

UHI Research Database pdf download summary

Salal (*Gaultheria shallon*) and aronia (*Aronia melanocarpa*) fruits from Orkney: Phenolic content, composition and effect of wine-making

Mcdougall, G.J.; Austin, C.; Van Schayk, E.; Martin, P.

Published in:
Food Chemistry

Publication date:
2016

Publisher rights:

This document was originally uploaded as the AAM and made available accordingly. However, Peter noticed today it was not the AAM so a new document has been uploaded today and this returned to 'other'.

The re-use license for this item is:
CC BY-NC-ND

The Document Version you have downloaded here is:
Other version

The final published version is available direct from the publisher website at:
[10.1016/j.foodchem.2016.03.025](https://doi.org/10.1016/j.foodchem.2016.03.025)

[Link to author version on UHI Research Database](#)

Citation for published version (APA):

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 Chemistry*, 205, 239-247. <https://doi.org/10.1016/j.foodchem.2016.03.025>

General rights

Copyright and moral rights for the publications made accessible in the UHI Research Database are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights:

- 1) Users may download and print one copy of any publication from the UHI Research Database for the purpose of private study or research.
- 2) You may not further distribute the material or use it for any profit-making activity or commercial gain
- 3) You may freely distribute the URL identifying the publication in the UHI Research Database

Take down policy

If you believe that this document breaches copyright please contact us at RO@uhi.ac.uk providing details; we will remove access to the work immediately and investigate your claim.



Salal (*Gaultheria shallon*) and aronia (*Aronia melanocarpa*) fruits from Orkney: Phenolic content, composition and effect of wine-making



G.J. McDougall^{a,*}, C. Austin^a, E. Van Schayk^b, P. Martin^c

^a Environmental and Biochemical Sciences Group, Enhancing Crop Productivity and Utilisation Theme, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

^b Orkney Wine Company, Lamb Holm, Orkney, KW17 2SF, UK

^c Agronomy Institute, Orkney College UHI, East Road, Kirkwall, Orkney, KW15 1LX, UK

ARTICLE INFO

Article history:

Received 8 December 2015

Received in revised form 22 February 2016

Accepted 8 March 2016

Available online 9 March 2016

Keywords:

Salal fruits
Wine-making
Anthocyanins
Polyphenols
Stability

ABSTRACT

The polyphenol content and composition of salal and aronia fruits from plants established in Orkney was examined. The composition of the salal fruits has not previously been recorded, and they contained anthocyanins, flavonols, hydroxycinnamates and proanthocyanins. The aronia fruits contained anthocyanins, hydroxycinnamates and flavonols as previously described.

Although salal fruits had half the anthocyanin content of aronia fruits, salal wine had higher anthocyanin content, probably due to the relative stability of diglycoside pentose anthocyanins. The wines contained components suggestive of anthocyanin and flavonol degradation, but there was no consistent pattern to stability within phenolic sub-classes. Indeed, the wine made from equal amounts of salal and aronia fruits had patterns of recovery of individual phenolic components which could not be predicted from recoveries in wines from single fruits. This strongly suggests that stability of individual phenolic constituents during wine-making is influenced by the presence and relative stability of other components.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Located about 10 km off the north coast of Scotland at 59°N, the Orkney archipelago has a hyper-oceanic climate (Crawford, 2000), with high rainfall, frequent strong winds, relatively mild winters and a cool summer growing season with a long day length. Climatic and environmental factors make Orkney a challenging location for outside fruit production, particularly because of low summer temperatures for fruit ripening, soil waterlogging over the winter and damage to bushes and fruit from wind, which often carries salt from the sea. The most successful outside fruit crops in Orkney are blackcurrants (*Ribes nigrum*) and gooseberries (*Ribes uva-crispa*), while strawberries (*Fragaria x ananassa*) and raspberries (*Rubus idaeus*) grow well under polythene. There are few reports, however, about the success of less common fruit species on these islands. As part of a programme to screen species for potential use by the local food and drink industry, the University of the Highlands and Islands' Agronomy Institute planted observation plots of several novel fruit species between 2002 and 2006 on agricultural land at Orkney College UHI. Amongst the species tested, aronia (*Aronia melanocarpa*) and salal (*Gaultheria shallon*) were two of

the most successful, fruiting within 2–3 years from planting and consistently producing crops annually.

Salal is a coarse-leaved, evergreen shrub belonging to the Ericaceae, which is native to western North America and usually grows in the forest understorey (USDA Forest Service, 2013; <http://plants.usda.gov/core/profile?symbol=GASH>). It is particularly common in the Pacific Northwest and produces purple/black fruit which were an important part of the diet of coastal Indians before European settlement (Keely, Martinsen, Hunn, & Norton, 1982). The fruits were eaten fresh or as a dried cake over the winter and are reported to have a high ascorbic acid content (4.87 mg/g dry weight; Keely et al., 1982). Acuna, Atha, Ma, Nee, and Kennelly (2002) and Einbond, Reynertson, Xiao, Basile, and Kennelly (2004) demonstrated that salal fruit extracts had high antioxidant activity and noted that they appeared to contain anthocyanins, although little more was established about their phenolic composition. Economically, the fruit is now of only minor local importance in North America, although the foliage is wild harvested to supply a valuable market for cut evergreens (i.e. "lemon leaf"; Hobby, Dows, & MacKenzie, 2010). It was introduced to Britain in the 19th century as an ornamental shrub, on shooting estates, but has now become naturalised in some areas.

Aronia or black chokeberry is a member of the Rosaceae and is native to eastern North America, where it was used by indigenous people as a food resource and traditional medicine. It forms a

* Corresponding author.

E-mail address: Gordon.mcdougall@hutton.ac.uk (G.J. McDougall).

multi-stemmed deciduous shrub and produces shiny black fruits noted for high anthocyanin content (Kähkönen et al., 1999; Strigl, Leitner, & Pfannhauser, 1995). Although originating in North America, commercial growing of aronia fruit was pioneered in the Soviet Union and Eastern Europe (Kask, 1987) and a number of improved cultivars are now available. Aronia berries are extensively used for the production of jams, preserves, juices and food colourants (Ochmian, Grajkowski, & Smolik, 2012) and a number of health benefits have been ascribed to their intake (e.g. Kardum et al., 2015).

As a result of the promising agronomic performance of salal and aronia, they were selected for study of their phenolic composition and, in collaboration with an Orkney fruit wine producer, to determine their potential for providing locally-sourced fruit to make distinctive fruit wines. The objective of the current study was to examine the phenolic content and composition of salal and aronia fruits, and of wines made from salal, aronia and an equal mixture of these berries.

2. Materials & methods

2.1. Plant material, extraction and wine-making process

Fruit were obtained from plants grown at Orkney College UHI in the summer of 2012. Aronia bushes (*A. melanocarpa* cv. 'Viking') were planted in 2006, in rows 2.0 m apart, with spacing of 1.0 m within rows. In 2012, plants were 1.2 m tall with a canopy spread of about 1.4 m. Ten salal (*G. shallon*) plants, (obtained from Barwinock Herb Nursery, Barrhill, South Ayrshire, UK), were planted in 2003, in a single row, with 0.5 m between plants. A further 10 plants were added in 2006. By 2012, plants had grown together to form a hedge, about 9 m long and 0.7–0.9 m wide. Both the aronia and salal were surrounded by polyethylene wind break netting and on two sides by a willow wind break. For each species, fruit were picked, when ripe, throughout the 2012 harvesting season and stored frozen. Three representative sub-samples of the total harvest for each fruit were transported frozen to the James Hutton Institute.

