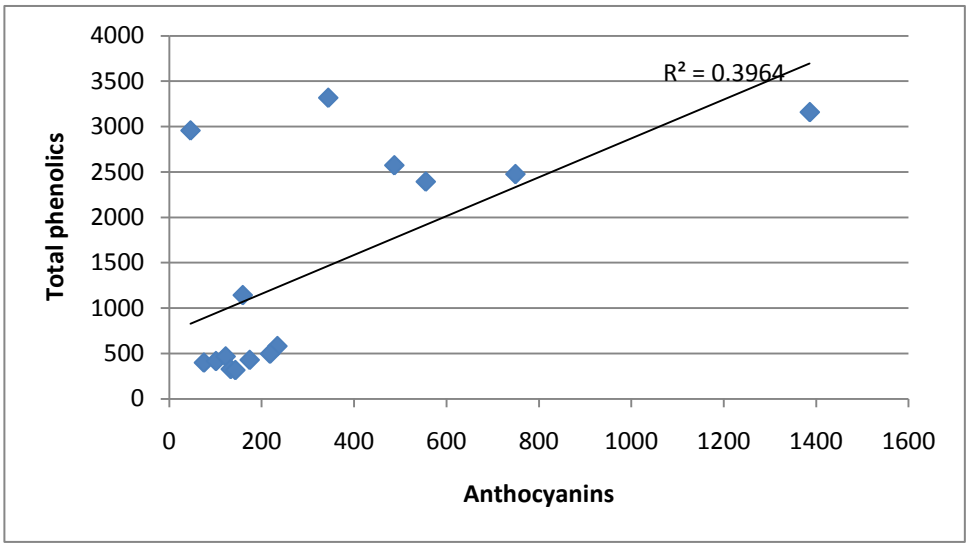
Appendix 3: Relationships between components

A. Relationship between anthocyanins and total phenolics

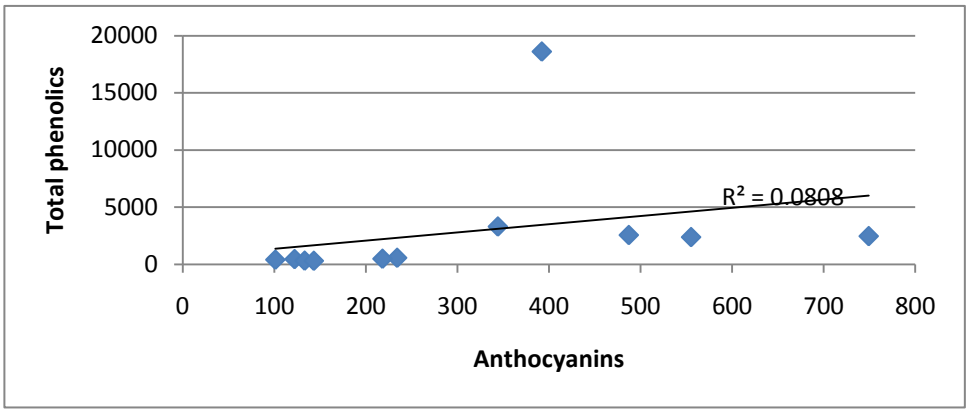
a) For all berry fruit samples (n=16)



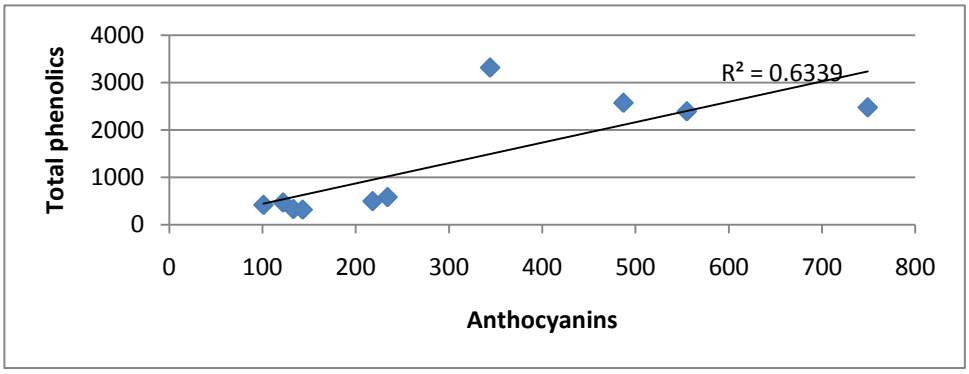
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)

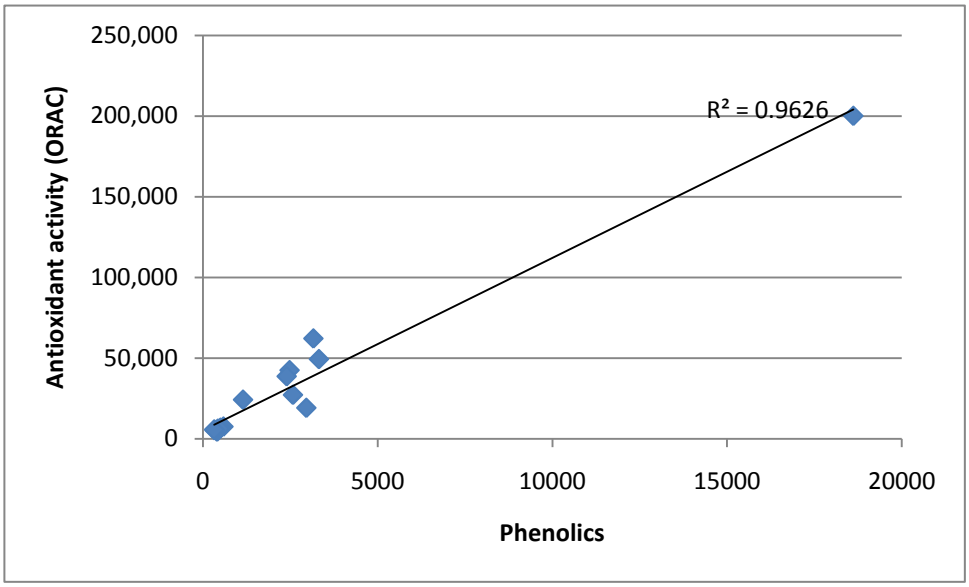


d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)

