Phytochemical analysis of salal berry (*Gaultheria shallon* Pursh.), a traditionally-consumed fruit from western North America with exceptionally high proanthocyanidin content

Andrew Ferguson[1], Elisabete Carvalho[2], Geraldine Gourlay[1], Vincent Walker[1], Stefan Martens[2], Juha-Pekka Salminen[3], and C. Peter Constabel[1]*

[1] Centre for Forest Biology & Department of Biology, University of Victoria, Box 3020 Stn CSC, Victoria, BC, Canada,V8W 3N5

[2] Fondazione Edmund Mach, Istituto Agrario di San Michele all'Adige (IASMA), San Michele all'Adige, Italy

[3] Natural Chemistry Research Group, Department of Chemistry, University of Turku, Vatselankatu 2, FI-20500 Turku, Finland

*corresponding author

Address for correspondence:

Centre for Forest Biology, Department of Biology
University of Victoria,
Box 3020, Stn CSC
Victoria, B.C., Canada
Tel: (250) 472-5140 Fax: (250) 721-6611

Abstract

Salal (*Gaultheria shallon* Pursh.) is a wild perennial shrub of the Ericaceae and common in coastal forests of western North America, and its berries were an important traditional food for First Nations in British Columbia. Salal berries were investigated for phytochemical content and antioxidant capacity over the course of fruit development. The proanthocyanidin content was extremely high in young berries (280.7 mg/g dry wt) but dropped during development to 52.8 mg/g dry wt. By contrast, anthocyanins accumulated only at the late berry stages. Total antioxidant capacity, as measured by the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method, reflected both proanthocyanidin and anthocyanin content, and in mature berries reached 36 mmol Trolox equivalents/100g dry wt. More detailed phytochemical analysis determined that delphinidin 3-*O*-galactoside is the dominant anthocyanin, and that the berries are also rich in procyanidins, including procyanidin A2 which has been implicated in anti-adhesion activity for uropathogenic *E. coli*. Proanthocyanidins were 60% prodelphinidin, and overall concentrations were higher than reported for many *Vaccinium* species including blueberry, lingonberry, and cranberry. Overall, the phenolic profile of salal berries indicates that these fruit contain a diversity of health-promoting phenolics.

1. Introduction

Plants produce an impressive number of phenolic secondary plant metabolites with diverse ecological roles that allow plants adapt to their environment. A prominent group within the phenolics are the flavonoids, which include the anthocyanins, flavonols, flavones, isoflavonoids, flavan-3-ols, and proanthocyanidins (PAs; *syn.* condensed tannins). While most flavonoids share a common three-ring structure and are synthesized via the general flavonoid pathway, they have diverse bioactivities and functions. For example, anthocyanins are common red and blue plant pigments, which function to attract pollinators and seed dispersers to flowers and fruit (Davies et al., 2012). By contrast, the biosynthetically related PAs are known to bind proteins and act as defenses against mammalian herbivores and fungal pathogens (Barbehenn and Constabel, 2011). They also have general antimicrobial activity (Scalbert, 1991); when deposited in soils as leaf litter, PAs can inhibit microbial activity and nutrient cycling (Schweitzer et al., 2004). In most species, PAs consist of a mixture of oligomeric and polymeric flavan-3-ols with varying mean degrees of polymerization (mDP), ranging from 2-30 or higher (Porter, 1988). They may be linked via several types of C-C linkages; most common are C4 → C8 linkages, but others such as double-linked A2 type oligomers are also found. PAs are particularly abundant in trees and woody plants, and occur at high concentrations in roots, bark, as well as leaves (Porter, 1988). They are considered the most widespread secondary plant metabolite.

The PAs are also common phytochemical constituents of berry fruits (Prior and Gu, 2005; Rasmussen et al., 2005). Fruits are thought to be the biggest source of PAs in western diets, although cereals, beans and nuts are also important. Comprehensive surveys indicate a wide range in both PA content and structure in commercially grown fruits (Gu and Prior, 2005; Hosseinian et al., 2007). Cranberry, blueberry, and strawberry show among the highest PA contents, accumulating 150 - 420 mg/ 100g fr. wt. Wild berries also demonstrate a broad range of PA concentrations. In a detailed analysis of twelve native berries from northern Canada, Dudonné et al. (2015) found the greatest PA concentrations (700 mg/100g fr. wt) in highbush cranberry (*Viburnum trilobum* Marsh.). Anthocyanins are also prevalent in many dark berries, with concentrations as high as 500 mg/ 100g fr. wt in black crowberry (*Empetrum nigrum* L.). Other phenolic compounds such as flavonols or hydroxycinnamic acids including chlorogenic acid, also occur in many berries, but generally in lower concentrations (Dudonné et al., 2015).

Phenolic phytochemicals, in particular the PAs and anthocyanins, have been studied intensively as antioxidants and for their benefits to human health (Quideau et al., 2011; Prior and Gu, 2005).

Many phenolics have strong *in vitro* free radical scavenging capacity (Quideau et al., 2011; Hagerman et al., 1998), since a phenolic ring with at least two hydroxyls in the *ortho* position is typically an effective antioxidant (Quideau et al., 2011). Commonly occurring examples are the flavonol quercetin, anthocyanins such as cyanidin, and flavan-3-ols, for example catechin and PA. The radical scavenging and antioxidant ability of PAs is further increased in polymers due to the large number of aromatics and hydroxyls in close proximity to each other (Hagerman et al., 1998). Antioxidant capacity of phenolics is readily measured using the common ABTS (2,2'- azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay, which has facilitated surveys demonstrating the substantial antioxidant capacity of many fruits and vegetables (Re et al., 1999; Määttä-Riihinen et al. 2005; Prior et al., 2005).

In parallel, epidemiological studies suggest that consumption of foods high in PAs has long-term benefits to human health, including a reduced risk of cardiovascular and neurodegenerative diseases (Prior and Gu, 2005, Santos-Buelga and Scalbert, 2000), as well as hypoglycaemic and anti-inflammatory effects (Grace et al., 2013; Esposito et al., 2014). Other potential health benefits include increasing serum antioxidant capacity (Serafini et al., 1998) and antihypertensive benefits (Furuuchi et al., 2012). Whether the well-established *in vitro* antioxidant capacity of PAs and other phenolics is mechanistically linked to their health-promoting function is still an open question, since this is likely not their only effect. It is important to note that the PA content of a food does not reflect its bioavailability *in vivo;* this varies with the specific structures involved, and depends on its propensity to be absorbed by the intestine as well as metabolic conversions, and interactions with gut flora (Santos-Buelga and Scalbert 2000). However, despite incomplete knowledge of their interactions with human metabolic processes, the potential beneficial effects of dietary PAs continues to drive investigations of the phytochemistry of fruit.

Salal (*Gaultheria shallon* Pursh.) is an ericaceous shrub common in temperate rainforests of the Pacific Northwest (Tappeiner et al., 2001). Salal berries are similar in shape and size to blueberries, huckleberries and other *Vaccinium* species*;* they are a traditional food of coastal First Nations, who consumed the berries fresh, or mashed and dried into cakes for consumption throughout the winter (Turner and Bell, 1971; 1973). Salal forms a dense understory, was once thought compete with reforestation by outcompeting seedlings (Tappiener et al., 2001). An earlier survey found that salal berries exhibit high antioxidant capacity (Acuña et al., 2002; Einbond *et al.*, 2004), and contain a range of simple phenolics including notably high levels of caffeic acid (Towers et al., 1966). Recently, two reports identified additional phenolic constituents, including small oligomeric PAs,

anthocyanins, and flavonols in salal fruit (McDougall et al., 2016), and measured total antioxidant activity, anthocyanins, and phenolic content (Martin et al., 2015). These authors did not include polymeric PAs in their analysis, and did not provide quantitative data on individual compounds.