A portion from each of the three sub-samples of the ripe fruit was taken for extraction (Martin, Wishart, McDougall, & Brennan, 2015). The fruits were weighed and then extracted with an equal volume to weight of acetonitrile containing 0.2% formic acid. The samples were homogenized by hand using a hand held glass tissue homogenizer with a PTFE pestle and then centrifuged at 13,000 rpm for 5 min. The centrifugation was repeated and the supernatant retained. These extracts were stored at -80°C .

Separate test wines were made from representative 2 kg samples of the harvested fruit for each species. A third wine was prepared in the same way but used a mixture of 1 kg each of salal and aronia fruit. The fruit were homogenised with 4.0 l water and 1.5 kg Demerara sugar, and 0.5 ml each of Trenolin® Super DF and Trenolin® Color DF (Erbslöh, Gessenheim Germany) was added. Yeast (strain EC-1118, Lallemand Inc., Burton upon Trent, UK) was added and left to ferment for 10 days. The juice was then separated by straining the entire mixture through a cotton cloth which was twisted vigorously. The juices, which contained some fruit fragments, were then put in demijohns, fitted with airlocks, and left for about 4 months, until fermentation had completely ceased. No adjustment was made to the natural pH of the fermenting wines and after they were racked, three sub-samples were taken, frozen and transported frozen to the Hutton for analysis.

2.2. Total anthocyanin, total phenol and FRAP assays

The total anthocyanin concentration was estimated by the pH differential absorbance method (Deighton, Brennan, Finn, &

Davies, 2000). The absorbance value was related to anthocyanin content using the molar extinction coefficient calculated for cyanidin-3-*O*-glucoside (purchased from ExtraSynthese Ltd., Genay, France). Phenol content was measured using a modified Folin-Ciocalteu method (Deighton et al., 2000). Phenol contents were estimated from a standard curve of gallic acid as gallic acid equivalents (GAE). Samples were dried in aliquots to constant phenol content using a SpeedVac prior to LC-MS analysis.

A manual FRAP assay was used based on the method described previously (Deighton et al., 2000). FRAP values are presented as micromolar ferrous ion (ferric reducing power) of the extracts. For the TPC, TAC and FRAP assays, extracts from the three sub-samples (for fruit and wines) were assayed in triplicate. Statistical significance between samples was assessed using analysis of variance by means of GENSTAT software (14th edition, VSN International, Hemel Hempstead, UK) with a blocking statement that described the sampling method.

2.3. Liquid chromatography–mass spectrometry (LC-MS)

Samples (containing 20 μg GAE) were analyzed on a LCQ-DECA system, comprising Surveyor autosampler, pump, photodiode array detector (PDAD) and a ThermoFinnigan ion-trap mass spectrometer. The PDAD scanned three discrete channels at 280, 365 and 520 nm. Solvent A was ultra-pure water containing 0.1% formic acid and solvent B was acetonitrile containing 0.1% formic acid. After a 5 min hold at 5% solvent B, samples were eluted with a gradient of 5% solvent B to 45% solvent B on a C18 column (Synergi Hydro C18 with polar end capping, 2.0 mm \times 150 mm, Phenomenex Ltd.) over 25 min at a flow rate of 200 $\mu\text{l}/\text{min}$. Wine extracts were separated using 2% solvent B held for 5 min and then a linear gradient to 40% solvent B at 35 min. The LCQ-DECA liquid chromatography–mass spectrometer was fitted with an electrospray ionization interface, and all samples were analyzed in both positive and negative-ion mode. There were two scan events: full-scan analysis followed by data-dependent MS/MS of the most intense ions. The data-dependent MS/MS used collision energies (source voltage) of 45% in wide-band activation mode. The MS detector was tuned against cyanidin-3-*O*-glucoside (positive mode) and against rutin (negative mode). Polyphenol components were detected and putatively identified using their PDA, MS and MS² properties using data gathered in-house and from literature.

Peak areas for specific components were obtained by two different methods. For anthocyanins, the LCMS data was searched for the relevant masses (Supplementary data, Fig. S1) and peak areas calculated using the resident Xcalibur software. MS data was used to confirm the identity of each peak. For flavonols, relevant peak areas were estimated from the traces obtained in channel B of the PDAD at 365 nm using the resident Xcalibur software. MS data was used to confirm identity for each peak. Data was obtained from LCMS runs from the three sub-samples for wine and fruit and are expressed as means \pm standard error ($n = 3$). Statistical significance of values was assessed using ANOVA. As all samples were run at an equivalent phenol content (20 μg GAE/injection), they are comparable in terms of relative recovery.

3. Results and discussion

3.1. Phenolic content and composition of fruits

The dark blue-black salal fruits produced an extract with total phenol content (TPC) of 975 ± 9 mg GAE/100 g and total anthocyanin content (TAC) of 256 ± 5 mg CGE/100 g (Table 1). These figures fit with our studies on year-to-year variation in salal fruits (Martin et al., 2015) and the TPC is in the range commonly

Table 1
Total phenol and anthocyanin contents of fruits and wines: Estimated recovery of components in wines.

Sample	FRUIT				WINE				
	Phenol Content (mg/100 g FW)	Anthocyanin Content (mg/100 g FW)	Total PC ¹	Total AC ¹	Phenol Content (µg/ml)	Anthocyanin Content (µg/ml)	Volume (L)	PC Recovery ¹ (%)	AC Recovery ¹ (%)
Aronia	^a 1045 ± 13	^a 467 ± 3	20,900	9340	^a 966 ± 12	^a 18.4 ± 0.6	4.47	4318 (20.6%)	82.3 (0.88%)
Salal	^b 975 ± 9	^b 256 ± 5	19,500	5120	^b 1413 ± 14	^b 44.1 ± 0.8	4.64	4181 (21.4%)	204.6 (4.00%)
Salal + Aronia	NA	NA	<i>20,200</i>	<i>7230</i>	^c 1122 ± 14	^c 33.0 ± 1.1	4.40	4937 (24.4%)	145.2 (2.01%)

¹ Total figures in mg. NA = values not available. Figures in italics were estimated based on equal amounts of the two fruit types. Recoveries in wines are expressed as % of the total PC and AC values available from the fruit. Values are averages ± standard error ($n = 9$) and those with different letters in columns were significantly different at $p < 0.001$ (ANOVA).