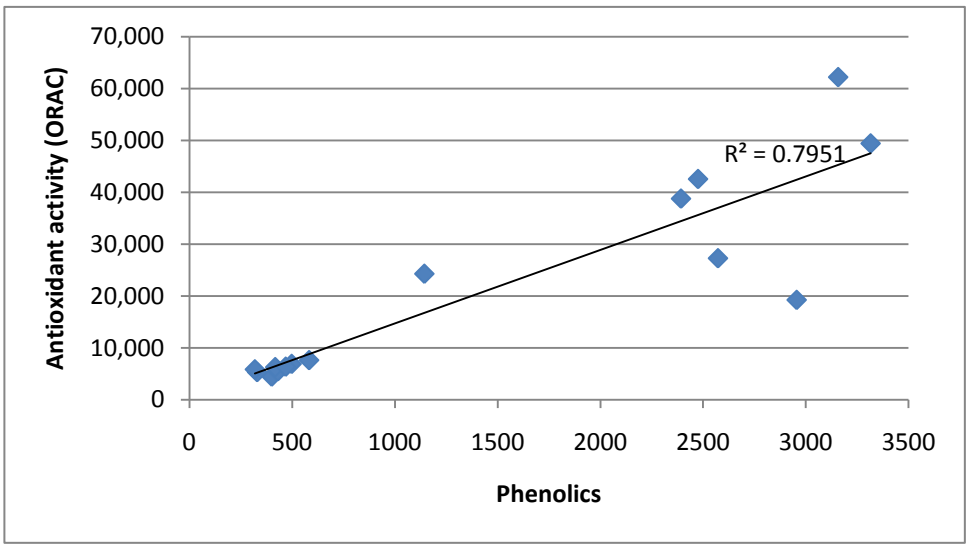


B. Relationship between total phenolics and antioxidant capacity as determined by the ORAC assay

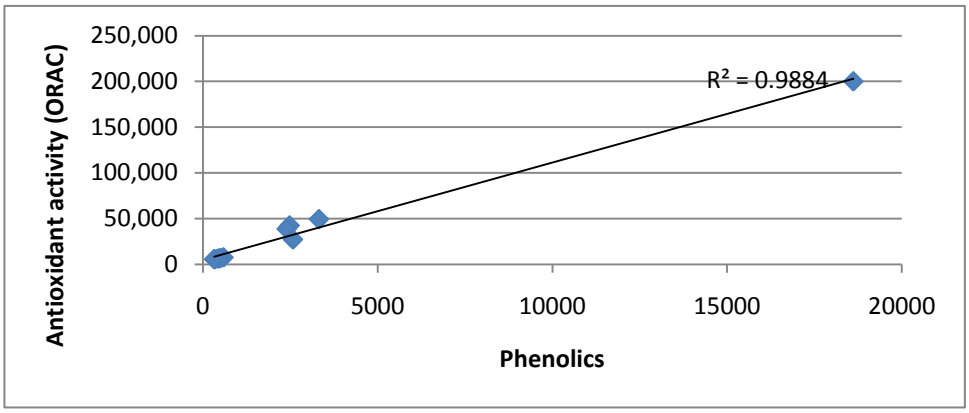
a) For all berry fruit samples (n=16)



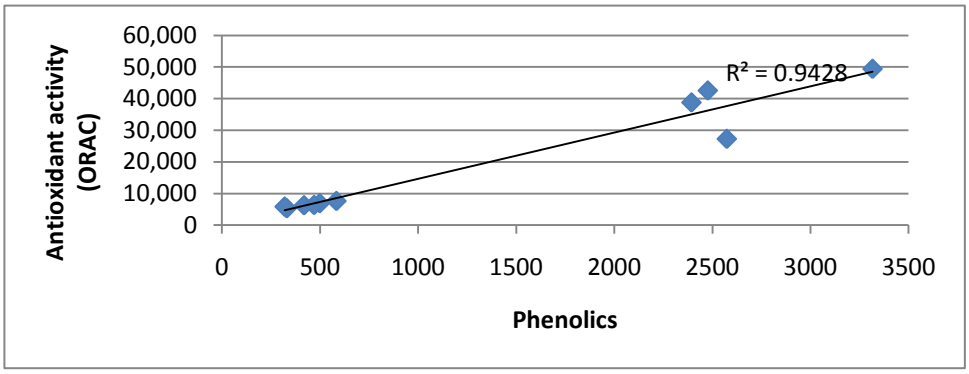
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)

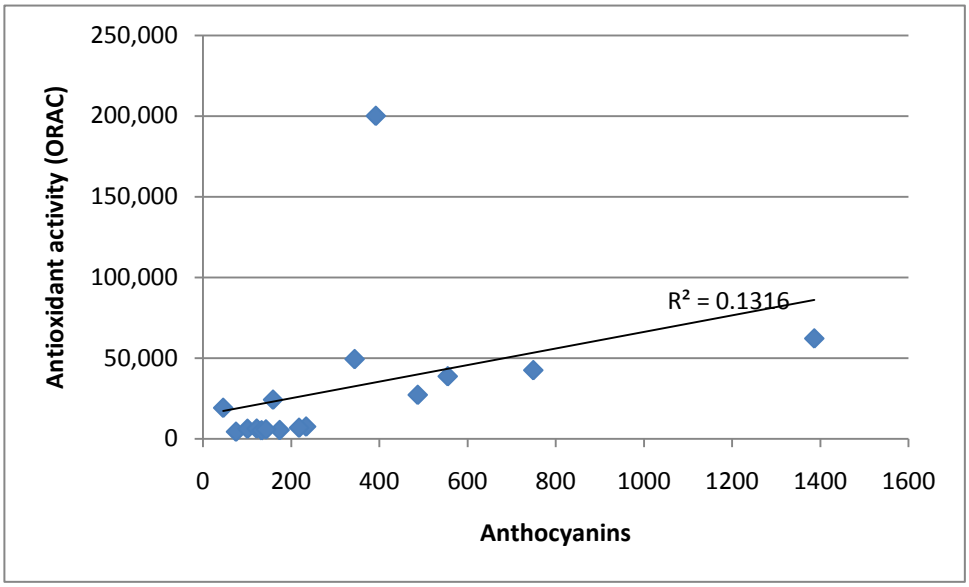


d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)

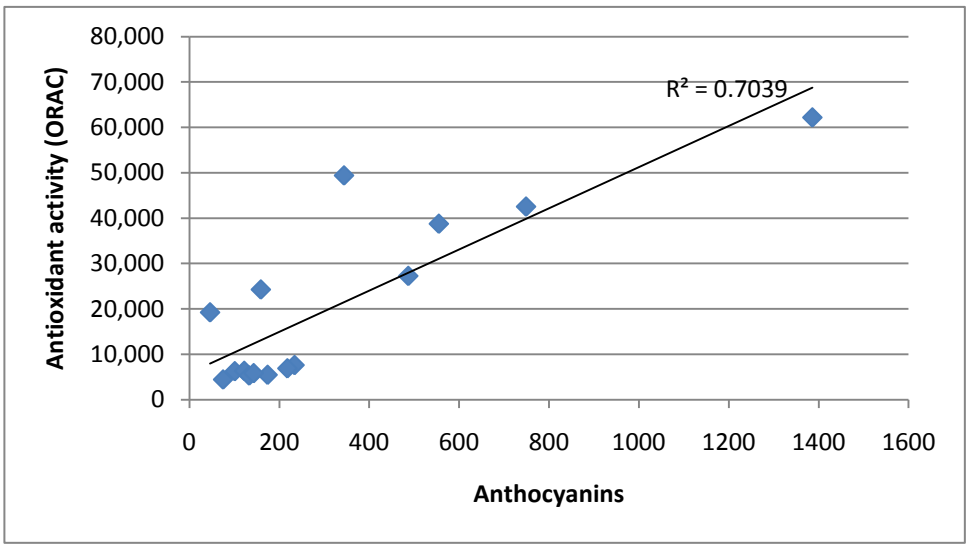


C. Relationship between anthocyanins and antioxidant capacity as determined by the ORAC assay

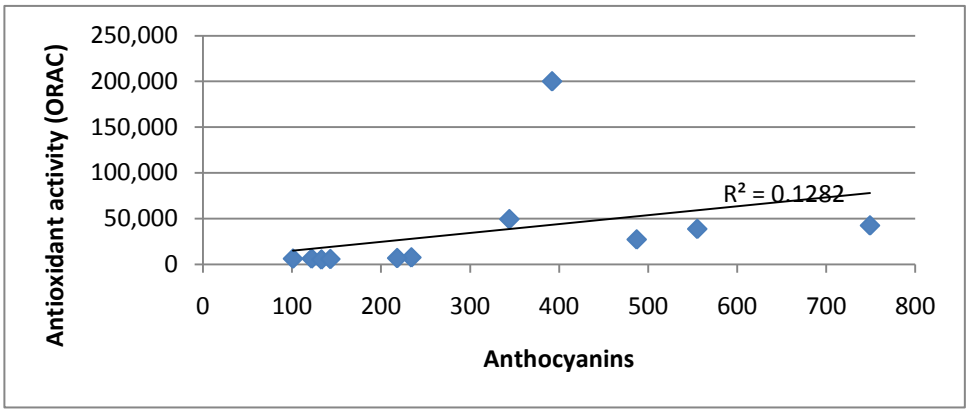
a) For all berry fruit samples (n=16)