The suggestion of strong antioxidant activity, reports of high PA concentrations in salal leaves (Preston, 1999), and traditional First Nations use of salal berries prompted us to investigate these fruit in detail. We hypothesized that the reported antioxidant capacity of salal berry is due to PA concentrations, and that the berries could thus be an important source of dietary PAs. Furthermore, we took a developmental approach and profiled PAs and other phytochemicals throughout fruit development. Our aim was to understand patterns of antioxidant capacity and phytochemicals over time, which ultimately should reflect their biological function.

2. Results

2.1 Developmental profile of salal anthocyanins, proanthocyanidins, and antioxidant capacity

Salal berries from the eight stages of berry development and ripening showed a predictable increase in weight and size (Fig. 1a, b). Anthocyanins were detected in all stages of flower and berry, but were lowest in the white open flower stage (Fig. 1c). A low concentration of total anthocyanins was maintained until stage B7, when their concentration increased dramatically. Mature salal berries (B8) contained very high levels of anthocyanins, more than 1500 mg/ 100g dry wt of total anthocyanins.

To determine how berry antioxidant capacity changed over the profile, we carried out antioxidant assays of MeOH extracts using the colourimetric ABTS method (Re at al., 1999). Antioxidant capacity of salal extracts was highest in the flower and young berry stages, with maximal levels reaching 106 mmol TE/100g dry weight at stage B2 (Fig. 2)**.** As berries matured, antioxidant activity diminished several-fold, to approximately 36 μmol TE/100g dry weight. The reduction in antioxidant capacity from B4 to B5 coincided with a rapid increase in mean berry mass, presumably diluting out the antioxidant levels. For comparison, we tested antioxidant capacity of mature highbush blueberries (cv. Rubel), and measured 13 μmol TE/100g dry weight, approximately one-third the capacity as mature salal berries.

Since the antioxidant levels of berries did not follow the anthocyanin concentrations, we investigated the PA concentrations using the butanol-HCl assay (Fig. 3). Using a purified salal leaf PA standard, we determined that salal berries contained very high concentrations of PAs in younger stages, which declined several fold thereafter to a mean of 5280 mg/100 g dry wt in mature berries (stage B8). High concentrations of PAs were also found in the flowers. In general, the PA concentration profile paralleled the antioxidant activity, suggesting this activity is mostly due to salal berry PAs. Indeed, we determined a strong correlation between PA concentration and antioxidant capacity of samples (R^2= 0.969) (Supplemental Fig. S1). When PA content was replotted on a per berry basis, it became apparent that total PAs continued to accumulate during ripening (Fig. 3). The PA concentration profile is thus the balance of biosynthesis and dilution by berry expansion. Overall, the PA concentration of mature berries is almost ten-fold higher than those we measured in blueberries (Zifkin et al., 2012), suggesting that salal could be an excellent source of dietary PAs.

2.2. UHPLC-MS/MS analysis of individual salal phenolics

The high antioxidant and flavonoid content of salal prompted us to investigate the phytochemistry of these berries in more detail. We subjected a selection of berry stages to a more detailed analysis of anthocyanins, flavan-3-ols, and small phenolics using established UHPLC-MS/MS methods (Arapitsas et al., 2012, Vrhovsek et al., 2012). These methods are based on the scanning of specific ions and their respective fragments for flavonoids and other phenolics, and have been used to characterize several fruits and berries (Zoratti et al., 2014; Oertel et al., 2017). This analysis provided quantitative data for a wide range of specific anthocyanins and phenolics commonly found in berries. For comparison, we also analyzed immature and mature blueberry fruit.

As expected, anthocyanins were detected in significant quantity only in ripe fruit (Table 1). Salal berries contained predominantly delphinidin derivatives, but no methylated anthocyanins (malvidin, peonidin) as are found in blueberry. The most abundant delphinidin was delphinidin-3-*O*-galactoside, which was also the most abundant anthocyanin in blueberry. A diversity of cyanidin glycosides was detected, but at lower in levels than the delphinidins. We detected fewer types of anthocyanins in salal berry compared to blueberry, although anthocyanins were more abundant in salal relative to blueberry. At maturity, salal berries are a deep black colour, reflecting the high concentration of anthocyanins (Fig. 1).

Consistent with the high levels of PAs in salal berry, substantial concentrations of monomeric and dimeric flavan-3-ols were also detected (Table 2). Paralleling the PA profile, these compounds were more abundant in the unripe berry stages, and decreased as the berries matured. The most abundant flavan-3-ol in salal berry was catechin, a common plant flavonoid and potential precursor of PAs. Gallocatechin were also present at significant concentrations. The dimeric procyanidins B1, B2/B4, B3, and A2 were all detected, but procyanidin B1, a catechin-epicatechin dimer, was the most abundant. Procyanidins B3 and A2 were also present in salal, but not blueberry. Procyanidin A2 in particular may be important for certain health-promoting activities: its abundance in American cranberry (*Vaccinium macrocarpon* Ait) is important for counteracting urinary tract infections (see Discussion). Three caffeoyl quinic acids were present in salal at all stages of development, with neochlorogenic acid (5-*O*-caffeoyl quinic acid) being the most prominent. By contrast, blueberry accumulated primarily chlorogenic acid (3-*O*-caffeoyl quinic acid); beside the PAs, this is the most abundant phenolic in blueberry.

Our MS/MS method also quantified a diversity of flavonols, but these were relatively minor components of the salal extracts (Table 2). Quercetin glycosides were the most prominent, including quercetin-3-*O*-glucuronide and quercetin-3-*O*-galactoside. Lesser amounts of myricitrin (myricetin-3-*O*-rhamnoside) were measured, as well as a flavone (luteolin-7-*O*-glucoside). Blueberry contained similar amounts of both quercetin derivatives as salal. We also measured low concentrations of kaempferol and isorhamnetin glucosides in blueberry (data not shown), which were not detected in salal. Total concentrations of flavonols were similar in ripe salal and blueberry.

2.3. Structural analysis of PAs

The presence of flavan-3-ols with trihydroxylated B-rings, in particular gallocatechin, suggested that the PA oligomers and polymers could have a significant prodelphinidin content. To test this idea, we employed a recently developed MS/MS method for determining prodelphinidin and procyanidin content of PAs in complex mixtures. This method relies on in-source fragmentation of PA oligomers and polymers by a series of increasing cone voltages followed by selective multiple reaction monitoring (MRM) quantification of both terminal and extension units of prodelphinidin and procyanidin subunits thus generating characteristic PA fingerprints for the samples analysed (Engström et al., 2014). The results indicate that salal berry PA contains a slightly greater proportion of prodelphinidin than procyanidin subunits (Fig. 4), with 60% prodelphinidin in B8 berries. We also determined that the mDP of mature salal berry PAs was 13.3, whereas in open flowers, the mDP was 9.2..

3. Discussion

The Ericaceae family contains a number of widely consumed berries, including blueberry, cranberry, and other *Vaccinium* species known for their antioxidant phenolic phytochemicals and health-promoting properties. Here we report a detailed phytochemical analysis of salal berry, an Ericaceous plant from western North America. We found that these berries have a remarkably high antioxidant capacity, which is correlated with high PA and anthocyanin concentrations. The concentration of PA was highest in young salal berries, dropping approximately five-fold as the berries expanded and matured. By contrast, anthocyanin accumulated only towards the end of ripening. The PA concentration decreased to approximately 5300 mg PAs/100 g dry wt for mature berries, but on a per-fruit basis, PA content continued to increase (Fig. 3). This suggests on-going PA synthesis during all stages of maturation. There are few phytochemical studies of berries across a developmental profile; however, our earlier work on blueberry, as well as studies in Saskatoon berry (*Amelanchier alnifolia Nutt.*) showed that PA synthesis followed the same pattern: high initial PA levels are diluted out by berry expansion, while *de novo* synthesis continues throughout maturation (Zifkin et al, 2012; Jin et al., 2015).

