Research Article

Lipophilic components in black currant seed and pomace extracts

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The nature of the fatty acids and other lipophilic components in extracts from black currant seed and pomace (containing seed) were investigated, with a view to highlighting any potential uses. The same non-hydroxylated fatty acids were the major components in both types of extract, but total levels were less in pomace (75 582 mg 100 g^{-1} oil) than in seed alone (90 972 mg 100 g^{-1} oil) and there were less unsaturated fatty acids, including GLA (8653 and 12 625 mg 100 g^{-1} oil, respectively), but long chain *n*-20:0 – *n*-30:0 fatty acids (4080 and 437 mg 100 g^{-1} oil, respectively) were greatly increased in pomace. Phytosterols (mainly β-sitosterol), saturated *n*-20:0 – *n*-30:0 policosanols, ω-hydroxy fatty acids (mainly 16-hydroxy 16:0) and 2-hydroxy fatty acids (mainly 2-hydroxy 24:0) were present at much greater levels in pomace (2496, 2097, 958 and 46 mg 100 g^{-1} oil, respectively) than in seed (553, 108, 161, and 1 mg 100 g^{-1} oil, respectively). The pomace extract is a useful source of fatty acids, phytosterols and policosanols with potential functional properties.

Practical applications: The study investigated the lipophilic components in isohexane extracts from black currant seed and pomace (containing seed). Only pomace extracts had substantial amounts of phytosterols and policosanols that have potential as cholesterol-lowering agents, whereas fatty acids such as GLA, that has anti-inflammatory properties, are mainly in the seed.

Keywords: Black currant / Fatty acids / Phytosterols / Policosanols / Pomace

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1 Introduction

Black currant (*Ribes nigrum* L.) is grown primarily for processing into juices, cordials and jams [1], and the seed and pomace are by-products for which uses in the functional food [2–4] and human health [5, 6] sectors are being studied. After pressing the juice from the black currant, the pomace, including seed, is air-dried and the seed is selectively sieved out, leaving the seedless pomace waste consisting of skins and varying amounts of stalks and leaves. The seed oil can then be obtained by either solvent extraction with hexane or by a combination of solvent extraction and cold pressing.

Black currant seed oil contains linoleic acid (LA) as the major fatty acid together with some nutritionally important fatty acids, ALA, stearidonic acid (SA) and especially GLA [7–9] and, similar to borage and evening primrose oils, is used as a dietary supplement [1]. The levels of GLA normally range between 14 and 19% of the total fatty acids, and are thus intermediate between evening primrose oil (8–14%) and borage oil (21–25%) [1], although values of greater than 20% have been recorded [8, 9]. GLA has anti-inflammatory properties and has been used to treat a variety of conditions including atopic eczema and rheumatoid arthritis [10] and

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Abbreviations: LA, linoleic acid; **MSTFA,** *N*-methyl-*N*-(trimethylsilyl)-trifluroacetamide; **OA,** oleic acid; **PA,** palmitic acid; **SA,** stearidonic acid; **TMS,** trimethylsilyl

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cancer [11]. Black currant oil also contains significant amounts of phytosterols, tocopherols and tocotrienols [1, 7], compounds implicated with exhibiting beneficial health effects with respect to reduced risk of cardiovascular disease [12] oxidative stress and inflammation [13] and neuroprotection [14], respectively.

Black currant pomace and the seed residue remaining after oil extraction are generally viewed as low-value waste products. There are few reports specifically focussed on black currant pomace and information is especially lacking on the lipophilic components. The majority of these studies have focussed on the polyphenol component and predominantly the anthocyanins together with flavonols and hydroxycinnamic acids, that are more abundant in the pomace than the juice [2, 15, 16]. A detailed characterisation of the polysaccharide content showed mainly cellulose and seed-derived mannans [17]. The content of various phytochemicals, including vitamin C and many lipophilic components (tocopherols, tocotrienols, carotenoids, phytosterols and fatty acids), have been determined in black currant seed press residues [18]. The cutin monomers of black currant seed residues have also been investigated and were dominated by epoxy-substituted C_{18} ω-hydroxy acids [19, 20] whereas in berry residues they were mainly C_{16} ω-hydroxy acids with either another hydroxy or oxo group in the 10-position, and a C_{18} ω-hydroxy acid with a double bond in the 9-position [21].

As part of the EU FP7 funded project BrainHealthFood (www.uku.fi/brainhealthfood/) to valorise black currant pomace for use as a potential dietary and/or nutraceutical bioactive component to reduce the risk or ameliorate the consequences of neurological oxidative damage, we have investigated the content and composition of some lipophilic components, including fatty acids and phytosterols, in extracts of pomace (containing seed) and have compared the compositions to those of the oil from the seed only.