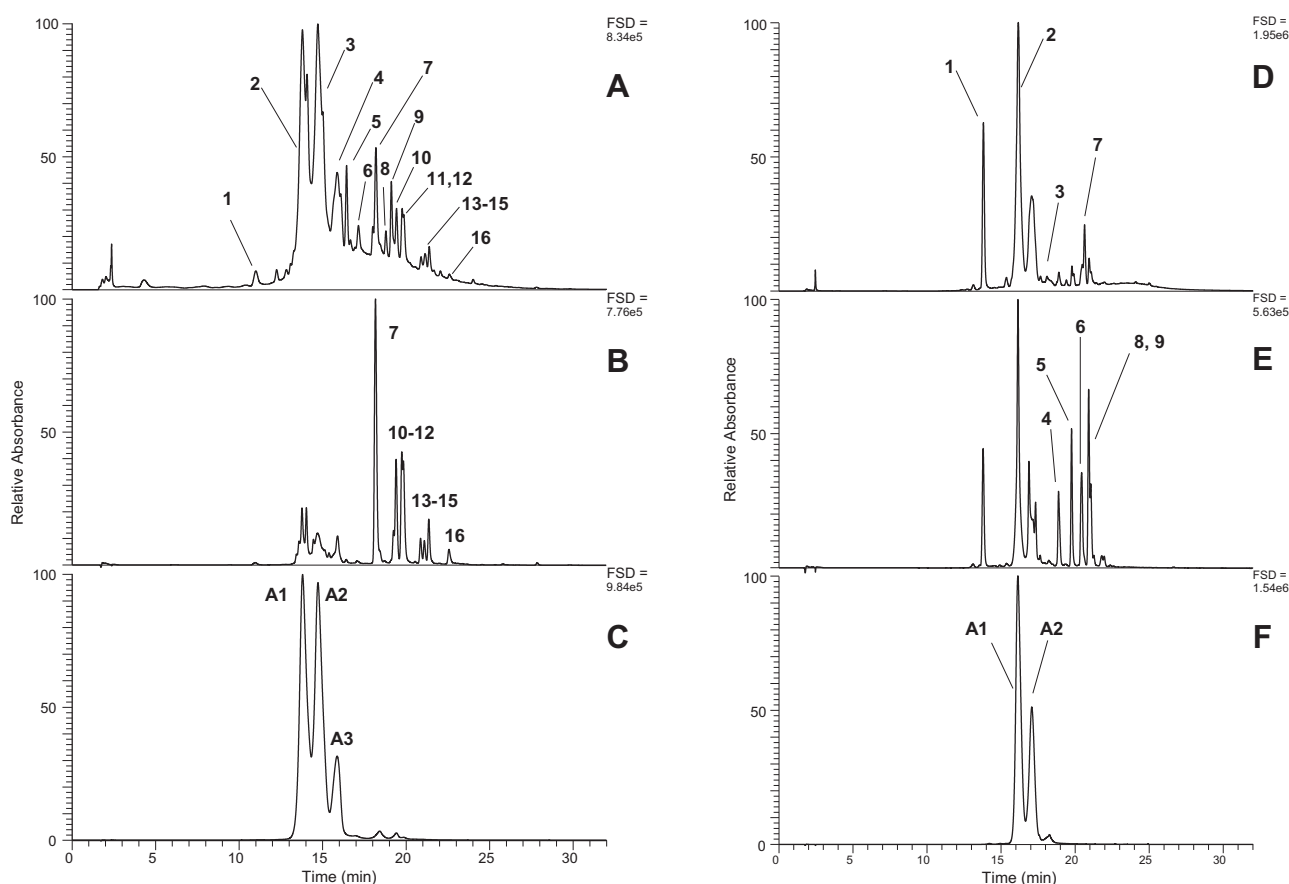


Fig. 1. PDAD traces of salal and aronia fruit extracts. Panel A shows PDAD trace at 280 nm; Panel B at 365 nm and Panel C at 520 nm for the salal fruit. Panel D shows PDAD trace at 280 nm; Panel E at 365 nm and Panel F at 520 nm for the aronia fruit. The full scale deflection for the PDAD is given in the top right corner of each panel. Peak numbers are discussed in Section 3.1 and in Table 2.

achieved by black currant varieties (600–1000 mg GAE/100 g) but is greater than noted for strawberry and raspberry varieties (~200 mg/100 g, exceptionally up to 400 mg/100 g; Deighton et al., 2000, 2002) and blueberry varieties (around 100 to 190 mg GAE/100 g; Kalt et al., 2003; Wang, Chen, Camp, & Ehlenfeldt, 2012). The TAC was lower than the range found in blackcurrants (Deighton et al., 2002) or aronia (Slimestad, Torskangerpoll, Nateland, Johannessen, & Giske, 2005) but was higher than raspberry or strawberry (Deighton et al., 2002). The antioxidant potential as measured by FRAP was also relatively high ($101,225 \pm 4159 \mu\text{M Fe}^{3+}$) but was in proportion with the TPC, as noted previously (Deighton et al., 2000; Martin et al., 2015). However, comparisons between species can be of limited value as the

variety, agronomy and environmental conditions at each growing location can markedly influence these parameters (e.g. Wang & Lin, 2000).

The salal fruit samples were relatively rich in anthocyanins with a total anthocyanin/phenol ratio of ~0.26, but this was lower than that of aronia (0.45) (Table 1). Anthocyanins were apparent in the UV profiles of salal fruits as evidenced by the three major peaks at 520 nm (compare Fig. 1a and c). There were also considerable amounts of flavonols (see Fig. 1b), some hydroxycinnamic acid derivatives were apparent and there was evidence for the presence of proanthocyanidin components (Table 2).

The major anthocyanin peaks were composed of a mixture of partly separated anthocyanin species. Peak A1 (or peak 2) was

Table 2
Phenolic components in salal and aronia fruit extracts.

Peak	TR	PDA max	m/z [M–H] [–]	MS ²	Putative ID
<i>Salal</i>					
1	11.00	324	353.0	191.0 , 179.0	Chlorogenic acid
2 (A1)	13.78	522, 278	597.0+	303.2*	Delphinidin pentose-hexose
			465.0+	303.2*	Delphinidin hexose
3 (A2)	14.70	523, 278	581.1+	287.2*	Cyanidin pentose-hexose
			567.1+	303.2*	Delphinidin dipentose
			449.0+	287.2*	Cyanidin hexose
			435.0+	303.2*	Delphinidin pentose
4 (A3)	15.86	520, 278	551.0+	287.2*	Cyanidin dipentose
			419.0+	287.2*	Cyanidin pentose
5	16.43	278	863.1	711.1, 693.1, 575.0 , 559.0	PAC trimer
6	17.13	278	1151.1	multiple	PAC tetramer
7	18.18	355	479.0	317.2*	Myricetin hexose
			493.1	317.2*	Myricetin glucuronide
8	18.78	270	561.8+	521.8	Unknown
9	19.10	280	586.8+	None	Unknown
10	19.25	355	449.1	317.2*	Myricetin pentose
11	19.41	355	463.0	317.2*	Myricetin rhamnoside
12	19.75	355	463.1	301.1*	Quercetin hexose
			479.0	301.1*	Quercetin glucuronide
13	20.88	355	433.1	301.1*	Quercetin pentose
14	21.13	355	433.1	301.1*	Quercetin pentose
15	21.37	355	447.0	301.1*	Quercetin rhamnoside
16	22.58	365	none	ND	Unknown
<i>Aronia</i>					
1	13.80	324	353.1	191.0	Neochlorogenic acid
2	16.15	325	353.1	191.0	Chlorogenic acid
A1	16.15	520, 278	449.1+	287.2*	Cyanidin hexose
A2	17.06	520, 278	419.1+	287.2*	Cyanidin pentose
3	18.11	278	865.1	577.0	PAC trimer
			694.9	500.9	Unknown
4	18.90	350	625.1	301.1* , 463.0	Quercetin dihexose
5	19.97	355	595.1	301.1* , 463.0	Quercetin pentose hexose (vicianoside)
6	20.46	350	609.2	301.1*	Quercetin rutinoside
7	20.63	280	464.9, 289.1	None	Eriodictoyl glucuronide
8	20.93	355	463.1	301.1*	Quercetin hexose ^a
9	21.08	355	463.1	301.1*	Quercetin hexose ^b

+ denotes positive mode data. Values in bold are the main MS² fragments, those marked with * were also present as characteristic in-source fragments arising from aglycones.