When converted to a fresh weight basis to facilitate comparisons with the literature, we estimated that salal contained 1200 mg PA/ 100g fr. wt, substantially more than the 250 mg/ 100g fr. wt we measured in blueberry fruits (Zifkin et al., 2012). For comparison, among wild berries from northern Canada, only highbush cranberry (*V. trilobum*) and lingonberry (*Vaccinium vitis-idaea* L.) approach the high PA concentrations of salal, with 700 mg/g fr. wt and 250 mg/g fr. wt, respectively (Dudonné et al., 2015). Similarly, Shiraz grape berry skins contain 500 mg PA /100g fr. wt, with the seed accumulating three-fold higher levels (Downey et al., 2003).

Salal berries are also notable for their flavan-3-ol concentrations (Table 2); when converted to a fresh weight basis, these are equivalent to 50 mg/100g fr. wt. Both catechin and epicatechin, as well as gallocatechin and epigallocatechin, were detected at higher levels than blueberry (Table 2). This exceeds concentrations reported for any of the Canadian wild berries except for highbush cranberry (*V. trilobum*; Dudonné et al., 2015). Our analysis also included the dimeric procyanidins, which have health-related importance since unlike larger PAs they can be absorbed across the gut lining. Salal contained similar concentrations of procyanidin B1 as mature blueberry, but also accumulated additional procyanidin types. Interestingly, we detected procyanidin A2, a less common procyanidin typical of *Vaccinium* species including cranberry (*V. oxycoccus* L.), lingonberry (*V. vitis-idaea* L.), and bilberry (*V. myrtillus* L.) (Määttä-Riihinen et al., 2005; Jungfer et al., 2012). Procyanidin A2

and other A-type PAs are characterized by a two distinct linkages (C4-C8 and C2-O-C7). A-type PAs inhibit the adhesion of uropathogenic P-fimbriated *E. coli*, a causative agent of urinary tract infections; work with American cranberry (*V. macrocarpon*) extracts demonstrated that these anti-adhesive effects were specific for A-linked PAs (Foo et al., 2000).

The high concentration of anthocyanins in the skin of salal berry lead to a distinct dark black appearance. Among the northern Canadian berries analysed by Dudonné et al. (2015), only black crowberry (*E. nigrum*) and chokecherry (*Prunus virginiana* L.) have similarly high anthocyanin content. The major anthocyanins in salal are delphinidin derivatives, in particular delphinidin-3-*O*-galactoside. Overall, the anthocyanin profile is simpler than that seen in *Vaccinium* species, which additionally contain malvidin and petunidin glycosides (Table 1; Dudonné et al., 2015). A phytochemical analysis reported by McDougall et al. (2016) also determined a preponderance of delphinidins. Flavonols appear to be a relatively minor component of salal flavonoids, although we detected both quercetin and myricetin glycosides.

Our development profile suggests that high antioxidant activity in salal berry is due primarily to PAs with some contribution of anthocyanins. We noted that the antioxidant capacity of fruit extracts generally decreased during development from the B2 stage as these mature, closely following the PA concentration. In the later ripening stages (B7-B8), however, antioxidant capacity decreased less steeply than the PA concentration (compare Figs. 2 and 3). This likely reflects the rapid synthesis of anthocyanins in stages B7 and B8. Like the PAs, the anthocyanins are well known as effective *in vitro* antioxidants (Prior et al, 1998). On a Trolox equivalent basis, the total antioxidant capacity of salal is greater than most other berries surveyed: compared to published antioxidant activity using of *Vaccinium* berries (*V. corymbosum, V. myrtillus*) as measured by the same ABTS assay, salal has at least two-fold greater antioxidant capacity (Bakowska-Barczak et al., 2007). Whether overall *in vitro* antioxidant capacity necessarily predicts health benefits is an open question; anthocyanins are known to have poor bioavailability (Lila et al., 2016), while small PAs (dimers and trimers) can be absorbed and are found in plasma (Santos-Buelga and Scalbert, 2000; Rasmussen et al., 2005). Larger PA polymers may act without being absorbed by modulating the gut microbiome (Etxeberria et al. 2013). In human volunteers, blueberry extract was shown enhance the abundance of Bifidobacteria (Vendrame et al., 2011) which are among the most beneficial probiotic gut microbes. In this context, it is interesting that the mean degree of polymerization (mDP) for mature salal berry PAs was 13.3; this is greater than what has been reported for many berry species (Dudonné et al.,

2015) including *V. corymbosum* (mDP of 8,5; Zifkin et al., 2012), and could suggest an enhanced probiotic effect of salal PAs.

Other structural features of the PAs are likely to impact health benefits and potential ecological functions. The LC-MS/MS analysis indicated that salal PAs contain more prodelphinidin than procyanidin subunits, with a mean prodelphinidin content of 60% for mature berries. For comparison, our earlier analysis of blueberry PAs by phloroglucinolysis indicated 3% prodelphinidin (Zifkin et al, 2012). The functional and nutritional implications of the prodelphinidin: procyanidin ratio of PAs still need to be determined, and how this influences absorption and bioactivity. Laaksonen et al. (2015) observed that higher prodelphinidin content of blackberry juices impacts the sensation of PAs and is associated with the coarse astringency of the taste, whereas high procyanidin content is important for the 'puckering' sensation produced by tannins. In an ecological context, high-prodelphinidin PA have been associated with greater anti-herbivore activity (Ayres et al., 1997). Tannins with greater prodelphinidin content were also more inhibitory to soil N mineralization than those rich in procyanidins (Nierop et al., 2006).

A long-standing question on PAs and other non-pigment phenolics in fruit is how they influence ecological interactions with consumers, which are both negative (herbivory) and positive (seed dispersal). Deterring the herbivory of immature fruit is one likely function of PAs, but their persistent presence in mature fruit in substantial concentrations could also suggest a post-ripening function. For example, PAs are typically antimicrobial (Scalbert, 2000), and thus may help protect against moulds or pathogens. Since salal is a widespread and common native plant in the coastal forests of western North America, in addition to further investigating itsbeneficial health impacts, it would be an excellent system to investigate the ecological role of PAs in fruit.

4. Conclusions

Salal berries contained high concentrations of PAs, anthocyanins, procyanidins, and flavan-3-ols, but only moderate amounts of cholorogenic acids and flavonols. The PA content was highest in young fruit and declined with maturation, and correlated well with the substantial the antioxidant capacity of these berries. This phytochemical profile and high PA concentration could benefit their persistence and dispersal in nature, and if present in the human diet, would be predicted to have beneficial effects on health.

5. Experimental

5.1. Plant Material

Salal (*Gaultheria shallon Pursh.*) was collected from an exposed clear-cut location near Sooke, British Columbia (48°23'27.6" N, 123°52'33.5" W). Flower and immature berries were collected on July 16th, 2012, and all stages were collected on August 9th, 2012. Since salal berries mature unevenly and any one plant will have berries of a wide range of stages, samples were collected from more than 50 plants from the same locale in order to obtain enough material of all stages. Material was roughly sorted into stages on site, frozen in liquid nitrogen, then stored at -80° C. Flowers were sorted into two stages, closed flower (CF) and open flower (OF), based on whether or not the corolla was open at the apex. Frozen berries were later sorted on dry ice into eight stages based on size, then colour (Ozga et al., 2006; Zifkin et al., 2012). Immature berries were small, hard, and green, and progressed towards a more delicate, deep purple berry when mature (Fig. 1). The first five stages were diameter-based (B1, 2-4 mm; B2, 4-5.5 mm; B3, 5.5-7 mm; B4, 7-8 mm; B5, 8-10 mm). The last three stages were greater than 10 mm in diameter and sorted by colour (B6, some green remaining; B7, completely red-deep red; B8, completely purple). High-bush blueberry (*Vaccinium corymbosum*) material for comparative analyses was obtained from Zifkin et al. (2012) from the 2009 collection period.