2 Materials and methods

2.1 Chemicals

Standard chemicals, tricosanoic acid methyl ester (*n*-23:0), 16-hydroxyhexadecanoic acid (16-OH 16:0), 1-eicosanol (*n*-20:0 ol), 1-docosanol (*n*-22:0 ol), 1-octacosanol (*n*-28:0 ol), triacontanol 30:0 ol) and cholesterol were from Sigma-Aldrich Co. Ltd. (Poole, UK). A 2-hydroxy FAME mixture of 2-hydroxytetradecanoic (2-OH 14:0), 2-hydroxyhexadecanoic (2-OH 16:0), 2-hydroxyoctadecanoic (2-OH 18:0), 2-hydroxyeicosanoic (2-OH 20:0), 2-hydroxydocosanoic (2-OH 22:0), 2-hydroxytricosanoic (2-OH 23:0) and 2-hydroxytetracosanoic (2-OH 24:0) acid methyl esters was obtained from Matreya LLC (Pleasant Gap, PA). A characterised FAME mixture from fish oil and a sterol mixture (obtained by hydrolysis followed by aluminium oxide solid-phase extraction chromatography and finally thin-layer chromatography) from BCR-162R soya/maize oil blend (LGC, Teddington, UK) were used as reference standards. *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was from Thermo Fisher Scientific (Cramlington, UK). Solvents were of HPLC grade and were supplied by Fisher Scientific UK (Loughborough, UK). Florisil^{TM} was obtained from VWR International Ltd. (Lutterworth, UK).

2.2 Black currant pomace preparation

Black currant pomace was supplied by VIN-KON S.A (Konin, Poland) and was composed of skins and seeds, as well as stalks and leaves, resulting from the pressing of black currant cultivars Ben Lomond and Ben Tirran from the 2009 harvest. On a laboratory scale, samples of pomace were either air-or freeze-dried. Thin layers of pomace were spread on trays and dried in an air oven at 50°C to give a residual moisture content of 7 or 11%. Alternatively the pomace was freeze-dried to give a residual moisture content of 8 or 16%. Pomace samples that had been either air- or freeze-dried were then ground in batches of around 50 g with an electrical kitchen chopper (Speedy Pro GVA 1, Krups GmbH, Offenbach am Main, Germany) for 1 min (3×20 s to avoid warming of the samples). From similar unground dried pomace samples, the seeds were separated out with a 500 μm sieve. All samples were immediately packed in bags under vacuum and kept until used.

2.3 Oil extraction

Around 10 g of seeds or pomace were weighed and ground in an electric coffee grinder (Russell Hobbs 12149-10, Manchester, UK) for 30 s, and the oil was extracted with isohexane (130 mL) by Soxhlet extraction for 5 Soxhlet cycles over approximately 30 min using a Buchi B-811 extraction system (Buchi UK Ltd., Oldham, UK). The residue was re-ground and the extraction repeated once more using the same solvent for another five cycles. The bulk of the solvent was removed on the extraction system by heating under an atmosphere of nitrogen, and the remainder under nitrogen on a heating block at 100°C for 30 min until constant weight was reached. The oil content was determined gravimetrically and expressed as a weight percent of the seed or pomace.

2.4 Preparation of FAME

FAME were prepared from oils using a procedure based on that of Joh et al. [22]. In triplicate, oils (10–20 mg) were weighed accurately into culture tubes and internal standards of *n*-23:0 acid methyl ester (49.5 mg in 10 mL toluene; 100 μL) and cholesterol (25.6 mg in 100 mL toluene; 100 μL) were added to two of the tubes. Internal standards were not added to the third tube so that the possible presence of these compounds in the samples could be subsequently assessed by GC, and allowed for in quantitative determinations, if necessary.

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Toluene (0.5 mL) and 2% v/v methanolic sulphuric acid were added and the tubes were heated at 60°C for 36 h on a tube heater. Isohexane/diethyl ether (1:1 v/v; 3 mL) and 5% w/v aqueous sodium chloride (5 mL) were added and, after shaking, the upper isohexane/diethyl ether layer was removed to a clean tube and was shaken with 2% w/v potassium hydrogen carbonate (3 mL). The isohexane/diethyl ether layer was put through a short column of anhydrous sodium sulphate [prepared in a Pasteur pipette and prewashed with isohexane/diethyl ether (1:1)]. The column was washed with isohexane/diethyl ether (1:1 v/v; 2 mL) and the combined isohexane/diethyl ether extracts were taken to dryness.