^a Possibly galactoside.

^b Possibly glucoside.

composed of two anthocyanin species with MS properties consistent with the presence of a delphinidin hexoside [M+H = 465; MS² = 303] and another delphinidin derivative [M+H = 597; MS² = 303] which was consistent with the neutral loss of 294, a pentose (–132) plus a hexose (–162). This may be a sambubioside-derivative of delphinidin as described previously in Maqui berries and red currants (Escribano-Bailón, Alcalde-Eon, Muñoz, Rivas-Gonzalo, & Santos-Buelga, 2006; Wu, Gu, Prior, & McKay, 2004). Peak A2 also contained a mixture of four anthocyanin species with MS properties consistent with the presence of a delphinidin pentose [M+H = 435, MS² = 303], a cyanidin hexose [M+H = 449, MS² = 287], a cyanidin sambubioside-like component [M+H = 581, MS² = 287] and a delphinidin component with two pentose groups [M+H = 567, MS² = 449 and 303, two neutral losses of 132 amu]. The third peak (peak A3) contained two main species with properties consistent with a cyanidin pentose [M+H = 419, MS² = 287] and a cyanidin dipentoside [M+H = 551, MS² = 287, neutral loss of 264]. These components eluted in a predictable order with delphinidin derivatives eluting before their less polar cyanidin equivalents and pentoside derivatives being more strongly retained than hexoses (Maatta-Riihinen, Kamal-Eldin, Mattiila, Gonzalez-Paramas, & Torronen, 2004; McDougall, Gordon, Brennan, & Stewart, 2005). There were also two late-eluting and less polar peaks at 520 nm which, in analogy with other fruits (e.g. black currant), could be anthocyanins acylated with hydroxycinnamic acids (McDougall et al., 2005). However,

MS data did not allow the identification of these more minor components.

The major peak absorbing at 365 nm (peak 7; Fig. 1b) contained two components [M+H = 481 and 495, both giving MS² = 319] which is consistent with the presence of hexose and glucuronide derivatives of myricetin (Borges, Mullen, & Crozier, 2010). In fact, a set of glucuronide, hexose, pentose and rhamnose derivatives of both myricetin and quercetin were present (Table 2).

The anthocyanins and flavonols were the major phenolic components but there were distinct peaks with MS properties consistent with proanthocyanidin (PAC) components (Table 2). Other less distinct signals were evident between the main anthocyanin and flavonol peaks and indicated the presence of a series of procyanidin-B-type PACs (with M-H values of 863, 1151, 1441 & 1729). The poor resolution of PACs has been noted previously in other berry species, such as lingonberry, where similar PACs were noted (McDougall, Kulkarni, & Stewart, 2009).

The aronia fruit extracts had a TPC of 1045 ± 13 mg/100 g FW but were relatively enriched in anthocyanins with a TAC/TPC ratio of 0.45 (Table 1). The FRAP value was lower than the salal fruits (at 80,645 ± 2137 μM Fe³⁺). They had a characteristic phenolic composition which was similar to previous examinations of aronia species (e.g. Slimestad et al., 2005; Strigl et al., 1995). The profile was relatively simple and was dominated by two major anthocyanin peaks and two chlorogenic acid isomers (Fig. 1d–f, Table 2) with a range of flavonol components present at lower levels.

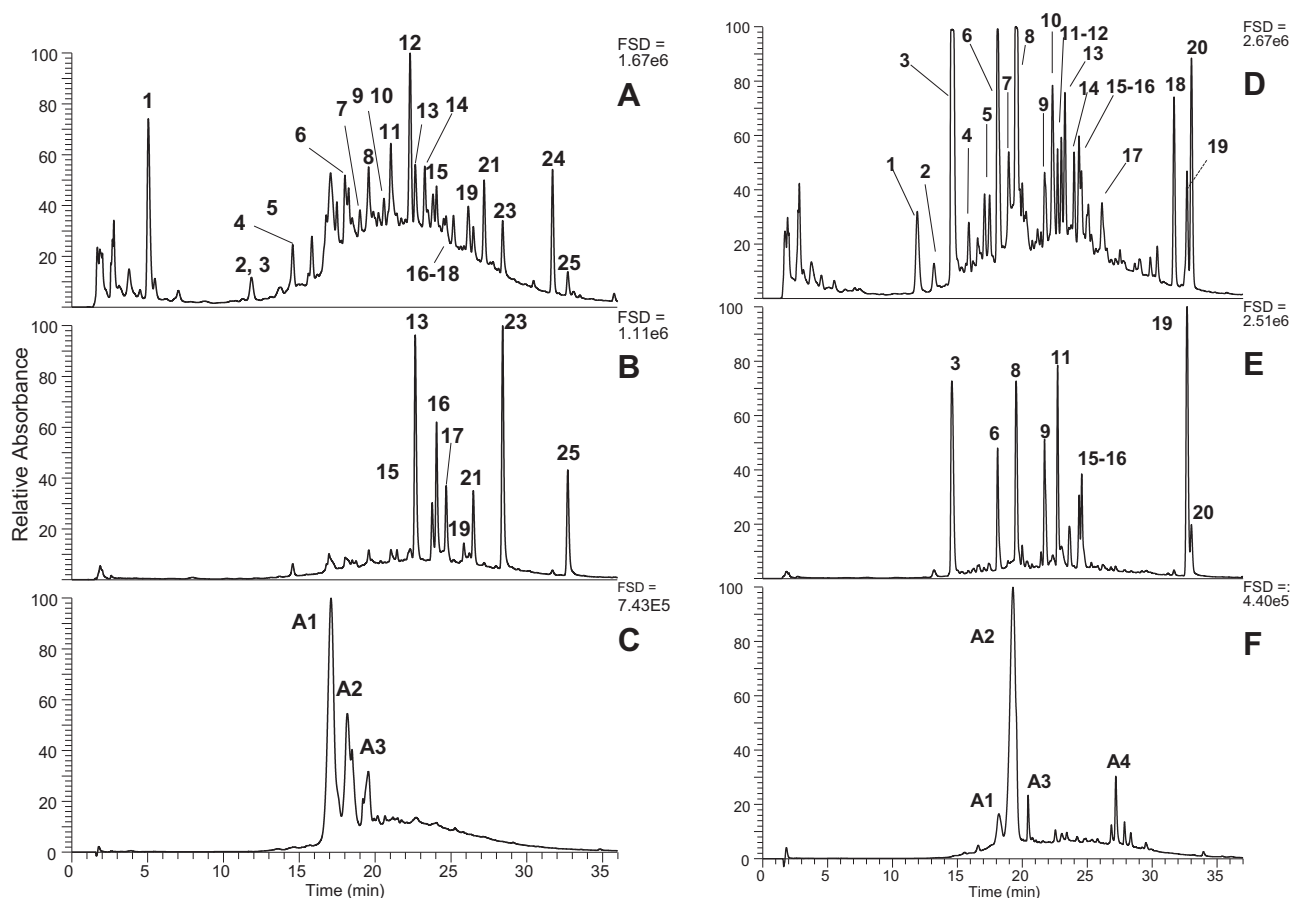


Fig. 2. PDAD traces of salal and aronia wines. Panel A shows PDAD trace at 280 nm; Panel B at 365 nm and Panel C at 520 nm for the salal wine. Panel D shows PDAD trace at 280 nm; Panel E at 365 nm and Panel F at 520 nm for the aronia wine. The full scale deflection for the PDAD is given in the top right corner of each panel. Peak numbers are discussed in Section 3.2 and in Table 3.