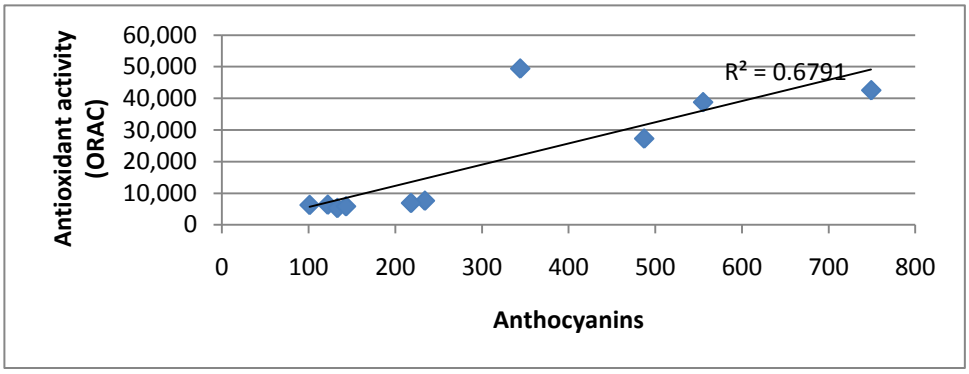
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)

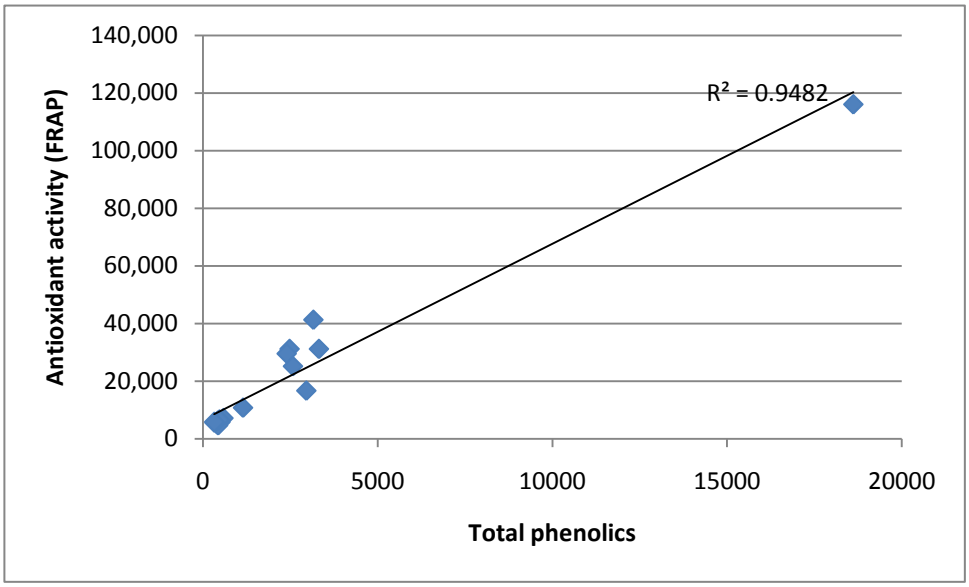


d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)

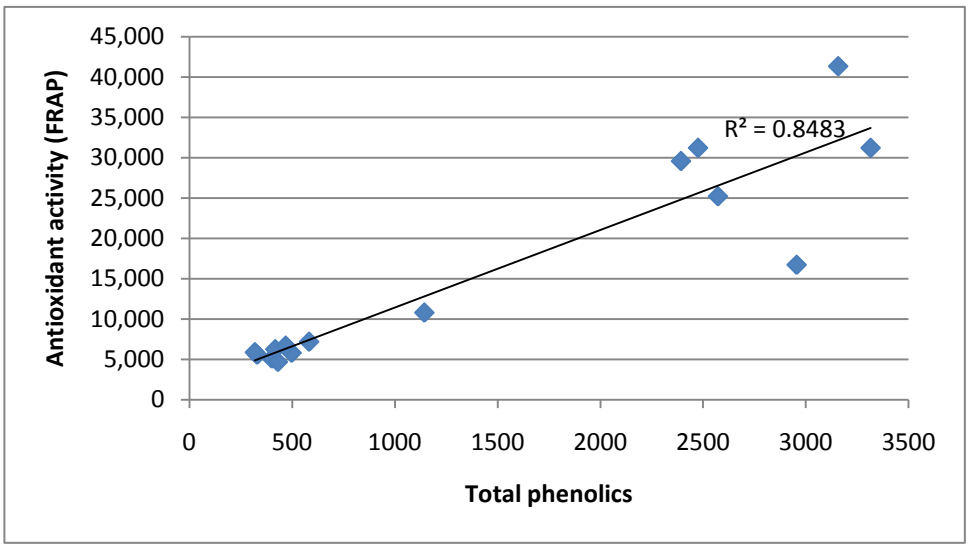


D. Relationship between total phenolics and antioxidant capacity as determined by the FRAP assay

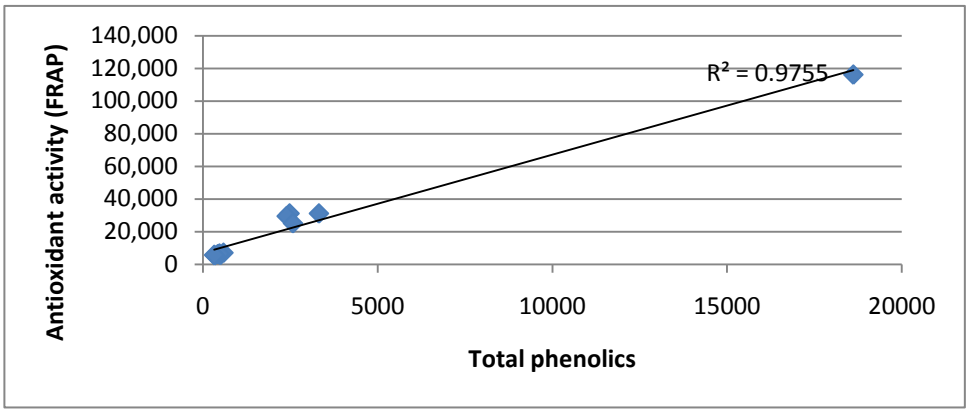
a) For all berry fruit samples (n=16)



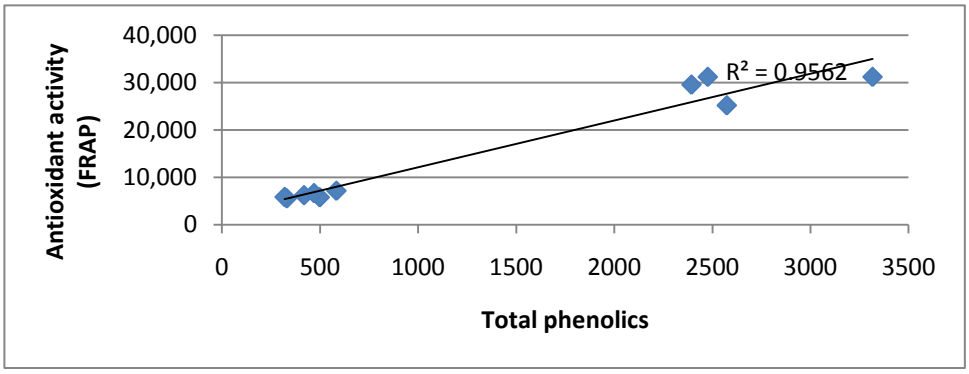
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)