2.5 Separation of non-hydroxylated FAME from other components

Methyl esterified samples in isohexane/diethyl ether (90:10 v/v) were loaded on to short (3 cm) columns of Florisil™, prepared in a Pasteur pipette and prewashed with isohexane/diethyl ether (90:10 v/v; 3 mL). Non-hydroxylated FAME were eluted with isohexane-diethyl ether (90:10 v/v, 10 mL) and other components, including hydroxylated FAME, long chain fatty alcohols and sterols, were eluted with diethyl ether (10 mL) [23].

2.6 Trimethylsilylation

Trimethylsilyl (TMS) ether derivatives of hydroxylated compounds in unfractionated samples (after formation of methyl esters) and in fractions from Florisil™ columns were prepared by dissolving the samples in chloroform (40 μL), adding pyridine (10 μL) and MSTFA (40 μL) and heating at 37°C for 30 min.

2.7 GC of FAME and TMS ethers of hydroxylated components

GC of FAME and TMS ethers of hydroxylated compounds was performed on an Agilent model 6890 gas chromatograph. Non-hydroxylated FAME up to *n*-24:0 were analysed on a CP-Wax 52CB™ as previously described [9]. Due to limitations in the upper temperature of the CP-Wax 52CB™ column, non-hydroxylated FAME of chain length greater than *n*-24:0 and TMS ethers of hydroxy FAME, fatty alcohols and sterols were analysed on an HP-5™ capillary column (0.25 mm id × 30 m in length, 0.25 μm film thickness; Agilent, Stockport, UK). After holding the temperature at 160°C for 2 min, the column was temperature-programmed at 5°C min^{-1} to 325°C, then was held at this point for a further 15 min. Other conditions were as described earlier [9] except that the carrier gas was at a constant flow rate of 2 mL min^{-1}, and the injector was at 325°C. An Agilent Chemstation (Agilent, Stockport, UK) was used for data acquisition.

2.8 GC-MS of FAME and TMS ethers of hydroxylated components

Non-hydroxylated FAME up to *n*-24:0 were analysed by GC-MS on an Agilent 7890A GC coupled to an Agilent 5975C inert XL MSD mass spectrometer. A capillary column of fused silica coated with Supelcowax™10 (0.25 mm id × 30 m in length, 0.25 μm film thickness) was used, and other chromatographic conditions were as for GC of non-hydroxylated FAME on a CP-Wax 52CB™ column [9] except that helium was the carrier gas. The MS was operated in electron impact (EI) mode with ionisation at 70 eV, solvent delay 4 min, source temperature 230°C, mass range 50–550 amu at 3 spectra s^{-1}. The GC-MS interface temperature was 280°C. Non-hydroxylated FAME of greater chain length than *n*-24:0 and TMS ethers of hydroxy FAME, fatty alcohols and sterols were analysed on a Thermo Finnigan Trace DSQ II GC-MS. A capillary column of fused silica coated with DB5-MS™ (0.25 mm id × 15 m in length, 0.25 μm film thickness) was used, and helium was the carrier gas at a constant flow rate of 1 mL min^{-1}. After holding the temperature at 160°C for 2 min, the column was temperature-programmed at 5°C min^{-1} to 320°C, then held at this point for a further 15 min. The programmable temperature vaporising injector, used with a split of 40:1, and MS conditions were as previously described [24]. Data were acquired using the Xcalibur™ software package V. 2.07.

2.9 Quantification of lipophilic components

Samples fractionated on Florisil™ columns were used for quantifying non-hydroxylated fatty acids and phytosterols by GC, and for aiding identification of all components by GC and GC-MS, but hydroxy fatty acids and policosanols were quantified by GC in unfractionated samples.

Individual non-hydroxylated fatty acid contents, in mg of free fatty acids per 100 g of oil, were measured from GC-FID analyses using the equation:

$$\frac{A_{XA} * CF_X * W_{IS} * 100\,000}{A_{IS} * W_A * 1.04}$$

where A_{XA}, area count of FAME; A_{IS}, area count of *n*-23:0 methyl ester internal standard; W_{IS}, mass (mg) internal standard added to sample; W_A, mass (mg) of oil used; CF_X, theoretical correction factor relative to *n*-23:0 methyl ester internal standard according to Christie [23]. The factor of 1.04 is to convert from FAME to free fatty acids.