5.2. Sample extraction for proanthocyanidin and antioxidant assays

Freeze-dried samples (45-150 mg) were weighed into 2 mL tubes with three to six stainless steel pellets. Each sample was an aggregate of 1-5 berries or flowers, depending on the stage; mature berry samples consisted of 1-2 fruit each, while immature stages consisted of 3-5 berries or flowers. At least seven samples were extracted and analyzed per time point. Samples were homogenized in 1 mL of 100% MeOH in a Precellys 24 (Bertin Technologies) twice for 45 s. Tubes were sonicated for 10 min and centrifuged for 5 min at 15000 rpm. The supernatant was removed and filtered through a 0.45 μm filter into a pre-weighed glass tube. Another 1 mL of MeOH was added to the sample tube, followed by vortexing, sonication and centrifugation to reextract the tissue. Extraction was repeated a third time, for a total of 3 mL of extraction solvent. The supernatant in glass tubes was dried overnight in a SpeedVac Plus coupled to a Universal Vacuum System (ThermoSavant), at medium heat. The dried extract was resuspended in 100% MeOH to a standard extract concentration and

stored in 1.5 mL Eppendorf tubes sealed with Parafilm at -20° C until analysis. These extracts were used for PA, anthocyanin, and ABTS assays.

5.3. Butanol-HCl assay for proanthocyanidins

The butanol-HCl assay for PAs was based on the method of Porter et al. (1986). Briefly, 100 μL of sample extract, 80 μL of distilled water, 220 μL of MeOH, 2 mL of butanol: concentrated (38%) HCl (95:5 v/v), and 67 μL of iron reagent (2% w/v $NH_4Fe(SO_4)$ in 2N HCl) were combined in a 15-mL conical tube. Tubes were vortexed and 200 μL of solution was transferred into a 96-well plate in duplicated wells, and sealed with Parafilm, to be used as unheated controls. The tubes with remaining extract were sealed and heated in a water bath at 95° C for 40 min, then removed and allowed to cool for 20 min. 200 μL of heated solution was removed into duplicate wells of a second 96-well plate, and the absorbance of both heated and unheated samples was read at 550 nm on a Multispec reader. Corrected absorbance was determined by subtracting the A_{550} of unheated samples from A_{550} of the heated samples. PA concentrations were calculated using a purified salal leaf tannin standard, described in Kraus et al. (2003) and provided by Dr. Caroline Preston, Natural Resources Canada, Pacific Forestry Centre, in Victoria B.C.

5.4. Total anthocyanin analysis

Total anthocyanin content of extracts was determined based on the method of Wrolstad (1976), with modifications from Vagiri et al. (2012) using buffered solutions with different pH (0.4M sodium acetate, pH 4.5, and 0.025M potassium chloride, pH 1.0). A 25 μL aliquot of sample extract was added to 1 mL of each buffer and mixed by pipetting. After 15 minutes, the absorbance of each solution was read at 700 nm and at 516 nm. The corrected absorbance of the sample was determined as $A = (A_{516} - A_{700})_{pH1.0} - (A_{516} - A_{700})_{pH4.5}$. Anthocyanin content was calculated as cyanidin-3-*O*-glucoside equivalents (CGE) using the molar absorptivity of cyanidin-3-*O*-glucoside ($\varepsilon = 26900$). Analyses were performed in duplicate for each sample.

5.5 Antioxidant assays

The antioxidant assay method was performed as described by Re at al. (1999). Briefly, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; 7mM final concentration) was allowed to

react with (2.45 mM final concentration) potassium persulfate (dipotassium peroxdisulphate; 2.45 mM final concentration) in ddH_2O water. The solution was left in the dark at room temperature for 12-16 hours to generate $ABTS^+$. Prior to the assays, the solution was diluted with ethanol to an A_{734} of 0.75 +/- 0.05. A 10 μL aliquot of sample extract was combined with 1 mL of diluted $ABTS^+$ solution, mixed by pipetting, and the A_{734} again measured after 6 min. A standard curve was prepared using a Trolox standard, and results are reported in Trolox equivalent antioxidant capacity (TEAC).

5.6. UPLC-MS/MS analysis of berry phenolics

UHPLC-MS/MS analysis was carried out at the Edmund Mach Foundation (San Michele all'Adige, Italy) using in-house pipelines previously developed for berry fruit analysis (Arapitsas et al., 2012; Vrhovsek et al., 2012). For this analysis, pooled berry samples corresponding to each ripening stage were lyophilized and ground in a mortar. Four replicate samples per stage were weighed (100±10 mg) into a 2.0 mL microcentrifuge tubes, and extracted with 1.5 mL of methanol (80%) on an orbital shaker (15 min at room temperature). The samples were centrifuged (5 min, 12000 rpm) and the supernatant was transferred to a 5 mL volumetric flask. The pellet was re-extracted as above, the joint supernatants were brought to volume with 80% methanol. The extracts where filtered on 0.22 μm PFTE membrane into glass vials.

The analysis of anthocyanidin was performed using the UHPLC−MS/MS method described in Arapitsas et al. (2012) using conditions as described. Multiple reaction monitoring (MRM) parameters for the analytes detected are listed in Supplementary Table S1. As MRM methods for galactoside and arabinoside derivatives of anthocyanidins were not included in Arapitsas et al. (2012), these respective MRM transitions were taken from data in the literature. Where authentic standards were not available quantification was achieved relative to the nearest isomer (e.g. delphinidin-3-*O*-galactoside was quantified using delphinidin-3-*O*-glucoside standard). Other polyphenols were quantified as described previously (Vrhovsek et al., 2012). Quantification was carried out using external calibration curves with authentic standards of each of the detected compounds (Arapitsas et al., 2012; Vrhovsek et al., 2012). Data processing was performed using the MassLynx Target Lynx Application Manager (Waters).

Analysis of prodelphinidin and procyanidin content in PAs of two technical replicates from a pool of lyophilized and ground berry tissue was carried out at the University of Turku, using the UPLC-MS/MS method of Engström et al. (2014), as described in James et al. (2017).

Acknowledgements

We thank Laura Zorrati for technical assistance and Panagiotis Arapitsas for support in setting up UPLC-MS/MS for anthocyanin analysis, Anne Koivuniemi for the help in UPLC-MS/MS analyses of PAs, and Caroline Preston for the gift of purified salal PA. This work was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery and CREATE grants to CPC), the Autonomous Province of Trento (ADP 2011-2017 project to SM), and the Academy of Finland (298177 to JPS).

List of Supplemental Material

Supplemental Table 1: Anthocyanidin identification and quantification using LC-MS/MS

Supplemental Figure S1: Correlation between PA concentrations and Trolox equivalent antioxidant activity (TEAC).

References

Acuña, U.M., Atha, D.E., Ma, J., Nee, M.H., Kennelly, E.J., 2002. Antioxidant capacities of ten edible North American plants. Phytother. Res. 16, 63-65.

Arancibia-Avila, P., Toledo, F., Werner, E., Suhaj, M., Leontowicz, H., Leontowicz, M., Martinez-Ayala, A.L., Pasko, P., Gorinstein S., 2011. Partial characterization of a new kind of Chilean Murtilla-like berries. Food Res. Int. 44, 2054-2062.

Arapitsas, P., Perenzoni, D., Nicolini, G., Mattivi, F., 2012. Study of Sangiovese wines pigment profile by UHPLC-MS/MS. J Agric. Food Chem. 60, 10461-10471.

Ayres, M.P., Clausen, T.P., MacLean, S.F., Redman, A.M., Reichardt, P.B., 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78, 1696-1712.