Peak A1 (Fig. 1f; Table 2) had MS properties consistent with the presence of a cyanidin hexose [$M+H = 449$; $MS^2 = 287$]. Peak A2 had MS properties consistent with the presence of a cyanidin pentose [$M+H = 419$; $MS^2 = 287$]. By comparison with previous work (Slimestad et al., 2005; Strigl et al., 1995), it is probable that peak A1 contained both glucosides and galactosides and peak A2 contained both arabinosides and xylosides. There were two major peaks with PDA maxima around 320 nm (Fig. 1e) and MS properties [$M-H = 353$, $MS^2 = 191$, 179; Table 2] which are consistent with neochlorogenic and chlorogenic acids, as identified before (Slimestad et al., 2005). There were also a range of quercetin flavonols, including the unusual vicianoside [$M-H = 595$, $MS^2 = 301$, 465], an arabinosylglucosyl derivative (Slimestad et al., 2005). There was also a flavonol component with MS properties consistent with a quercetin dihexose ($M-H = 625$, $MS^2 = 301$, 463) and a peak putatively identified as the flavonone derivative, eriodictyol glucuronide, again as previously identified (Slimestad et al., 2005).

3.2. Phenolic content and composition of fruit wines

Wine made from the salal fruits had a TPC of 1413 $\mu\text{g GAE/ml}$ and a TAC of 44.1 $\mu\text{g/ml}$, whereas the aronia wine gave a TPC of 966 $\mu\text{g GAE/ml}$ and a TAC of 18.4 $\mu\text{g/ml}$ (Table 1). The salal + aronia wine gave intermediate figures for TPC and TAC (TPC of 1122 $\mu\text{g GAE/ml}$ and TAC of 33.0 $\mu\text{g/ml}$). The TPC figures compare reasonably well with figures for red (TPC mean 2150 $\mu\text{g GAE/ml}$; range 730–4180), rosé (mean 820 $\mu\text{g GAE/ml}$ range 340–1300) and white wines (320 $\mu\text{g GAE/ml}$; range 90–850; obtained from <http://www.phenol-explorer.eu/contents/food/138#folin-assay>).

The total recovery of phenolics was 21.4% for salal, 20.6% for aronia and 24.4% for the mixed wine, whereas anthocyanins were recovered at $\sim 4.00\%$ (salal), $\sim 0.88\%$ (aronia) and $\sim 2.01\%$ (salal and aronia). This difference in anthocyanin content was reflected in colour of the wines (see Image 1, Supplementary data) and large reductions in anthocyanin content have been noted previously for fruit wines (e.g. Czynowska & Pogorzelski, 2004).

The polyphenol profiles of wines were notably different from the fruit extracts. There was an increase in the complexity of the UV traces at 280 nm (see Fig. 2a; salal and Fig. 2d; aronia), 365 nm (Fig. 2b and e) and 520 nm (Fig. 2c and f) with variation in retention of certain components but also new components appearing in the wines (Table 3). Reduction in anthocyanin content was indicated by the change in TAC/TPC ratio from 0.26 to ~ 0.05 in salal and from 0.45 to < 0.02 in aronia wines, respectively. The main anthocyanin peaks present in the salal and aronia fruits were present in the respective wines (Fig. 2c; A1, A2 and A3; Fig. 2f, A1 and A2). In salal wine, peak A2 was greatly reduced relative to peaks A1 and A3 (Fig. 2c) Peak A1 was composed almost entirely of delphinidin sambubioside with only a small amount of delphinidin hexose. Peak A2 had almost separated into two peaks with the earlier eluting peak being composed of cyanidin sambubioside and the later composed of delphinidin dipentoside. Peak A3 consisted almost entirely of cyanidin dipentoside. In fact, the salal wine had “lost” virtually all of the pentose and hexose derivatives of cyanidin and delphinidin but the relative amounts of the dipentoside and sambubioside derivatives had increased (Fig. 3a). In aronia wine, the two main anthocyanin peaks (A1 and A2) were detected (Fig. 2f) but peak A1 was reduced compared to A2 due to a loss

Table 3
Phenolic components in salal and aronia wines.

Peak	TR	PDA max	m/z [M–H] [−]	MS ²	Putative ID
<i>Salal</i>					
1	4.92	270	169.1	125.0	Gallic acid
2	11.50	290, 260	593.1	467.1, 425.1, 407.0, 289.0	PAC dimer (EGC-EC)
3	11.50	260	153.0	125.1	Dihydroxybenzoic acid
4	14.40	320	353.1	191.0	Chlorogenic acid
5	15.68	280	200.9	157.0 , 111.0	Unknown
A1	17.13	525, 275	597.0+	303.1*	Delphinidin pentose-hexose
	Rear		465.0+	303.1*	Delphinidin hexose
6	17.80	280	289.1	245.1	Epicatechin
A2	18.18	525, 275	581.0+	287.1*	Cyanidin pentose-hexose
A2	18.53	525, 275	567.1+	303.1*	Delphinidin dipentoside
7	18.86	275	879.1	727.0 , 711.0, 589.0, 411.0, 289.0	Galloylated PAC
8	19.39	280	577.1	251.0, 424.9 , 407.0, 289.0	PAC dimer (EC2)
A3	19.50	525, 275	551.1+	287.1*	Cyanidin dipentoside
9	20.09	280	1151.1	multiple	PAC tetramer (EC4)
10	20.44	280	863.1	710.9 , 693.0, 573.0, 289.0	PAC trimer (EC3)
11	20.86	280–340	318.9	257.0, 193.0	Unknown
12	22.06	282	153.0 (front)	153.0 , 125.0	Phloroglucinaldehyde
			369.1 (rear)	369.1 , 206.9	Hexose of 207*
13	22.52	355	493.0	317.2*	Myricetin glucuronide
14	23.09	280–340	457.1	457.1 , 275.0	Unknown
15	21.13	355	449.0,	317.2*	Myricetin pentose
16	23.95	355	463.1	317.2*	Myricetin rhamnose
17	24.60	355	477.0,	301.0*	Quercetin glucuronide
18	25.00	280–340	634.9	482.9, 471.0 , 330.9	Galloylated PAC
19	25.75	355	433.1,	301.1*	Quercetin pentose
20	26.02	262	439.1	poor	Unknown
21	26.38	355	447.1	301.1*	Quercetin rhamnoside
22	27.02	285	606.9	poor	Unknown
23	28.25	365	317.1	178.9, 151.0	Myricetin
24	31.55	285	574.9, 287.0	286.9	Dimer of 287
25	32.57	365	301.1	178.9	Quercetin
<i>Aronia</i>					
1	11.86	260, 292	153.0	109.0	Dihydroxybenzoic acid
2	13.21	320	353.1	191.0, 179.0	Chlorogenic acid derivative (CGA1)
3	14.62	320	353.1	191.0, 179.0	Neochlorogenic acid (CGA2)
4	15.89	276	200.9	156.9 , 111.0	Unknown
			175.0	128.7 , 114.9	Unknown
5	17.48	285, 315	341.0	250.9, 179.0	Caffeic acid hexose
6	18.12	320	353.0	191.0 , 179.1	Chlorogenic acid (CGA3)
A1	18.26	520, 278	449.1+	287.2*	Cyanidin hexose
7	18.97	270	383.0	302.9 , 300.9, 257.0, 175.0	Unknown
A2	19.31	520, 278	419.1+	287.2*	Cyanidin pentose
8	19.53	325,	179.1	135.0	Caffeic acid
A3	20.48	500	486.9+	355.0*	pyranoCyanidin pentose
9	21.73	355	625.1	301.1 *, 463.0	Quercetin dihexose
10	22.35	290	153.0	153.1, 125.0	Phloroglucinaldehyde
11	22.74	355	595.1	301.1*	Quercetin vicianoside
12	23.03	268, 320	455.0	309.0 , 163.0	Hydroxycinnamic acid derivative
13	23.31	305	301.0	No MS ²	Unknown
14	23.65	350	609.2	301.1*	Quercetin rutinoside
15	24.39	355	463.1	301.1*	Quercetin hexose
16	24.60	355	463.1	301.1*	Quercetin hexose
17	26.16	263–310	370.9	234.9, 191.0	Unknown
A4	27.22	500	625.1 , 339.0	Poor MS	Pyranocyanidin derivative
18	31.72	285–340	574.8 , 287.0	286.9 , 575.0, 531.0, 508.9	Dimer of 287
19	32.72	365	301.1	301.1 , 178.9	Quercetin
20	33.06	320, 306	207.1	179.1 , 135.0	Ethyl caffeate