Individual hydroxy fatty acids, policosanol and phytosterol contents, in mg per 100 g of oil, were measured from GC-FID analyses using the equation:

$$\frac{A_{XA} * W_{IS} * 100\,000 * RCF}{A_{IS} * W_A}$$

where A_{XA}, area count of component; A_{IS}, area count of *n*-23:0 methyl ester (for hydroxy FAME TMS and policosanol

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TMS) or cholesterol TMS (for phytosterol TMS) internal standard, W_{IS} and W_A as above. RCF is a response correction factor for the compounds of interest relative to the relevant internal standard, and has a value of 1.00 for phytosterol TMS, 0.86 for policosanol TMS and 0.70 for hydroxy FAME TMS. The RCF for policosanol TMS and hydroxy FAME TMS, relative to *n*-23:0 methyl ester, were experimentally derived using TMS derivatives of *n*-22:0 and *n*-28:0 alkan-1-ols, and 2-OH 24:0 FAME, respectively.

2.10 Statistical evaluation

Differences in compound levels between seed and pomace were assessed by a Mann–Whitney *U*-test using Genstat™ for Windows, 13th Edition (VSN International Ltd., Hemel Hempstead, UK).

3 Results and discussion

3.1 Oil content

The oil content of black currant seeds was on average 14.5% w/dry w and was within the expected range [9] whereas that of pomace (containing seed) was 7.8%. In pomace, considering that the seed content was estimated as 44% on a dry weight basis, the seed contributed the majority of oil at about 6.4% of the pomace. The oil from the seeds alone was a near colourless clear liquid whereas the extract from pomace was waxy and dark green in appearance, presumably reflecting extracts from leaf, stalk and skin.

3.2 Lipophilic compound content

The levels of various lipophilic compounds were determined in samples of seed and pomace dried by different drying methods (air-dried or freeze-dried) and to different moisture levels. The differences in levels of all compounds between seed and pomace, irrespective of drying method or moisture content, were of much greater magnitude than between different drying methods or moisture levels for either seed or pomace, and therefore mean values of the different drying methods and moisture contents are presented (Tables 1–4).

3.2.1 Non-hydroxylated fatty acids

In agreement with other studies on the percent fatty acid composition of black currant seed oil [7–9], LA was the major fatty acid followed by ALA and GLA, palmitic acid (*n*-16:0) and SA (Table 1). All minor saturated, monounsaturated and diunsaturated fatty acids up to C_{24} had previously been detected in black currant oil. The fatty acid composition up to C_{20} in the pomace extract was not very different to that of the seed oil. However, pomace contained higher percentages of *n*-14:0 and *n*-16:0 and lower percentages of PUFA LA, GLA, ALA and SA, which must reflect differences in the fatty composition of non-seed pomace components compared to seed. Long chain saturated fatty acids from *n*-20:0 to *n*-30:0, with even carbon fatty acids predominating, were present almost exclusively in pomace, although *n*-24:0, the most abundant long chain fatty acid, was only 1.2% of the total fatty acids.

Table 1. Non-hydroxylated fatty acid compositions of black currant seed oil and pomace extract (weight percent of total fatty acids)

Fatty acid	Seed	Pomace
n-14:0[a)]	0.1	0.3
n-15:0[a)]	0.0	0.1
n-16:0[a)]	6.5	8.6
16:1(*n*-9)[b)]	<0.1	<0.1
16:1(*n*-7)[a)]	0.1	0.2
n-17:0[b)]	0.1	0.1
n-18:0[b)]	1.6	1.6
18:1(*n*-9)[a)]	10.2	9.8
18:1(*n*-7)[a)]	0.7	0.8
18:2(*n*-6) (LA)[a)]	49.1	45.6
18:3(*n*-6) (GLA)[a)]	13.9	11.4
18:3(*n*-3) (ALA)[a)]	13.4	12.6
18:4(*n*-3) (SA)[a)]	2.7	2.1
20:0[a)]	0.2	0.9
20:1(*n*-11)[b)]	0.1	0.1
20:1(*n*-9)[a)]	0.9	0.8
20:1(*n*-7)[b)]	<0.1	<0.1
20:2(*n*-6)[b)]	0.3	0.3
22:1(*n*-9)[b)]	<0.1	<0.1
n-21:0[a)]	<0.1	0.1
n-22:0[a)]	0.1	0.8
n-23:0[a)]	<0.1	0.1
n-24:0[a)]	0.1	1.2
n-25:0[a)]	<0.1	0.1
n-26:0[a)]	<0.1	1.0
n-27:0[a)]	<0.1	0.1
n-28:0[a)]	<0.1	1.0
n-30:0[a)]	<0.1	0.3

Values are mean values from samples that had either been air-dried or freeze-dried to two different moisture levels. Each of the four samples were analysed in duplicate.
[a)] Levels significantly different ($p<0.05$) between seed and pomace.
[b)] No significant difference ($p<0.05$) between levels in seed and pomace.