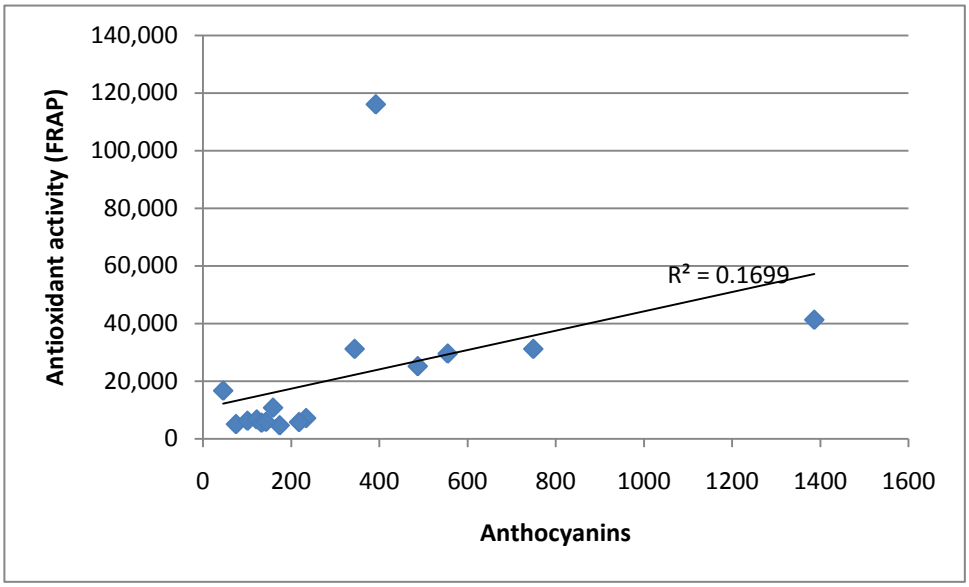


d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)

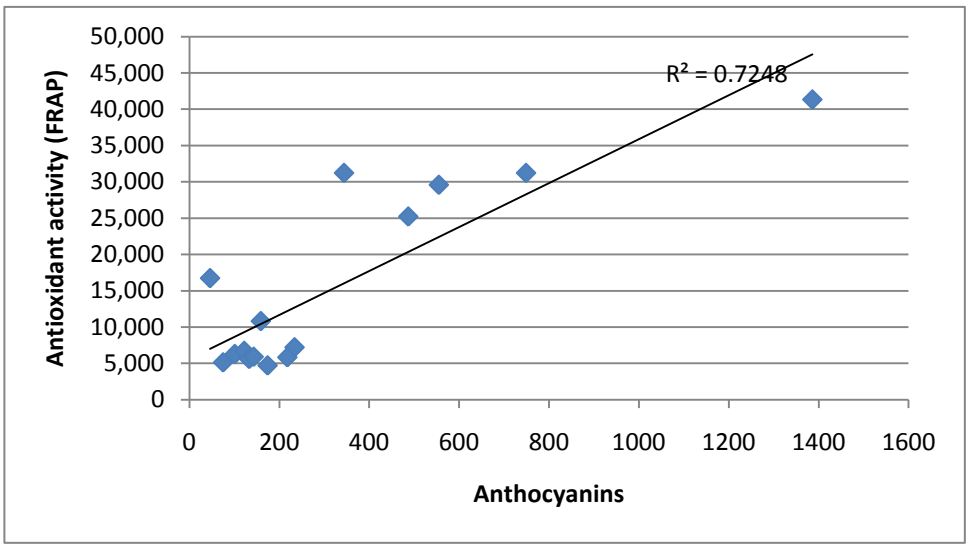


E. Relationship between anthocyanins and antioxidant capacity as determined by the FRAP assay

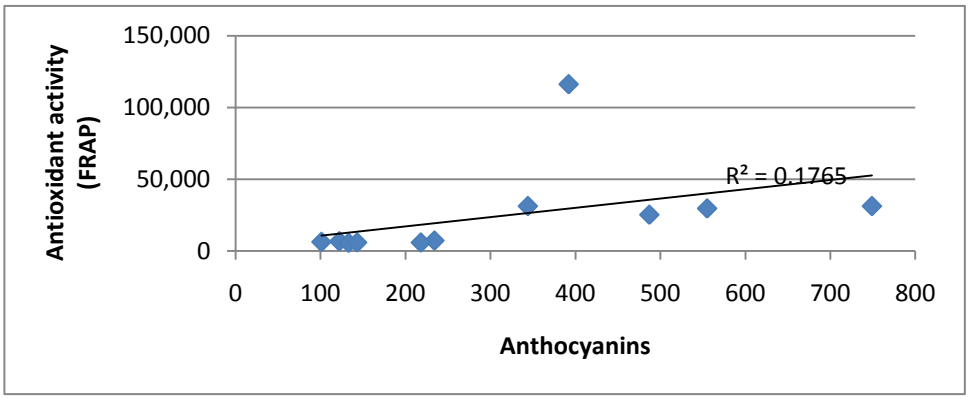
a) For all berry fruit samples (n=16)



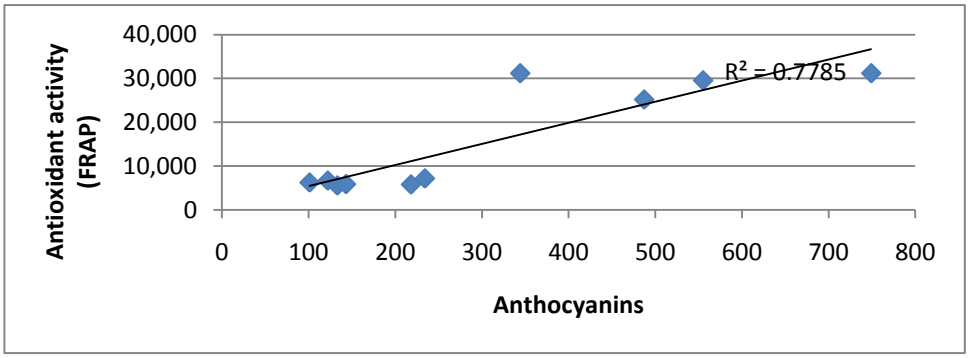
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)

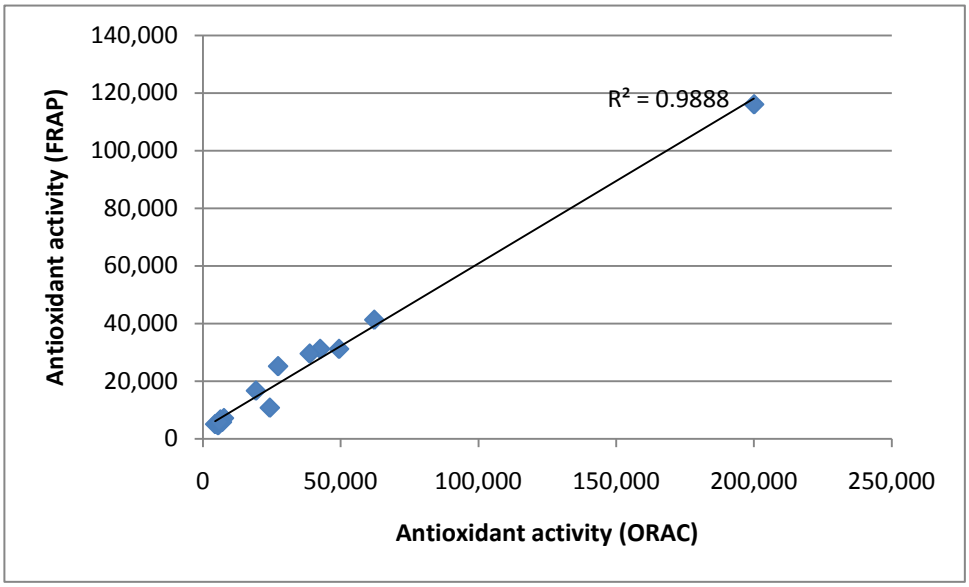


d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)