Values in bold are the main MS² fragments, those marked with * were also present as characteristic in-source fragments arising from aglycones. + = positive mode data. EGC = epigallocatechin, EC = epicatechin, PAC = proanthocyanidin.

* – the possible “aglycone” at m/z 207 elutes close to quercetin and is more apparent in Aronia wine.

of cyanidin hexose in peak A1 (to <2% of fruit levels), whereas cyanidin pentose was more stable (~23%). New anthocyanin-like derivatives that absorbed at 520 nm were also present. Peak A3 had MS properties [M+H = 487, MS² = 355, neutral loss of 132], which is consistent with a pentoside of a vitisin A derivative of cyanidin, a pyruvate adduct formed during wine making from grapes (Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002) and similar vitisin A derivatives have previously been found in blackberry fruit wines (Arozarena et al., 2010). Peak A4 gave poor MS and could not be positively identified.

It should be noted that the total amounts of anthocyanins were considerably reduced in the wines over the fruit extracts and the apparent increases only reflect their relative proportions in the wine anthocyanin profile. The particular instability of anthocyanin hexosides may be related to the substrate specificity of yeast β -glycosidases expressed during fermentation (Ugliano & Moio, 2008). Indeed, the yeast strain used for fermentation (EC-1118) has been shown to produce considerable β -glucosidase activity (Tate & Reynolds, 2006). In addition, although Trenolin® enzymes were added primarily as a source of pectinase activity,

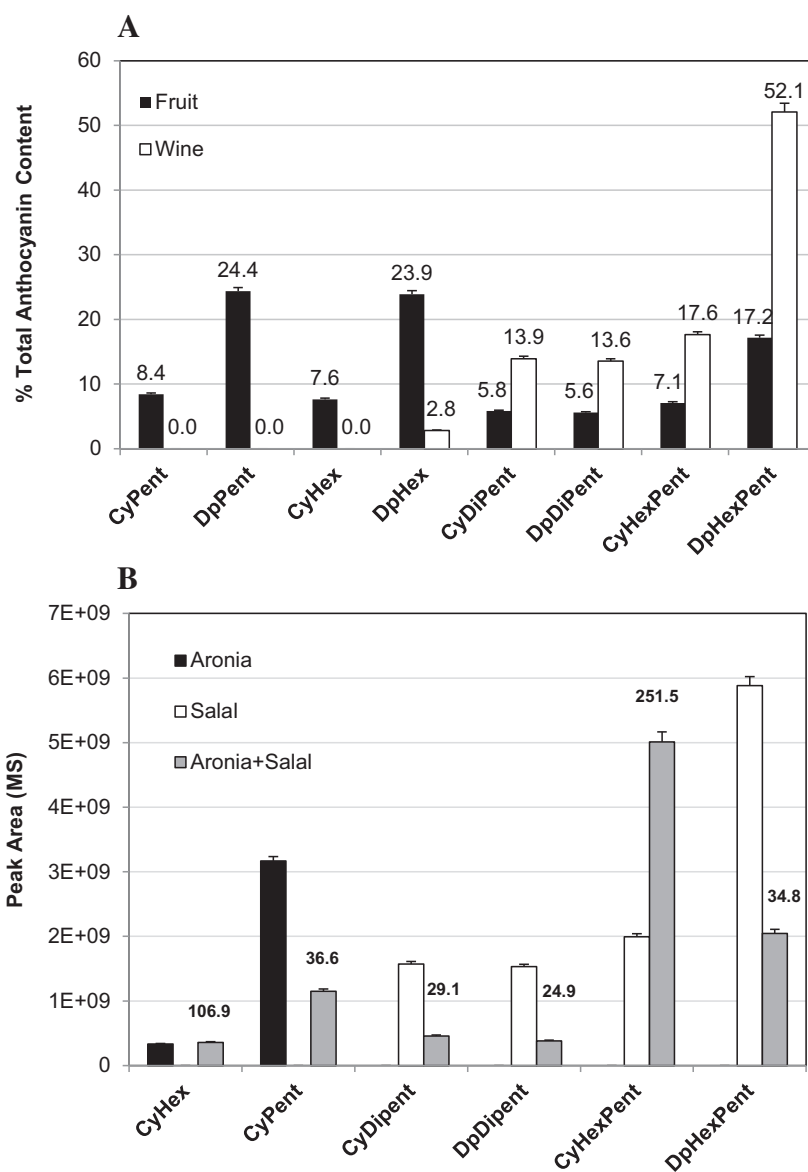


Fig. 3. Relative anthocyanin composition in salal fruits, salal wine and mixed wine. (A) Shows the relative anthocyanin composition of salal fruit extracts and salal wine. Peak areas were obtained by searching the LCMS data for the masses for the different anthocyanins and calculated using the resident Xcalibur software. The figures represent the relative contribution of each anthocyanin as % of the total peak area estimated for all anthocyanins. The figures are means from three different LCMS runs using the sub-sample extracts \pm standard error. As each sample was run at an equivalent phenol content (20 μ g GAE/injection), these figures are comparable and appropriate for comparison of the relative anthocyanin composition. (B) Shows the relative anthocyanin contents in salal, aronia and mixed wine. The bars are means of triplicate runs \pm SE. The peak areas for aronia + salal wine were multiplied by 2 to adjust for half amounts of berries being used. No figures were obtained for DpHex as this peak underlay the m/z 597 peak and could not be discerned from an in-source fragment of this peak. The % recovery figures inserted above the aronia + salal bars are based on the “expected” recovery of 100% if the recovery was the average of the recovery levels in the individual wines.

they may contain other glycosidases (e.g. glucosidases, rhamnosidases or arabinosidases) which expand the types of glycosidic linkages under threat. However, the apparent low recovery of anthocyanin hexosides may have been influenced by less effective extraction from the fruits during the wine-making procedure compared to that achieved by acetonitrile extraction, as hexosides have been shown to be less well extracted in sequential solvent extractions of black currants (Juedtz, Aberdein, Stewart, & McDougall, 2013). In contrast, in elderberry, cyanidin sambubioside levels were reduced 10-fold during wine making and all other cyanidin anthocyanins including cyanidin glucoside were much more stable (Schmitzer, Veberic, Slatnar, & Stampar, 2006).