Non-hydroxylated fatty acids were the most abundant class of compounds in both seed and pomace but the total levels were greater in seed (91 g 100 g^{-1} oil) than pomace (76 g 100 g^{-1} oil), as were many unsaturated fatty acids [18:1(*n*-9), LA, ALA, GLA, SA, 20:1(*n*-9), 20:1(*n*-11) and 20:2(*n*-6)] (Table 2). Considering that the pomace extract was mainly derived from seed, it follows that these fatty acids were mainly seed-derived. Conversely, in pomace compared to seed, the minor monounsaturated fatty acids 16:1(*n*-7), 20:1(*n*-7) and 22:1(*n*-9) were more abundant and there was a large increase in the levels of *n*-14:0 and long

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Table 2. Non-hydroxylated fatty acid compositions of black currant seed oil and pomace extract (mg 100 g^{-1} of oil)

Fatty acid	Seed	Pomace
n-14:0[a]	57	195
n-15:0[a]	10	40
n-16:0[b]	5923	6460
16:1(*n*-9)[b]	33	33
16:1(*n*-7)[a]	69	149
n-17:0[b]	57	65
n-18:0[a]	1428	1239
18:1(*n*-9)[a]	9282	7423
18:1(*n*-7)[b]	612	605
18:2(*n*-6) (LA)[a]	44 623	34 552
18:3(*n*-6) (GLA)[a]	12 625	8653
18:3(*n*-3) (ALA)[a]	12 194	9545
18:4(*n*-3) (SA)[a]	2434	1590
20:0[a]	145	672
20:1(*n*-11)[a]	88	66
20:1(*n*-9)[a]	821	629
20:1(*n*-7)[a]	6	31
20:2(*n*-6)[a]	255	194
22:1(*n*-9)[a]	16	35
n-21:0[a]	11	40
n-22:0[a]	83	605
n-23:0[a]	23	92
n-24:0[a]	69	859
n-25:0[a]	13	84
n-26:0[a]	45	713
n-27:0[a]	4	63
n-28:0[a]	28	705
n-30:0[a]	16	247
Total[a]	90 972	75 582

Values are mean values from samples that had either been air-dried or freeze-dried to two different moisture levels. Each of the four samples were analysed in duplicate.
[a] Levels significantly different ($p<0.05$) between seed and pomace.
[b] No significant difference ($p<0.05$) between levels in seed and pomace.

chain saturated fatty acids (C_{20}–C_{30}). These fatty acids must be at higher concentrations in the non-seed components of the pomace extract compared to seed oil.

Triacylglycerols are by far the most abundant lipid component of oilseeds, whereas in plant tissue, membrane phospholipids and glycolipids are likely to be major contributors to the fatty acid content. The extraction solvent, isohexane, will efficiently extract non-polar lipids such as triacylglycerols, but polar lipids, such as phospholipids and glycolipids, may require a more polar solvent for efficient extraction [23], hence the oil content and the fatty acid content of the oil extracted from pomace may well have been underestimated.

3.2.2 Phytosterols

According to Bakowska-Barczak et al. [7] the non-fatty acid components in black currant seed oil are mainly tocopherols and phytosterols. The major phytosterol in black currant seed oil was β-sitosterol followed by campesterol then cholesterol and Δ5,25-stigmastadienol (clerosterol) and Δ7-stigmastenol (Table 3). Δ5,23-Stigmastadienol, Δ7-campesterol, stigmasterol, Δ5-avenasterol and campestanol were detected as minor sterols. Sitostanol and Δ5,24-stigmastadienol were also detected at low levels but could not be quantified due to overlap with *n*-triacontanol (as TMS) and an unknown, respectively. A similar pattern of phytosterols was observed for the pomace extract but the levels were about five fold greater, indicating greater levels in the non-seed components of the pomace extract compared to seed oil.

Table 3. Phytosterol compositions of black currant seed oil and pomace extract (mg 100 g^{-1} of oil)

Sterol	Seed	Pomace
Cholesterol[b]	13	17
Campesterol[a]	44	184
Campestanol[a]	1	3
Stigmasterol[a]	3	14
Δ7-Campesterol[a]	4	26
Δ5,23-Stigmastadienol[a]	6	19
Clerosterol[a]	12	57
β-Sitosterol[a]	460	2116
Δ5-Avenasterol[a]	1	7
Δ7-Stigmastenol[a]	9	51
Total[b]	553	2496

Values are mean values from samples that had either been air-dried or freeze-dried to two different moisture levels. Each of the four samples were analysed in duplicate.
Sitostanol and Δ5,24-stigmastadienol were also detected but were not quantified due to overlap with *n*-30:0 fatty alcohol (TMS) and an unknown, respectively.
[a] Levels significantly different ($p<0.05$) between seed and pomace.
[b] No significant difference ($p<0.05$) between levels in seed and pomace.