Bakowska-Barczak, A.M., Marianchuk, M., Kolodziejczyk, P., 2007. Survey of bioactive components in western Canadian berries. Can. J. Physiol. Pharmacol. 85, 1139-1152.

Barbehenn, R.V., Constabel, C.P., 2011. Tannins in plant-herbivore interactions. Phytochemistry 72, 1551-1565.

Davies, K. M., Albert, N. W., Schwinn, K. E., 2012. From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning. Funct. Plant Biol., 39, 619-638

Downey, M.O., Harvey, J. S., Robinson, S.P., 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust. J. Grape Wine Res. 9, 15–27

Dudonné, S., Dubé, P., Anhe, F. F., Pilon, G., Marette, A., Lemire, M., Harris, C., Dewailly, E., Desjardins, Y., 2015. Comprehensive analysis of phenolic compounds and abscisic acid profiles of twelve native Canadian berries. J. Food Comp. Anal. 44, 214-224.

Einbond, L.S., Reynertson, K.A., Luo, X.-D., Basile, M.J., Kennelly, E.J., 2004. Anthocyanin antioxidants from edible fruits. Food Chem. 84, 23-28.

Engström, M. T., Palijarvi, M., Fryganas, C., Grabber, J. H., Mueller-Harvey, I., Salminen, J. P., 2014. Rapid qualitative and quantitative analyses of proanthocyanidin oligomers and polymers by UPLC-MS/MS. J. Agric. Food Chem. 62, 3390-3399.

Esposito, D., Chen, A., Grace, M. H., Komarnytsky, S., Lila, M. A., 2014. Inhibitory effects of wild blueberry anthocyanins and other flavonoids on biomarkers of acute and chronic inflammation in vitro. J. Agric. Food Chem. 62, 7022-7028.

Etxeberria, U., Fernandez-Quintela, A., Milagro, F. I., Aguirre, L., Martinez, J. A., Portillo, M. P., 2013. Impact of polyphenols and polyphenol-rich dietary sources on gut microbiota composition. J. Agric. Food Chem. 61, 9517-9533.

Fernandes, I., Faria, A., Calhau, C., de Freitas, V., Mateus, N., 2014. Bioavailability of anthocyanins and derivatives. J. Funct. Foods 7, 54-66.

Furuuchi, R., Sakai, H., Hirokawa, N., Watanabe, Y., Yokoyama, T., Hirayama, M., 2012. Antihypertensive effect of boysenberry seed polyphenols on spontaneously hypertensive rats

and identification of orally absorbable proanthocyanidins with vasorelaxant activity. Biosci. Biotechnol. Biochem. 76, 1694-1701.

Grace, M. H., Esposito, D., Dunlap, K. L., Lila, M. A., 2014. Comparative analysis of phenolic content and profile, antioxidant capacity, and anti-inflammatory bioactivity in wild Alaskan and commercial *Vaccinium* berries. J. Agric. Food Chem. 62, 4007-4017.

Hagerman, A.E., Reidl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L., .1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46, 1887-1892.

Hosseinian, F. S., Li, W., Hydamaka, A. W., Tsopmo, A., Lowry, L., Friel, J., Beta, T., 2007. Proanthocyanidin profile and ORAC values of manitoba berries, chokecherries, and seabuckthorn. J. Agric. Food Chem. 55, 6970-6976.

James, A. M., Ma, D. W., Mellway, R., Gesell, A., Yoshida, K., Walker, V., Tran, L., Stewart, D., Reichelt, M., Suvanto, J., Salminen, J. P., Gershenzon, J., Seguin, A., Constabel, C. P., 2017. Poplar MYB115 and MYB134 transcription factors regulate proanthocyanidin synthesis and structure. Plant Physiol. 174, 154-171.

Jin, A. L., Ozga, J. A., Kennedy, J. A., Koerner-Smith, J. L., Botar, G., Reinecke, D. M., 2015. Developmental profile of anthocyanin, flavonol, and proanthocyanidin type, content, and localization in Saskatoon fruits (*Amelanchier alnifolia* Nutt.). J. Agric. Food Chem. 63, 1601-1614.

Jungfer, E., Zimmermann, B. F., Ruttkat, A., Galensa, R., 2012. Comparing procyanidins in selected Vaccinium species by UHPLC-MS2 with regard to authenticity and health effects. J. Agric. Food Chem. 60, 9688-9696.

Kraus, T.E.C., Yu, Z., Preston, C.M., Dalhgren, R.A., Zasoski, R.J., 2003. Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. J. Chem. Ecol. 29, 703-730.

Laaksonen, O., Makila, L., Tahvonen, R., Kallio, H., Yang, B. R., 2013. Sensory quality and compositional characteristics of blackcurrant juices produced by different processes. Food Chem. 138, 2421-2429.

Lila, M. A., Burton-Freeman, B., Grace, M., Kalt, W., 2016. Unraveling anthocyanin bioavailability for human health. Annual Rev. Food Sci. Technol. 7, 375-393.

Määttä-Riihinen, K. R., Kahkonen, M. P., Törrönen, A. R., Heinonen, I. M., 2005. Catechins and procyanidins in berries of *Vaccinium* species and their antioxidant activity. J. Agric. Food Chem. 53, 8485-8491.

Martin, P., Wishart, J., McDougall, G., Brennan, R., 2015. Fruit production and polyphenol content of salal (*Gaultheria shallon* Pursh), a potential new fruit for northern maritime regions. Fruits 70, 377-383.

McDougall, G. J., Austin, C., Van Schayk, E., Martin, P., 2016. Salal (*Gaultheria shallon*) and aronia (*Aronia melanocarpa*) fruits from Orkney: Phenolic content, composition and effect of

wine-making. Food Chem 205, 239-247.

Nierop, K. G. J., Preston, C. M., Verstraten, J. M., 2006. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. Soil Biol. Biochem. 38, 2794-2802.

Oertel, A., Matros, A., Hartmann, A., Arapitsas, P., Dehmer, K. J., Martens, S., Mock, H. P., 2017. Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols. Planta 246, 281-297.

Ozga, J.A., Saeed, A., Reinecke, D.M., 2006. Anthocyanins and nutrient components of Saskatoon fruits (*Amelanchier alnifolia* Nutt.). Can. J. Plant Sci. 86, 193-197.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986) The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin. Phytochemistry 25, 223-230.

Preston, C.M., 1999. Condensed tannins of salal (*Gaultheria shallon* Pursh): A contributing factor to seedling "growth check" on northern Vancouver Island? In *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*, edited by Gross et al. Kluwer Academic/Plenum Publishers, New York.

Prior, R.L, Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C.M., 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46, 2686-2693.

Prior, R.L., Gu, L., 2005. Significance and biological significance of proanthocyanidins in the American diet. Phytochemistry 66, 2264-2280.

Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290-4302.

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouysegu, L., 2011. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew. Chem. 50, 586-621.

Rasmussen, S. E., Frederiksen, H., Krogholm, K. S., Poulsen, L., 2005. Dietary proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. Molec. Nut. Food Res. 49, 159-174.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation assay. Free Radical Biol. Med. 26, 1231-1237.

Santos-Buelga, C., Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effect on nutrition and health. J. Sci. Food Agric. 80, 1094-1117.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875-3883.

Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 130, 2073S-2085S.

Schweitzer, J. A., Bailey, J. K., Rehill, B. J., Martinsen, G. D., Hart, S. C., Lindroth, R. L., Keim, P.,

Whitham, T. G., 2004. Genetically based trait in a dominant tree affects ecosystem processes. Ecol. Lett. 7, 127-134.

Serafini, M., Maiani, G., Ferro-Luzzi, A., 1998. Alcohol-free red wine enhances plasma antioxidant capacity in humans. J. Nutr. 128, 1003-1007.