No anthocyanidins were detected in the salal or aronia wines, possibly because they have relatively low stability at $pH > 2.0$ and the final pH of fermentations was > 3.5 . However, components

identified in the wine could have resulted from anthocyanidin degradation. Dihydroxybenzoic acid and gallic acid in salal and dihydroxybenzoic acid in aronia (Table 3) are consistent with B-ring fragments of cyanidin and delphinidin, respectively, after C-ring cleavage (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Fleischhut, Kratzer, Reckemmer, & Kulling, 2007; Sadilova, Carle, & Stintzing, 2012) with phloroglucinaldehyde present in both wines as the A-ring fragment from both anthocyanidins (Sadilova et al., 2012).

The appearance of the flavonol aglycones, myricetin and quercetin, in the wines also suggests enzymic deglycosylation. Flavonols in wines showed specific patterns of recovery (see Supplementary data, Fig. S2, salal & S3, aronia). In salal wine, quercetin and myricetin hexosides were particularly reduced, with a lesser reduction in the pentoses and a relative increase in the

proportion of glucuronide and rhamnoside derivatives. In aronia wine, quercetin hexose and quercetin rutinoside were less stable than quercetin dihexose and quercetin vicinioside (see [Supplementary data, Fig. S3](#)) and the aglycone, quercetin [peak 19], made up a substantial proportion of the total flavonol content. The low recovery of flavonol hexoses also strongly suggests a role for yeast β -glucosidase activity.

Chlorogenic acid was apparent in salal wine (peak 4, salal, [Fig. 2b](#); [Table 3](#)) and chlorogenic acid, neochlorogenic acid plus a new CGA derivative were present in aronia wine (peaks 3, 6 and 2 resp; [Fig. 2e](#); [Table 3](#)) indicating the relatively high stability of these components. Caffeic acid (peak 8; [Fig. 2e](#)) was a major peak in aronia wine and may have arisen through anthocyanin degradation ([Nurmi et al., 2009](#)) but more probably through chlorogenic acid degradation. Aronia wine had a substantial peak late in the profile (peak 20) with properties that fitted with ethyl caffeate, which arises through esterification of caffeic acid with ethanol. Ethyl caffeate was also present, but at lower levels, in the salal wine and has been identified in white wine ([Boselli, Bendia, Di Lecce, Benedetti, & Frega, 2009](#)) and as an unidentified component in red wines ([Sun, Liang, Bin, Li, & Duan, 2007](#)), where it also eluted close to quercetin.

A number of proanthocyanidin peaks became more apparent in aronia wine and these components are known to survive fermentation and accumulate in red wines made from grapes ([Santos-Buelga & Scalbert, 2000](#)). Epicatechin (peak 6; [Fig. 2d](#)) also became apparent but this component may have been present at low levels, and was concentrated as a result of losses of other phenolic derivatives, particularly the anthocyanins, during vinification.

The wine made from equal amounts of salal and aronia fruits had a TPC at 1122 $\mu\text{g/ml}$ ([Table 1](#)) that was intermediate to the values of the wines made from the individual fruits (1413 $\mu\text{g/ml}$ salal and 901 $\mu\text{g/ml}$ aronia). The anthocyanin content at 33.0 $\mu\text{g/ml}$ was also between the values for the salal (44.1 $\mu\text{g/ml}$) and aronia wines (18.4 $\mu\text{g/ml}$). The composite wine appeared to have a phenolic composition by LCMS that was intermediate between the wines prepared from the individual berries (see [Supplementary Fig. S4](#)). If no other factors played a major part, one could assume that the recovery of individual components in the mixed wine would be close to the average of the values from the wines made with the individual fruits. However, this was not the case for the anthocyanins ([Fig. 3b](#)). Cyanidin hexose was recovered at around 100 % of the “expected value” but cyanidin pentose was recovered at 36%. Both cyanidin and delphinidin dipentose had similar recoveries (at 29% and 25%) but cyanidin sambubioside was recovered at much higher levels ($\sim 250\%$) than delphinidin sambubioside (35%). This indicates that stability of anthocyanins under wine-making conditions could not be predicted by anthocyanidin structure or glycosylation (e.g. [Fleschhut et al., 2007](#)). It seems likely that stability is influenced by the presence, and relative stability, of other components. Indeed, the estimated recovery of other major phenolic components also showed patterns that differed from the expected values ([Supplementary data, Fig. S5](#)). Quercetin rutinosides, pentoses and hexoses were recovered at higher than “expected” levels whereas quercetin rhamnoside levels were lower and quercetin glucuronide was not detected even though it was present in the salal wine ([Fig. 3e](#)). Again, this does not match the patterns in the individual wines where hexoses and rutinosides were the least stable. The myricetin derivatives found in the individual wines were either not detectable in the composite wine or were all below the expected recovery values. Chlorogenic acid (CGA2) was more stable than neochlorogenic acid (CGA1) but isomerisation of these acids can occur ([Clifford, 1999](#)). Caffeic acid and ethyl caffeate levels were also below “expected” levels.

4. Conclusions

The polyphenol content and composition of aronia and salal fruits grown in Orkney has been described. This is the first report of the polyphenol composition of salal fruits. The wines made from these fruits had relatively high polyphenol contents and antioxidant potential but showed substantial differences in polyphenol composition from the fruits. The anthocyanins in salal were more stable to vinification, possibly due to the presence of diglycoside pentose containing derivatives. Indeed, it would be interesting to note if other berries with similar anthocyanin components also retain more colour during wine-making. The salal fruits were grown in Orkney but their use could be more universal and applied anywhere they could be grown or sourced. Although there were shared patterns (i.e. in reduction in anthocyanin content and breakdown of flavonols) between wines made from different fruits, wines made from the mixture of fruits had a phenolic composition far from the average of the individual wines, which suggests that the stability of individual components is dependent on the presence of other phenolic components.

Acknowledgements

The Hutton acknowledges funding from the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) Division's Strategic Research Portfolio. The authors acknowledge Innovation Voucher funding from the Scottish Funding Council and the UK's Technology Strategy Board. We thank Susan Verrall (Hutton) and Christine Hackett (BioSS) for their advice on statistical matters. We also thank Grace McKenzie, a student at Harris Academy, who contributed to some of the analysis as part of her work experience project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.03.025>.