The total phytosterol levels in black currant seed oil were at the lower end of the range detected in black currant seed oil in one report [1] but considerably lower than those in another [25]. This may be a result of differences in germplasm, growth environment and/or analytical methodology. The same sterols were detected as found earlier [1] with the exception of brassicasterol, previously reported at very low levels, and Δ7-avenasterol. The latter sterol was also detected in two other studies [7, 25]. Although in the present study there was a component with a similar retention time to that of a Δ7-avenasterol standard, the mass spectrum indicated that it was not a sterol. Since the other studies did not utilise MS to confirm identities, this sterol may have been incorrectly assigned. Δ7-Stigmasterol was previously identified in two studies [7, 25] but was not found in another [1] or in this study. The converse occurred for Δ7-stigmastenol; it is possible that Δ7-stigmastenol had been misidentified as Δ7-stigmasterol.

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Table 4. Policosanol and hydroxy fatty acid compositions of black currant seed oil and pomace extract (mg 100 g^{-1} of oil)

Policosanol/hydroxy fatty acid	Seed	Pomace
n-20:0 ol[a)]	14	148
n-22:0 ol[a)]	20	510
n-23:0 ol[a)]	4	40
n-24:0 ol[a)]	32	823
n-26:0 ol[a)]	13	261
n-28:0 ol[a)]	25	315
Total[a)]	108	2097
2-OH 24:0[a)]	1	37
16-OH 16:0[a)]	131	780

Values are mean values from samples that had either been air-dried or freeze-dried to two different moisture levels. Each of the four samples were analysed in duplicate.
n-30:0 ol was also detected but was not quantified due to overlap with β-sitostanol.
[a)] Levels significantly different ($p<0.05$) between seed and pomace.

3.2.3 Policosanols

Low levels of long chain mainly even-carbon saturated fatty alcohols, or policosanols, ranging from *n*-20:0 – *n*-30:0 (major component *n*-24:0) were detected in black currant seed oil (Table 4). The same policosanols were detected in pomace although the levels were approximately 20-fold greater, again indicating greater levels in the non-seed component of the pomace extract compared to seed oil. The levels of policosanols in pomace were similar to those of long-chain *n*-20:0 – *n*-30:0 saturated fatty acids (Table 2) suggesting that they possibly exist naturally as wax esters. Indeed wax esters are major components of waxes present on the surfaces of leaves [26] and other aerial parts such as fruit skins [27].

Policosanols, usually from sugar cane wax, have been proposed to have potential in the prevention and treatment of cardiovascular disease and the evidence has been reviewed [28]. Effects include reducing platelet aggregation, endothelial damage and foam cell formation, and lowering total and LDL cholesterol. The highest policosanol content has been found in brown beeswax (12 g kg^{-1}) [29], and peanut oil (up to 0.54 g kg^{-1}) [30] and wheat germ oil (0.04 g kg^{-1}) [31] have considerably less. Black currant pomace extract has an even higher content (21 g kg^{-1}). With respect to the raw materials, the levels in various corn kernel [31], wheat [29] and sugar cane [29] fractions were 4–111, 0.2–164 and 17–270 mg kg^{-1}, respectively. In black currant pomace, considering that the extract was 7.8% of the pomace, the policosanol content is greater at about 1600 mg kg^{-1}.

The chain length distribution of policosanols differs between the various sources. *n*-24:0 was the major policosanol in black currant but the major components in beeswax [29], corn kernel [31], peanut oil [30] and sugar cane [29] were *n*-30:0, *n*-32:0, *n*-22:0 and *n*-28:0, respectively. Wheat germ oil would appear to be variable in composition; *n*-24:0, *n*-26:0 and *n*-28:0 [29], *n*-22:0, *n*-28:0 and *n*-30:0 [32] and *n*-28:0 [33] were the major components in three different studies. In view of *n*-28:0 being the major policosanol (usually from sugar cane wax) used in health studies [28, 34], any potential for black currant policosanols in this area would have to consider the effect of differences in chain length distribution (*n*-28:0 was the third greatest component in black currant) on the bioefficacy.