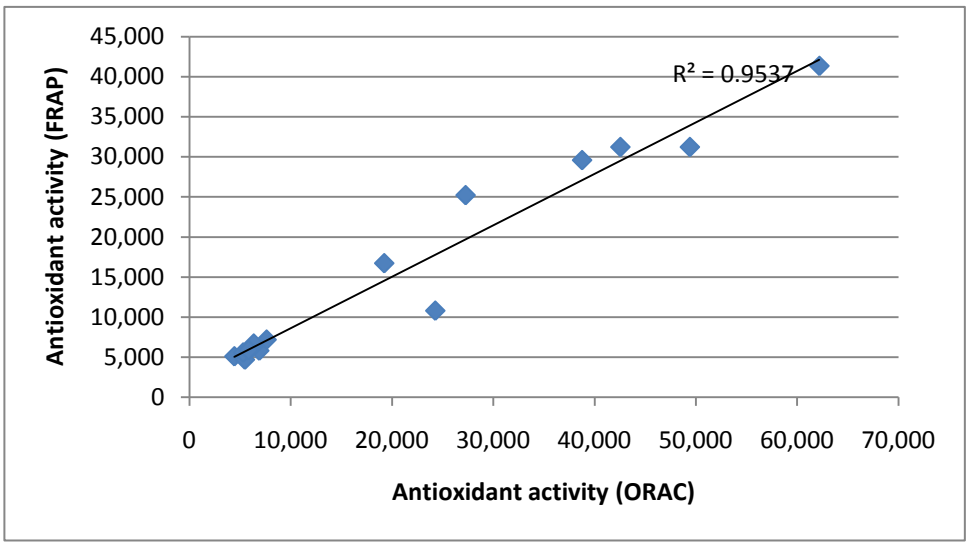


F. Relationship between antioxidant capacity as determined by the ORAC assay and antioxidant capacity as determined by the FRAP assay

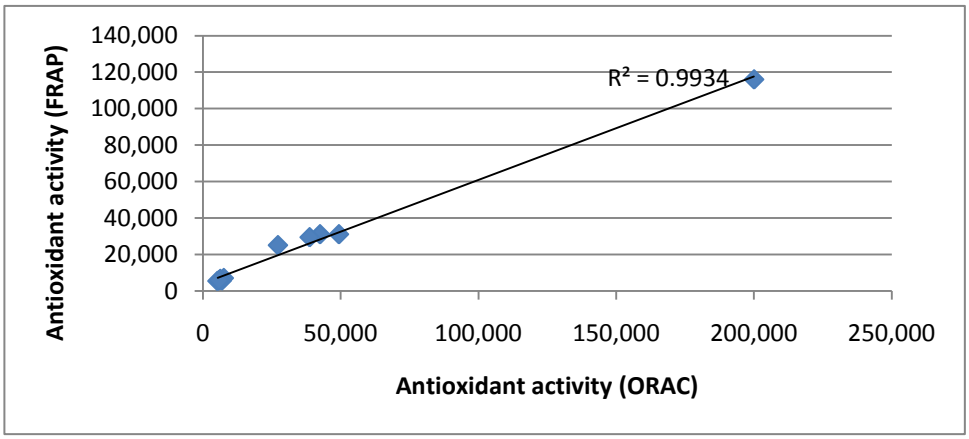
a) For all berry fruit samples (n=16)



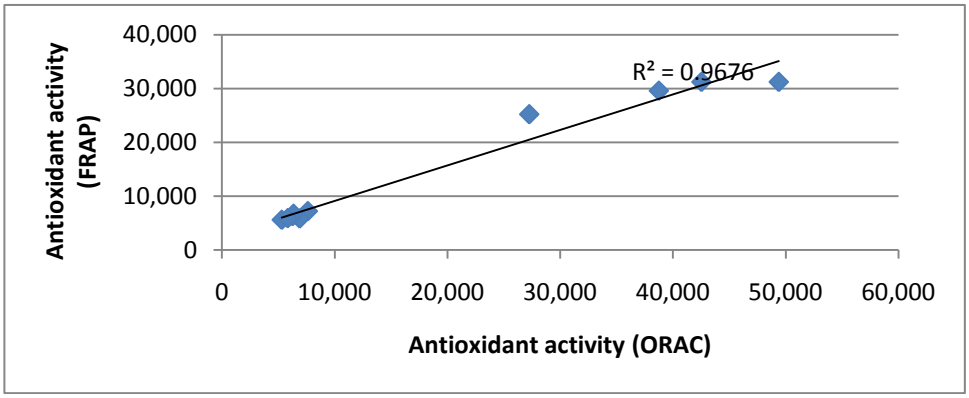
b) For all berry fruit samples excluding the Oxi-Fend powder (n=15)



c) For Boysenberry samples only (n=11)



d) For Boysenberry samples only excluding the Oxi-Fend powder (n=10)



Appendix 4: UPLC Figures

Figure 1a: Anthocyanins (530 nm)

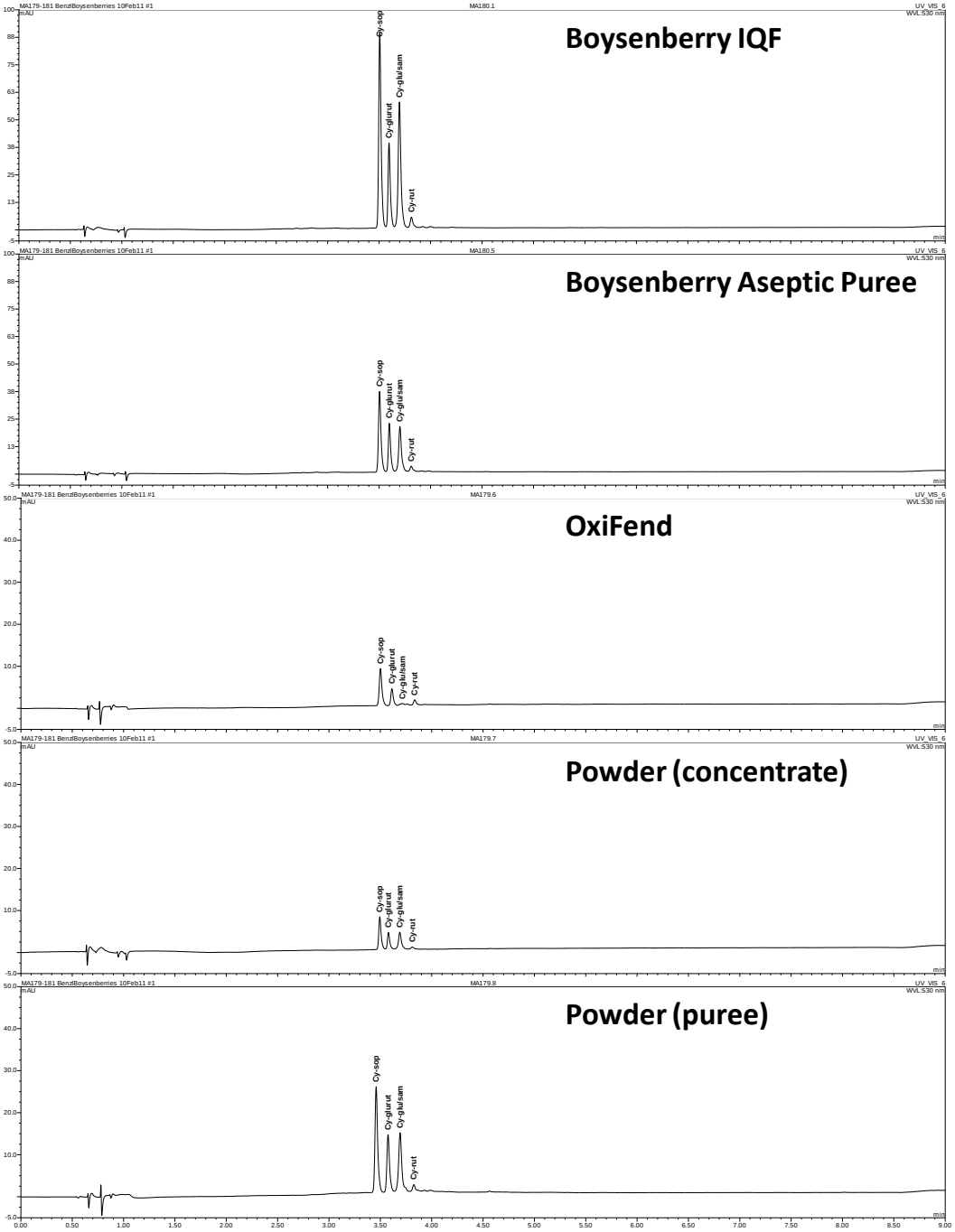


Figure 1b: Anthocyanins (530 nm)