References

- Acuna, U. M., Atha, D. E., Ma, J., Nee, M. H., & Kennelly, E. J. (2002). Antioxidant capacities of ten edible North American plants. *Phytotherapy Research*, *16*, 63–65.
- Arozarena, Í., Ortiz, J., Hermosín-Gutiérrez, I., Urretavizcaya, I., Salvatierra, S., Córdova, I., ... Marín-Arroyo, M. R. (2010). Colour, ellagitannins, anthocyanins, and antioxidant activity of Andean blackberry (*Rubus glaucus* Benth.) wines. *Journal of Agriculture and Food Chemistry*, *58*, 10143–10446.
- Borges, G., Mullen, W., & Crozier, A. (2010). Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. *Food & Function*, *1*, 73–83.
- Boselli, E., Bendia, E., Di Lecce, G., Benedetti, A., & Frega, N. G. (2009). Ethyl caffeate from Verdicchio wine: Chromatographic purification and *in vivo* evaluation of its anti-fibrotic activity. *Journal of Separation Science*, *32*, 3585–3590.
- Castañeda-Ovando, A., Pacheco-Hernández, M. D. L., Páez-Hernández, M.-E., Rodríguez, J.-A., & Galán-Vidal, C.-A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, *113*, 859–871.
- Clifford, M. N. (1999). Chlorogenic acids and other cinnamates – Nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, *79*, 362–372.
- Crawford, R. M. M. (2000). Ecological hazards of oceanic environments, Tansley Review No. 114. *New Phytologist*, *147*, 257–281.
- Czyzowska, A., & Pogorzelski, E. (2004). Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part II. Anthocyanins and flavanols. *European Food Research and Technology*, *218*, 355–359.
- Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild *Rubus* species. *Journal of the Science of Food and Agriculture*, *80*, 1307–1313.
- Deighton, N., Stewart, D., Davies, H. V., Gardner, P. T., Duthie, G. G., Mullen, W., & Crozier, A. (2002). Soft fruit as sources of dietary antioxidants. *Acta Horticulturae*, *585*, 459–465.

- Einbond, L. S., Reynertson, K., Xiao, D.-L., Basile, M. J., & Kennelly, E. J. (2004). Anthocyanin antioxidants from edible fruits. *Food Chemistry*, *84*, 23–28.
- Escribano-Bailón, M. T., Alcalde-Eon, C., Muñoz, O., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2006). Anthocyanins in berries of Maqui (*Aristotelia chilensis* (Mol.) Stuntz). *Phytochemical Analysis*, *17*, 8–14.
- Fleschhut, J., Kratzer, F., Rechkemmer, G., & Kulling, S. E. (2007). Stability and biotransformation of various dietary anthocyanins in vitro. *Molecular Nutrition & Food Research*, *51*, 1461–1471.
- Hobby, T., Dows, K., & MacKenzie, S. (2010). Commercial development of Salal on South Vancouver Island. *Journal of Ecosystems and Management, North America*, *11*. Available at: <<http://jem.forrex.org/index.php/jem/article/view/57>>.
- Juedtz, K., Aberdein, N., Stewart, D., & McDougall, G. J. (2013). Sequential extraction of black currant residues produces anthocyanin-rich extracts containing anthocyanin dimers. *Journal of Berry Research*, *3*, 53–64.
- Kalt, W., Lawand, C., Ryan, D. A. J., McDonald, J. E., Donner, H., & Forney, C. F. (2003). Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.) during ripening and storage. *Journal of the American Society for Horticultural Science*, *128*, 917–923.
- Kardum, N., Milovanović, B., Šavikin, K., Zdunić, G., Mutavdžin, S., Gligorijević, T., & Spasić, S. (2015). Beneficial effects of polyphenol-rich chokeberry juice consumption on blood pressure level and lipid status in hypertensive subjects. *Journal of Medicinal Food*, *18*, 1231–1238.
- Kask, K. (1987). Large-fruited black chokeberry (*Aronia melanocarpa*). *Fruit Varieties Journal*, *41*, 47.
- Kähkönen, M., Hopia, A. I., Vuorella, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agriculture and Food Chemistry*, *47*, 3954–3962.
- Keely, P. B., Martinsen, C. S., Hunn, E. S., & Norton, H. H. (1982). Composition of native American fruits in the Pacific Northwest. *Journal of the American Dietetic Association*, *81*, 568–572.
- Maatta-Riihinen, K. R., Kamal-Eldin, A., Matiila, P. H., Gonzalez-Paramas, A. M., & Torronen, R. (2004). Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *Journal of Agriculture and Food Chemistry*, *52*, 4477–4486.
- 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., Gordon, S., Brennan, R., & Stewart, D. (2005). Anthocyanin-flavanol condensation products from black currant (*Ribes nigrum* L.). *Journal of Agriculture and Food Chemistry*, *53*, 7878–7885.
- McDougall, G. J., Kulkarni, N. N., & Stewart, D. (2009). Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chemistry*, *115*, 193–199.
- Nurmi, T., Mursu, J., Heinonen, M., Nurmi, A., Hiltunen, R., & Voutilainen, S. (2009). Metabolism of berry anthocyanins to phenolic acids in humans. *Journal of Agriculture and Food Chemistry*, *57*, 2274–2281.
- Ochmian, I., Grajkowski, J., & Smolik, M. (2012). Comparison of some morphological features, quality and chemical content of four cultivars of chokeberry fruits (*Aronia melanocarpa*). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, North America*, *40*. Available at: <<http://www.notulaeobotanicae.ro/index.php/nbha/article/view/7181>>.
- Sadilova, E., Carle, R., & Stintzing, F. C. (2012). Thermal degradation of anthocyanins and its impact on colour and in vitro antioxidant capacity. *Journal of Agriculture and Food Chemistry*, *60*, 7463–7473.
- Santos-Buelga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds: Nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, *80*, 1094–1117.
- Schmitzer, V., Veberic, R., Slatnar, A., & Stampar, F. (2006). Elderberry (*Sambucus nigra* L.) wine: A product rich in health promoting compounds. *European Journal of Nutrition*, *45*, 7–18.
- Slimestad, R., Torskangerpoll, K., Nateland, H. S., Johannessen, T., & Giske, N. H. (2005). Flavonols from black chokeberries, *Aronia melanocarpa*. *Journal of Food Composition and Analysis*, *18*, 61–68.
- Strigl, A. W., Leitner, E., & Pfannhauser, W. (1995). Die schwarze Apfelbeere (*Aronia melanocarpa*) als natürliche Farbstoffquelle. *Deutsche Lebensmittel-Rundschau*, *91*, 177–180.
- Sun, J., Liang, F., Bin, Y., Li, P., & Duan, C. (2007). Screening non-colored phenolics in red wines using liquid chromatography/ultraviolet and mass spectrometry/mass spectrometry libraries. *Molecules*, *12*, 679–693.
- Tate, D., & Reynolds, A. G. (2006). Validation of a rapid method for measuring β -glucosidase activity in fermenting Muscat grape musts. *American Journal of Enology and Viticulture*, *57*, 60–68.
- Ugliano, M., & Moio, L. (2008). Free and hydrolytically released volatile compounds of *Vitis vinifera* L. cv. Fiano grapes as odour-active constituents of Fiano wine. *Analytica Chimica Acta*, *621*, 79–85.
- Vivar-Quintana, A. M., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2002). Anthocyanin-derived pigments and colour of red wines. *Analytica Chimica Acta*, *458*, 147–155.
- Wang, S. Y., & Lin, H.-S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agriculture and Food Chemistry*, *48*, 140–146.
- Wang, S. Y., Chen, H., Camp, M. J., & Ehlenfeldt, M. K. (2012). Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. *International Journal of Food Science and Technology*, *47*, 1540–1549.
- Wu, X. L., Gu, L. W., Prior, R. L., & McKay, S. (2004). Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. *Journal of Agriculture and Food Chemistry*, *52*, 7846–7856.