3.2.4 Hydroxy fatty acids

2-Hydroxy fatty acids were detected at very low levels in seed but at greater (but still low) levels in pomace. 2-OH 24:0 was the major component (Table 4), and although 2-OH 22:0 and 2-OH 16:0 were also detected they were not quantified due to overlap with other components. 2-Hydroxy fatty acids are ubiquitous moieties of ceramides and cerebrosides present in cell membranes, and 2-OH 24:0 is often the major fatty acid [35]. Therefore it is not surprising that they were present at higher levels in pomace compared to seed only. In cell membranes, 2-hydroxy fatty acids are amide-linked to sphingosine bases and therefore the actual levels may be higher than those detected because the analytical methodology adopted was not optimised for breaking amide bonds; harsher conditions than those used may be needed [35]. Also it is unlikely that there was complete extraction of ceramides, and especially sugar-containing cerebrosides, due to the non-polar nature of the solvent (isohexane) used.

ω-Hydroxy acids were also detected at greater levels in pomace compared to seed. 16-OH 16:0 was the major ω-hydroxy acid (Table 4). Other minor ω-hydroxy acids (18-OH 18:0, 18-OH 18:1, 20-OH 20:0 and 22-OH 22:0) were detected but were not quantified because they overlapped with other components. Complex mixtures of other hydroxy fatty acids were detected in both the seed and pomace but, in the absence of suitable standards, they could only be tentatively identified by comparison to library mass spectra. Possible identities include 9,10-hydroxymethoxy 18:0 dioic acids which could be an artefact due to acid methanolysis of 9,10-epoxy 18:0 dienoic acids [36]. This epoxy acid, together with 16-OH 16:0, has recently been reported in black currant seed cutin [19, 20]. It is possible that the monomers may be present in the extractable lipids.

4 Conclusions

Non-hydroxylated fatty acids were the major components in both black currant seed oil and pomace extracts, and there were only minor differences in the percent compositions up to C_{20} between the two types of extract (Table 1). Total levels and major unsaturated fatty acids including GLA were more abundant in seed oil than pomace extracts (Table 2). This is the first report of the lipophilic components in black currant pomace and it is interesting that it contained long chain

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normal saturated fatty acids (*n*-20:0 – *n*-30:0), phytosterols (major component β-sitosterol), policosanols (*n*-20:0 – *n*-30:0), and 2- and ω-hydroxy fatty acids at levels considerably greater than in the seed alone, indicating that they were derived mainly from the non-seed components of the pomace. The policosanols were present at higher levels than reported for other sources. Over 80% of the mass of the pomace extract was characterised leaving the components in about 20% still to be characterised. Some of these were unidentified hydroxy fatty acids.

The question arises as to whether a useful product could be extracted commercially from pomace that contains seed. As in the seed oil, the pomace extract contains appreciable amounts of the nutritionally important fatty acids GLA and SA but additionally there are other potentially useful components, namely phytosterols and policosanols, although there are also unwanted components such as long-chain saturated fatty acids. However, the waxy nature (possibly due to wax esters of the policosanols and long chain fatty acids) could cause handling problems, and further purification steps and treatment may be required. A hydrolysis step would be required for release of policosanols and phytosterols whereas the fatty acids would probably be more useful as the original triacylglycerols, the form in which they are available as a nutritional supplement. Additionally, GLA is targeted against inflammatory conditions such as atopic eczema whereas cardiovascular disease would be the potential target for policosanols and phytosterols, as they may have cholesterol-lowering activity. Separation of the seed (as a source of GLA and SA) from the pomace (as a source of policosanols and phytosterols) is therefore favourable and current efforts are geared towards further characterisation and exploitation of the seedless pomace.

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References

[1] Clough, P. M., in: Gunstone, F. D. (Ed.), *Structured and Modified Lipids*, Marcel Dekker, New York 2001, pp. 75–117.

[2] Kapasakaldis, P. G., Rastall, R. A., Gordon, M. H., Extraction of polyphenols from processed black currant (*Ribes nigrum* L.) residues. *J. Agric. Food Chem.* 2006, *54*, 4016–4021.

[3] Kapasakaldis, P. G., Rastall, R. A., Gordon, M. H., Effect of a cellulase treatment on extraction of antioxidant phenols from black currant (*Ribes nigrum* L.) pomace. *J. Agric. Food Chem.* 2009, *57*, 4342–4351.

[4] Tahvonen, R., Hietanen, A., Sankelo, T., Korteniemi, V.-M. et al., Black currant seeds as a nutrient source in breakfast cereals produced by extrusion cooking. *Zeitschrift Lebensmitteluntersuchung Forschung A* 1998, *206*, 360–363.

[5] Lengsfeld, C., Deters, A., Faller, G., Hensel, A., High molecular weight polysaccharides from black currant seeds inhibit adhesion of Helicobacter pylori to human gastric mucosa. *Planta Medica* 2004, *70*, 620–626.