Tappeiner, J.C., Zasada, J.C., Huffman, D.W., Ganio, L.M., 2001. Salmonberry and salal annual aerial stem production: the maintenance of shrub cover in forest stands. Can. J. For. Res. 31, 1629-1638.

Towers, G.H.N., Tse, A., Maass, W.S.G., 1966. Phenolic acids and phenolic glycosides of *Gaultheria* species. Phytochemistry 5, 677-681.

Turner, N.C., Bell, M.A.M., 1971. The ethnobotany of the Coast Salish Indians of Vancouver Island. Econ. Bot. 25, 63-104.

Turner, N.C., Bell, M.A.M., 1973. The ethnobotany of the Southern Kwakiutl Indians of British Columbia. Econ. Bot. 27, 257-310.

Vagiri, M., Ekholm, A., Andersson, S.C., Johansson, E., Rumpunen, K., 2012. An optimized method for analysis of phenolic compounds in buds, leaves and fruits of black currant (*Ribes nigrum* L.). J Agric. Food Chem. 60, 10501-10510.

Vendrame, S., Guglielmetti, S., Riso, P., Arioli, S., Klimis-Zacas, D., Porrini, M., 2011. Six-week consumption of a wild blueberry powder drink increases Bifidobacteria in the human gut. J. Agric. Food Chem. 59, 12815-12820.

Vrhovsek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R., Mattivi, F., 2012. A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. J. Agric. Food Chem. 60, 8831-8840.

Wrolstad, R.E., 1976. Color and pigment analyses in fruit products. Station Bulletin 624, Oregon State University.

Zoratti, L., Sarala, M., Carvalho, E., Karppinen, K., Martens, S., Giongo, L., Häggman, H., Jaakola, L., (2014). Monochromatic light increases anthocyanin content during fruit development in bilberry. BMC Plant Biol., 14, 377

Zifkin M., Jin A., Ozga, J.A., Zaharia, L.I, Schernthaner, J.P., Gesell, A., Abrams, S.R., Kennedy, J.A., Constabel, C.P. (2012) The expression and metabolite profiling of developing highbush blueberry fruit indicates transcriptional regulation of flavonoid metabolism and activation of abscisic acid metabolism. Plant Physiol. 158, 200-224.

Legends to Figures

Figure 1. Developmental profile, weight gain, and anthocyanin content of salal flower (CF,O) and berry at multiple stages of ripening (stages B1-B8). A, Image of representative berries. B, Mean dry weight of flowers and fruit. C, Mean anthocyanin content, as measured using a spectrophotometric method as described in Experimental. Error bars denote SE; n=7 for all samples except B8, where n=16. CF, closed flowers; OF, open flowers.

Figure 2. Mean antioxidant activity of salal flower (CF,O) and berry (B-B8) extracts. Methanolic extracts were assayed using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay as described. Error bars denote SE; n=7 for all samples except B8, where n=16.

Figure 3. Proanthocyanidin (PA) concentrations on a per 100g dry weight basis (dashed line) and per fruit basis (solid line) over the course of salal flower (CF,O) and berry (B-B8) development. The per fruit content was based on the average dry weight of fruit at each stage. Error bars denote SE. n=7 for CF-B7, n=16 for B8.

Figure 4. Average percent prodelphinidin (dark bars) and procyanidin (light bars) in proanthocyanidins in salal flower (OF) and fruit at stages as indicated. Analysis was carried out in duplicate using a UPLC-MS/MS as described in Engström et al. (2014). Error bars denote SE.

Table 1: Mean anthocyanin content of salal berry (*G. shallon*) at three developmental stages as defined in Fig. 1, in comparison with mature highbush blueberry fruit (*V. corymbosum* cv Rubel). Concentrations were determined by UHPLC-MS/MS as described and shown as mg/100 g dry wt ± S.E. (n=4 and n=2 for salal and blueberry fruit, respectively).

	salal berry stages			**blueberry**
Anthocyanins	**B5**	**B7**	**B8**	**mature**
Cyanidin-3-*O*-lathyroside	nd[1]	18 ± 1.5	57 ±3	nd
Cyanidin-3-*O*-arabinoside	nd	39 ± 3	83 ± 3.5	17.3 ± 0.6
Cyanidin-3-*O*-galactoside	nd	37 ± 3.5	130 ± 10	24.3 ± 0.3
Cyanidin-3-*O*-sambucoside	nd	9.9 ± 0.4	4.5 ± 0.2	nd
Cyanidin-3-*O*-glucoside	nd	12 ± 0.5	9 ± 1	21 ± 2
Delphinidin-3-*O*-arabinoside	nd	400 ± 30	700 ± 50	360 ± 7
Delphinidin-3-*O*-galactoside	nd	770 ± 35	2600 ± 100	850 ± 50
Delphinidin-3-*O*-glucoside	nd	280 ± 25	130 ± 10	350 ± 35
Petunidin-3-*O*-glucosides[2]	nd	0.3 ± 0.2	3.6 ± 0.4	330 ± 16
Malvidin-3-*O*-glucosides[3]	nd	nd	nd	440 ± 45
Peonidin-3-*O*-glucosides[4]	nd	nd	nd	11 ± 0.8

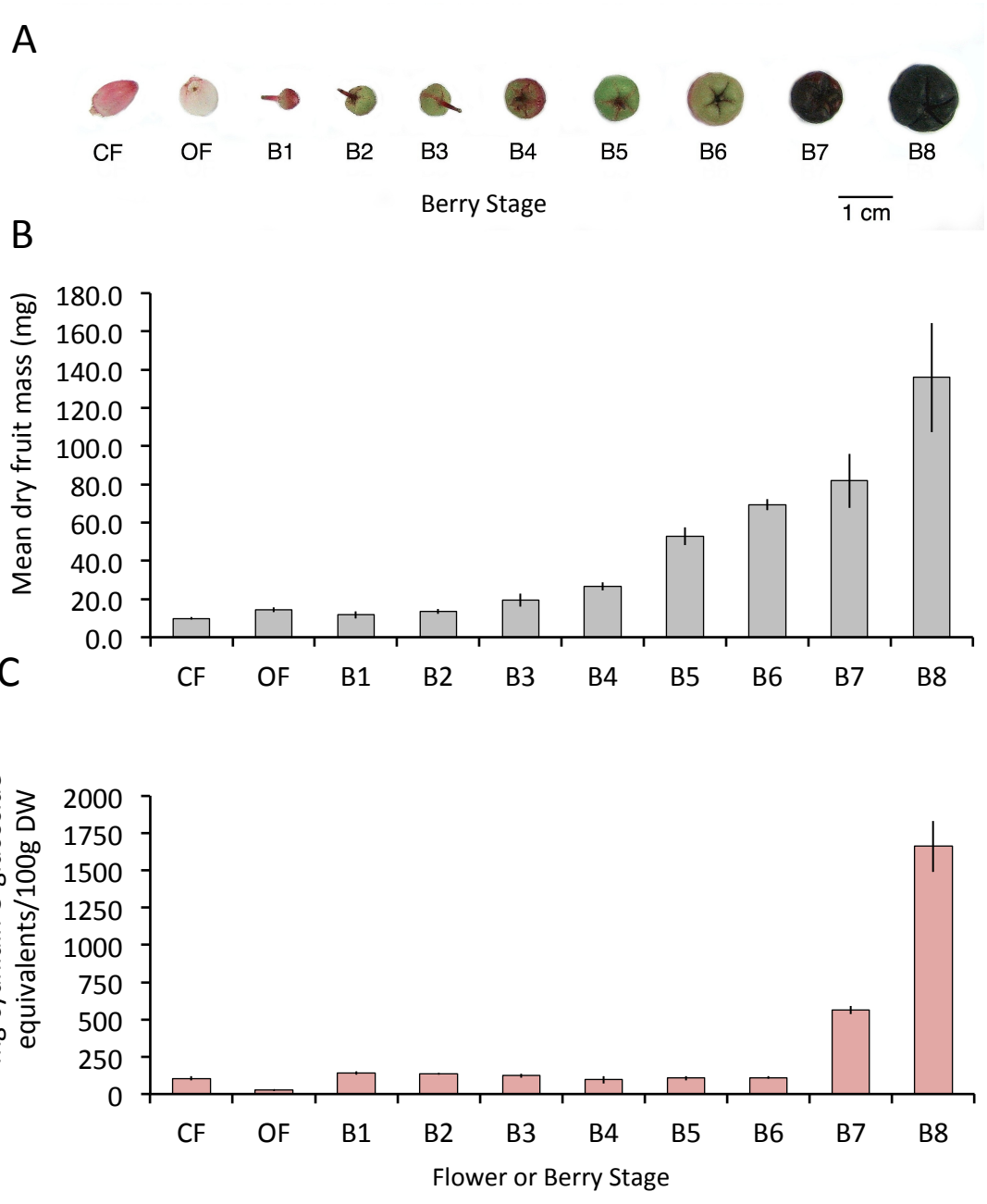
[1] not detected
[2] Sum of petunidin-*O*-galactoside, -*O*-arabinoside, and -*O*-glucoside. In salal, only petunidin-*O*-galactoside was detected.
[3] Sum of malvidin-*O*-galactoside, -*O*-arabinoside, and -*O*-glucoside, plus the acetyl and *p*-coumaroyl-glucosides
[4] Sum of peonidin-*O*-galactoside, -*O*-arabinoside, and -*O*-glucoside