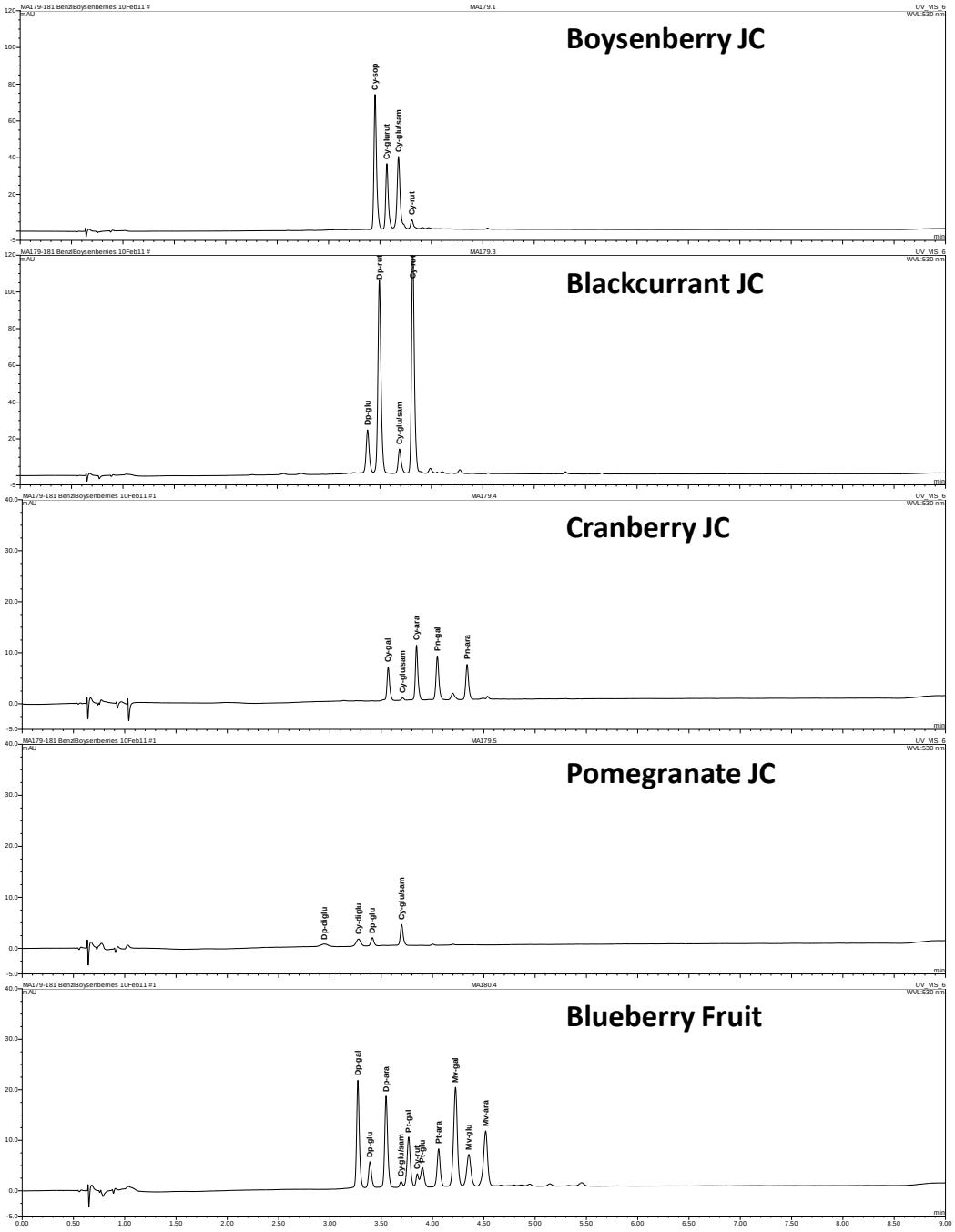


Figure 2a: Phenolics (280 nm)

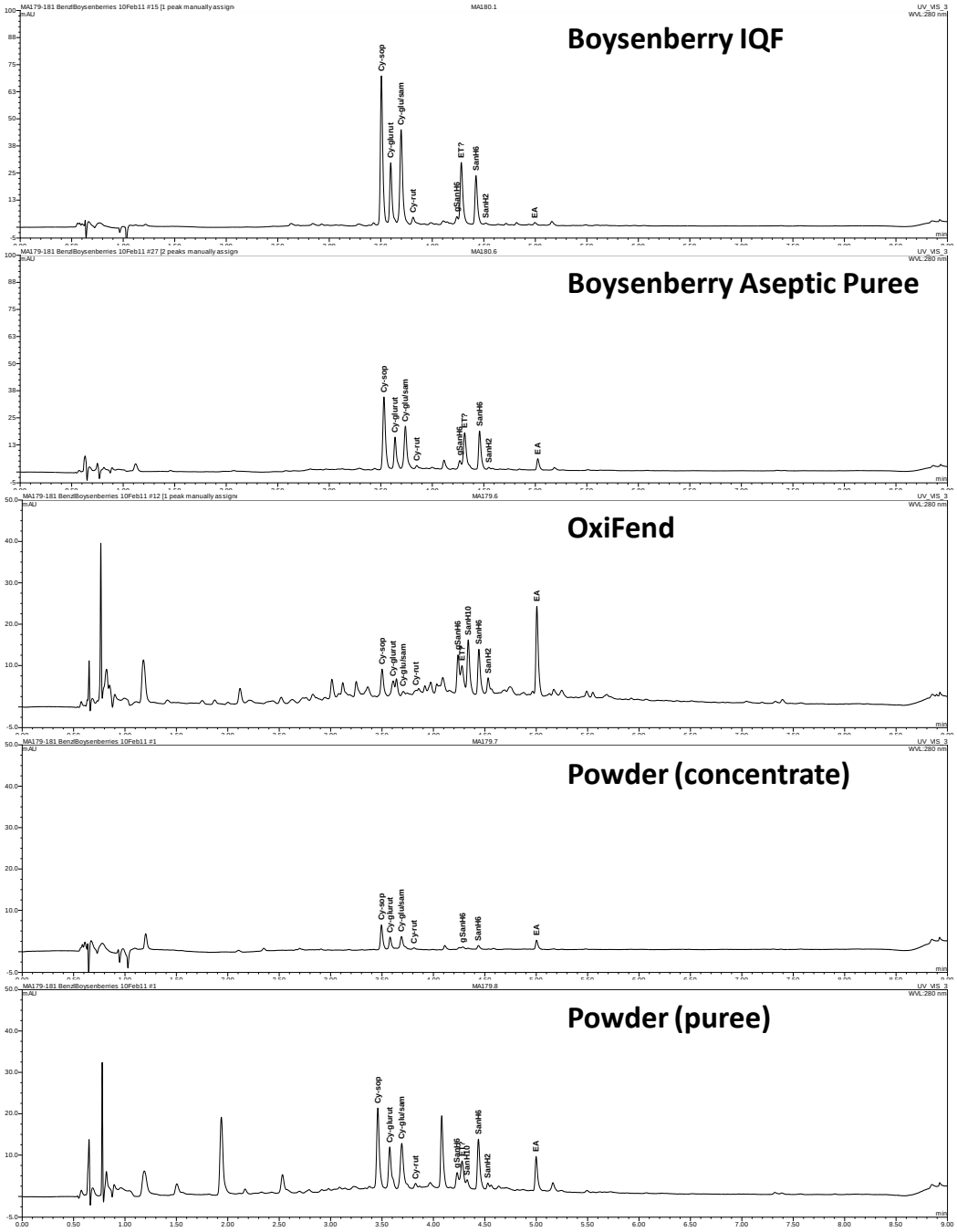


Figure 2b: Phenolics (280 nm)

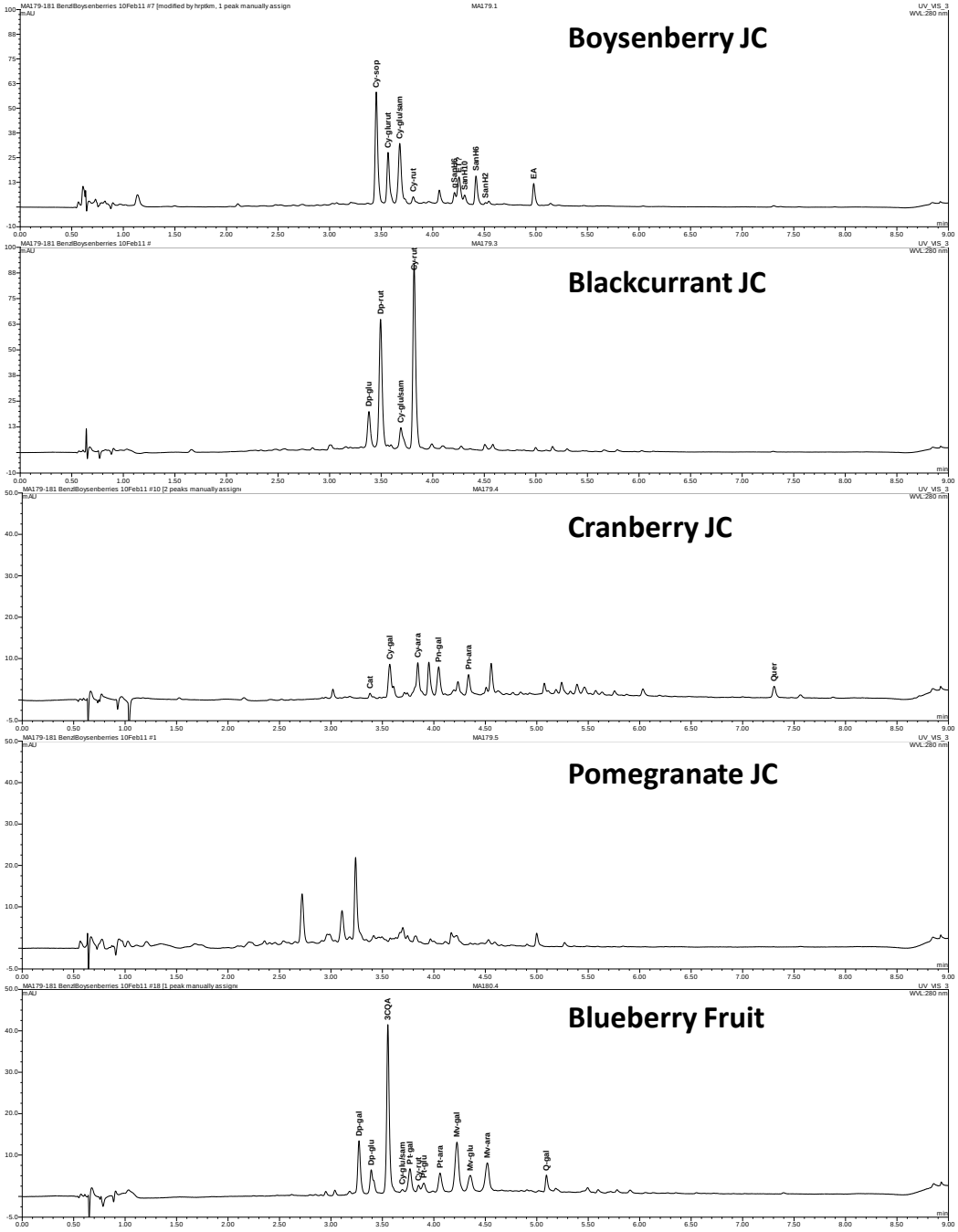


Figure 3a: Ellagitannins (210 nm)

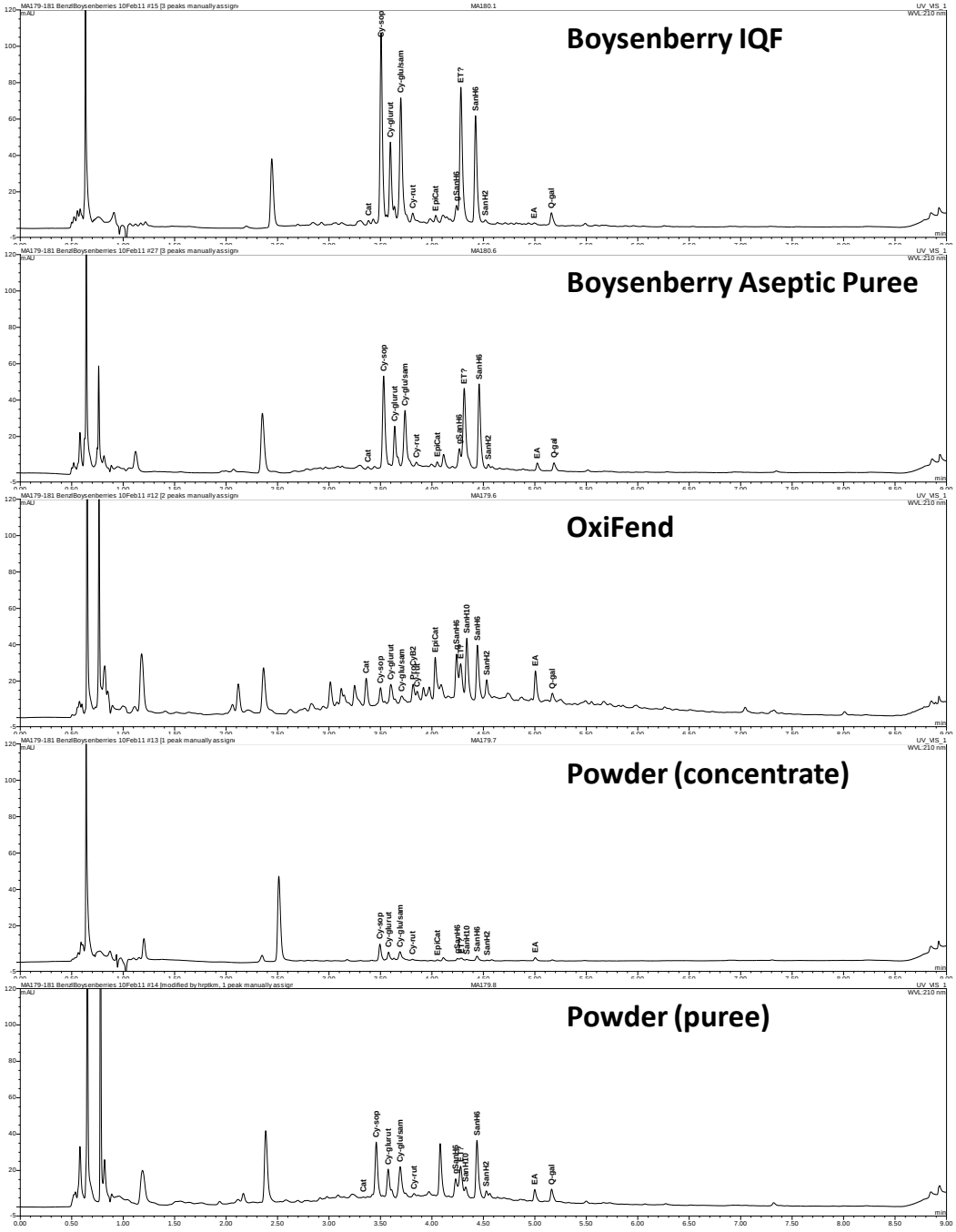


Figure 3b: Ellagitannins (210 nm)