Table 2. Phenolic content (mg / 100g dry wt) of salal (*G. shallon*) berries at four developmental stages (n=4) as defined in Fig. 1, compared with immature and mature highbush blueberry (*V. corymbosum* cv Rubel) (n=2). Means ± SE are shown n=4 (salal) or n=2 (blueberry).

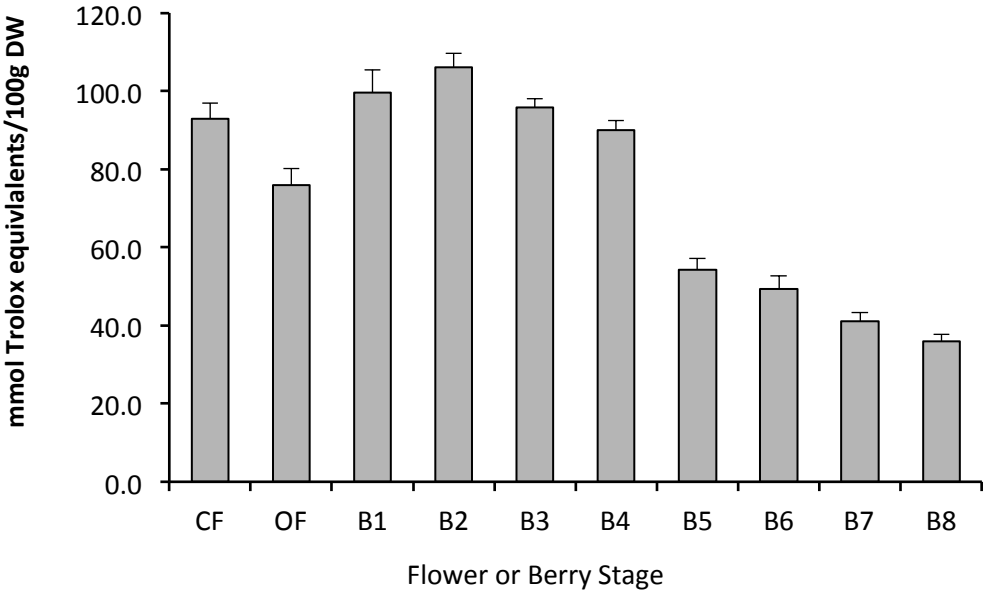
	Salal berry				Blueberry	
	B4	**B5**	**B7**	**B8**	**Immature**	**Mature**
Flavan-3-ols						
catechin	580 ± 40	260 ± 15	190 ± 25	150 ± 35	47 ± 4	12 ± 0.2
epicatechin	84 ± 2	35 ± 2	38 ± 2	27 ± 2	44 ± 2	4.0 ± 0.03
gallocatechin	49 ± 1	38 ± 1.2	23 ± 1	21 ± 1	1.3 ± 0.4	1.1 ± 0.2
epigallocatechin	5.5 ± 0.4	2.4 ± 0.3	2.7 ± 0.4	2.3 ± 0.1	0.3 ± 0.1	0.48 ± 0.02
Procyanidins						
procyanidin B1	76 ± 2	45 ± 1	38 ± 2	29 ± 1	220 ± 20	25 ± 3
procyanidin B2+B4	55 ± 3	26 ± 1	23 ± 1	14 ± 1	85 ± 2	7.1 ± 0.4
procyanidin B3	41 ± 1	23 ± 1	12 ± 1	8 ± 1	nd	nd
procyanidin A2	10 ± 0.4	5.5 ± 1	2.9 ± 0.2	2.1 ± 0.3	nd	nd
Chlorogenic acids						
chlorogenic acid	6.3 ± 0.2	6.4 ± 0.9	5.4 ± 0.2	4.2 ± 0.6	1900 ± 200	490 ± 10
neochlorogenic acid	42 ± 3	34 ± 2	34 ± 0.6	23 ± 2	12 ± 2	3.1 ± 0.07
cryptochlorogenic acid	0.2 ± 0.1	nd	0.04 ± 0.09	0.04 ± 0.09	16 ± 1	3.8 ± 0.9
Other flavonoids						
quercetin-3-*O*-rhamnoside	17 ± 4	7 ± 4	8.3 ± 0.6	5.8 ± 0.6	28 ± 4	12 ± 0.2
quercetin-3-*O*-galactoside	35 ± 5	19 ± 6	26 ± 5	22 ± 7	74 ± 9	30 ± 10
quercetin-3-*O*-glucuronide	46 ± 6	24 ± 2	25 ± 3	24 ± 2	nd	nd
quercetin-3-*O*-glucoside	tr[1]	tr	tr	tr	18 ± 8	5.0 ± 0.003
quercetin-3-*O*-sulfate	8 ± 0.7	3.9 ± 0.3	2 ± 0.2	nd	1.4 ± 0.2	0.85 ± 0.004
myricetin[2]	1 ± 0.6	0.1 ± 0.2	1.3 ± 0.5	4.1 ± 0.3	nd	0.3 ± 0.4
myricitrin	17 ± 4	11 ± 2	10 ± 2	6±2	nd	4.1 ± 0.5
apigenin	0.06 ± 0.04	0.03 ± 0.03	nd	nd	nd	nd
kaempferol-3-*O*-rutinoside	nd	nd	nd	nd	0.4 ± 0.3	nd
rutin	nd	nd	nd	nd	6.4 ± 1	1 ± 1
luteolin-7-*O*-glucoside	0.6 ± 0.1	0.6 ± 0.3	0.32 ± 0.08	0.38 ± 0.06	0.1 ± 0.1	0.45 ± 0.05
naringenin	0.1 ± 0.03	0.13 ± 0.04	0.37 ± 0.05	0.29 ± 0.07	nd	0.023 ± 0.004
naringenin-7-*O*-glucoside	26 ± 5	36 ± 1	6 ± 3	3 ± 2	nd	nd
isorhamnetin-3-*O*-rutinoside	nd	nd	nd	nd	2.9 ± 0.5	0.57 ± 0.04