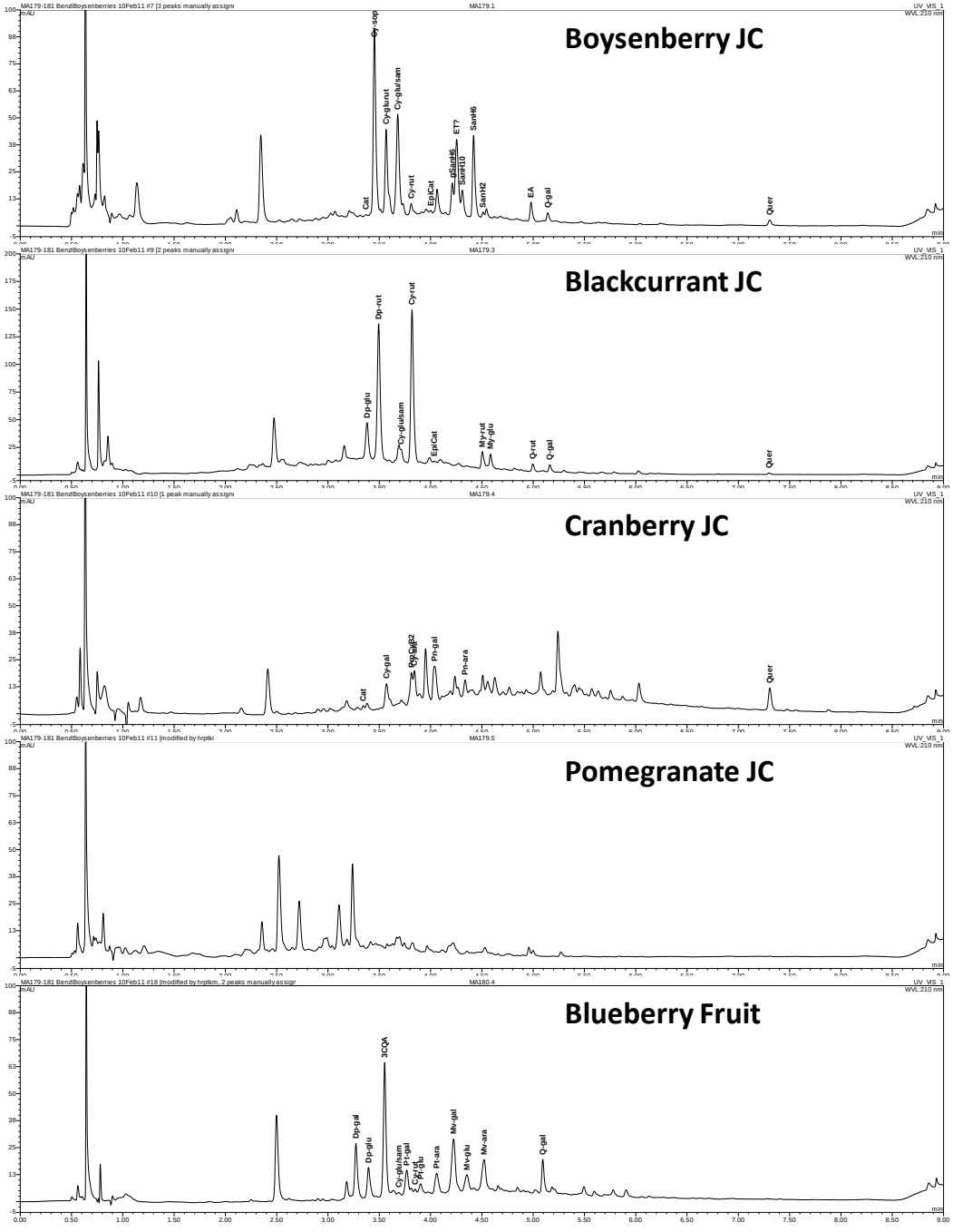
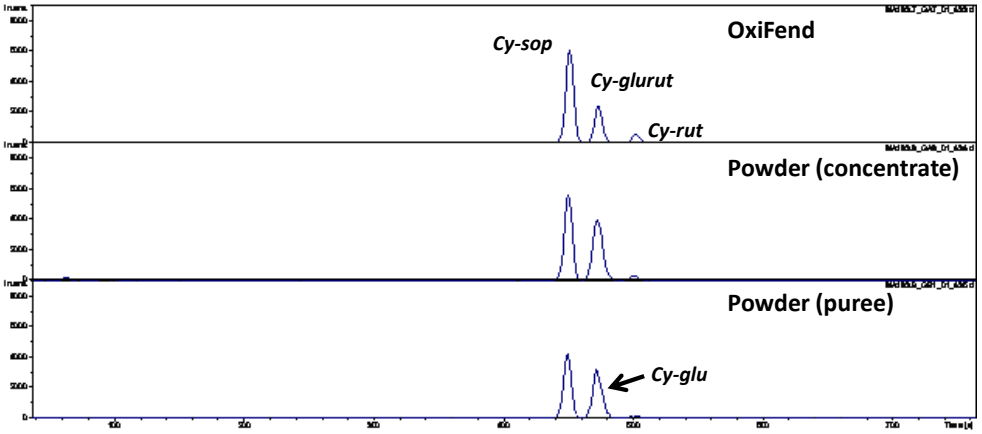
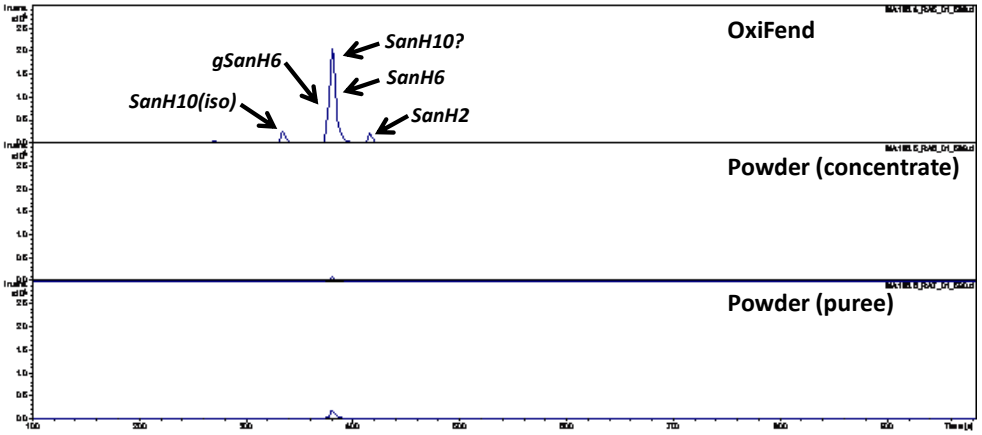


Figure 4: LCMS (Powders)

Anthocyanins



Ellagitannins



Polyphenols