[1] traces only detected in salal berries

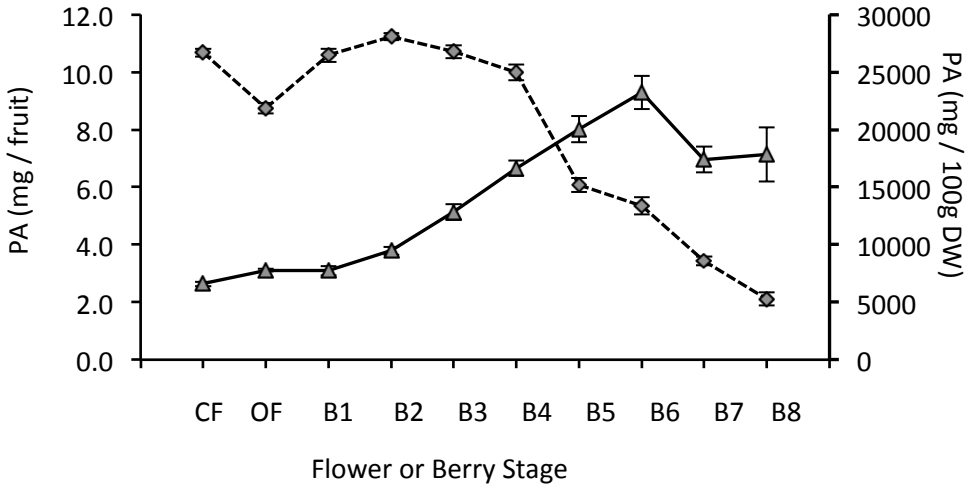
Ferguson et al., Figure 1



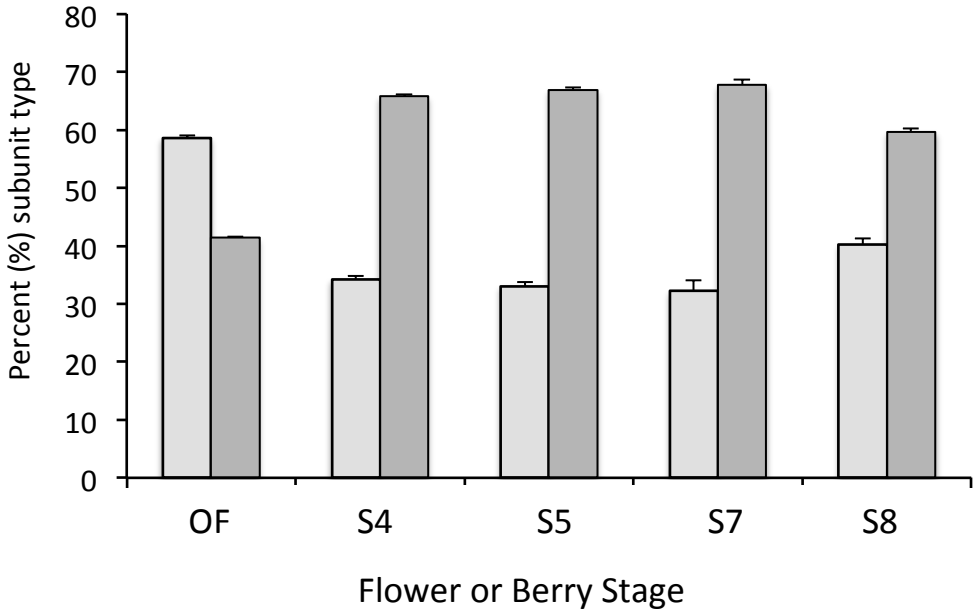
Ferguson et al., Figure 2

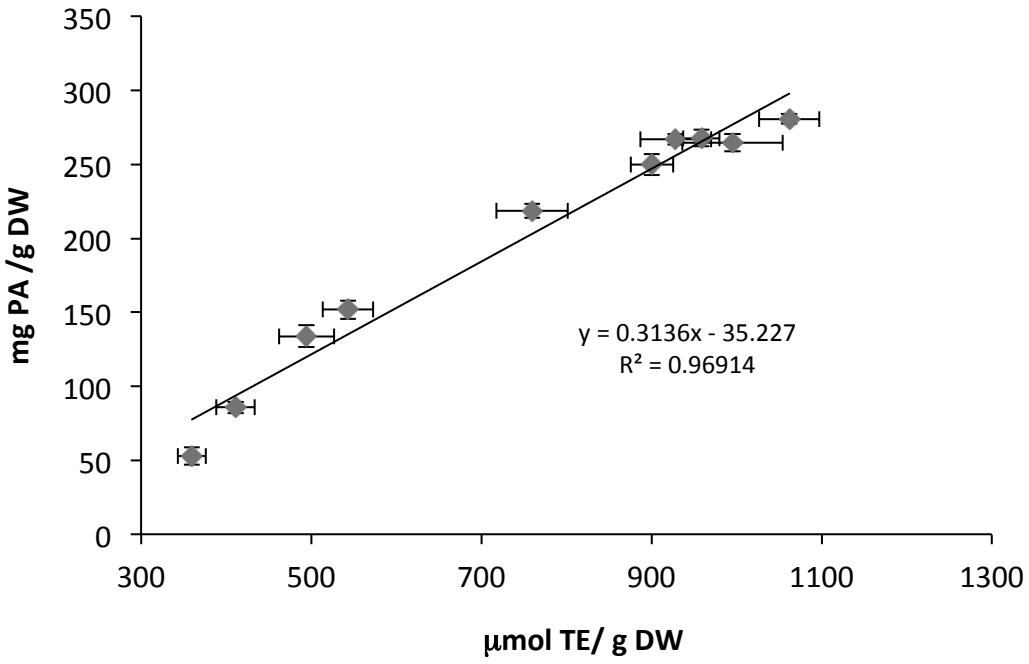


Ferguson et al., Figure 3



Ferguson et al., Figure 4





Supplementary Figure S1. Correlation between PA concentrations and Trolox equivalent antioxidant activity (TEAC). Bars denote SE, n=7.

Supplementary Table S1: Anthocyanidin identification and quantification data

Peak	Tentative Identification[a]	rt	MRM transition 1	MRM transition 1	CV	CE	quantified using[a,b]
1	Cy lath	2.93	581→137	581→287	30	66,28	Cy samb
2	Cy arab	3.36	419→137	419→287	26	52,54	Std
3	Cy gal	3.03	449→136	449→287	28	48,30	Std
4	Cy samb	3.15	581→137	581→287	30	66,28	Std
5	Cy glu	3.19	449→136	449→287	28	48,30	Std
6	Dp arab	2.99	435→303		20	22	Mv glu
7	Dp gal	2.66	465→229	465→303	20	58,22	Dp glu
8	Dp glu	2.82	465→229	465→303	20	58,22	Std
9	Mv arab	4.06	463→331		28	24	Mv glu
10	Mv gal	3.75	493→315	493→331	28	48,24	Mv glu
11	Mv glu	3.89	493→315	493→331	28	48,24	Std
12	Mv ac glu	5.01	535→315	535→331	30	50,26	Std
13	Mv pc glu	5.61	639→315	639→331	38	58,30	Std
14	Pl gal	3.36	433→121	433→271	26	58,36	Pl glu
15	Pn arab	3.93	433→301	433→286	26	22,40	Std
16	Pn gal	3.61	463→286	463→301	28	42,28	Std
17	Pn glu	3.76	463→286	463→301	28	42,28	Std
18	Pn pc glu	5.59	609→286	609→301	38	54,32	Std
19	Pt arab	3.58	449→317		28	30	Mv glu
20	Pt gal	3.25	479→302	479→317	28	42,30	Pt glu
21	Pt glu	3.39	479→302	479→317	28	42,30	Std

[a] dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; pl, pelargonidin; glu, 3-glucoside; arab, 3-arabinoside; gal, 3-galactoside; ac glu, 3-(6”-acetyl)-glucoside; pc glu, 3-(6”-p-coumaroyl)-glucoside; samb, 3-sambubioside; lath, 3-lathyroside

[b] Std: samples quantified using the respective authentic standard