[6] Parry, J., Su, L., Moore, J., Cheng, Z. et al., Chemical compositions, antioxidant capacities, and antiproliferative activities of selected fruit seed flours. *J. Agric. Food Chem.* 2006, *54*, 3773–3778.

[7] Bakowska-Barczak, A. M., Scheiber, A., Kolodziejczyk, P., Characterization of Canadian black currant (*Ribes nigrum* L.) seed oils and residues. *J. Agric. Food Chem.* 2009, *57*, 11528–11536.

[8] Ruiz del Castillo, M. L., Dobson, G., Brennan, R., Gordon, S., Genotypic variation in fatty acid content in blackcurrant seeds. *J. Agric. Food Chem.* 2002, *50*, 332–335.

[9] Ruiz del Castillo, M. L., Dobson, G., Brennan, R., Gordon, S., Fatty acid content and juice characteristics in black currant (*Ribes nigrum* L.) genotypes. *J. Agric. Food Chem.* 2004, *52*, 948–952.

[10] Zilberberg, M. D., Levine, C., Komaroff, E., Guldin, M. et al., in: Huang, Y-. S., Ziboh, V. A. (Eds.), *γ-Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications*, AOCS Press, Champaign, USA 2001, Ch. 10, pp. 90–104.

[11] Bakshi, A., Mukherjee, D., Bakshi, A., Banerji, A. K. et al., Gamma-linolenic acid therapy of human gliomas. *Nutrition* 2003, *19*, 305–309.

[12] Marangoni, F., Poli, A., Phytosterols and cardiovascular health. *Pharmacol. Res.* 2010, *61*, 193–199.

[13] Singh, U., Devaraj, S., Jialal, I., Vitamin E, oxidative stress, and inflammation. *Ann. Rev. Nutr.* 2005, *25*, 151–174.

[14] Sen, C. K., Rink, C., Khanna, S., Palm oil-derived natural vitamin E alpha tocotrienol in brain health and disease. *J. Am. College Nutr.* 2010, *29*, 314S–323S.

[15] Sandell, M., Laaksonen, O., Järvinen, R., Rostiala, N. et al., Orosensory profiles and chemical composition of black currant (*Ribes nigrum*) juice and fractions of press residue. *J. Agric. Food Chem.* 2009, *57*, 3718–3728.

[16] Sójka, M., Guyot, S., Kołodziejczyk, K., Król, B. et al., Composition and properties of purified phenolics preparations obtained from an extract of industrial blackcurrant (*Ribes nigrum L.*) pomace. *J. Hort. Sci. Biotechnol.* 2009, ISAFRUIT Special Issue, 100–106.

[17] Hilz, H., Bakx, E. J., Schols, H. A., Voragen, A. G. J., Cell wall polysaccharides in black currants and bilberries – characterisation in berries, juice, and press cake. *Carbohydr. Polym.* 2005 *59*, 477–488.

[18] Helbig, D., Bohm, V., Wagner, A., Schubert, R. et al., Berry seed press residues and their valuable ingredients with special regard to black currant seed press residues. *Food Chem.* 2008, *111*, 1043–1049.

[19] Järvinen, R., Kaimainen, M., Kallio, H., Cutin composition of selected northern berries and seeds. *Food Chem.* 2010, *122*, 137–144.

[20] Järvinen, R., Silvestre, A. J. D., Gil, A. M., Kallio, H., Solid state ^{13}C CP-MAS NMR and FT-IR spectroscopic analysis

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of cuticular fractions of berries and suberized membranes of potato. *J. Food Comp. Anal.* 2011, *24*, 334–345.

[21] Kallio, H., Nieminen, R., Tuomasjukka, S., Hakala, M., Cutin composition of five finnish berries. *J. Agric. Food Chem.* 2006, *54*, 457–462.

[22] Joh, Y.-G., Brechany, E. Y., Christie, W. W., Characterization of wax esters in the roe oil of amber fish, *Seriola aureovittata*. *J. Am. Oil Chem. Soc.* 1995, *72*, 707– 713.

[23] Christie, W. W., *Gas Chromatography and Lipids*, The Oily Press, Dundee, UK 1989, pp. 128–253.

[24] Shepherd, T., Dobson, G., Verrall, S. R., Conner, S. et al., Potato metabolomics by GC-MS: what are the limiting factors? *Metabolomics* 2007, *3*, 475–488.

[25] Zlatanov, M. D., Lipid composition of Bulgarian chokeberry, black currant and rose hip seed oils. *J. Sci. Food Agric.* 1999, *79*, 1620–1624.

[26] Walton, T. J., in: Harwood, J. L., Bowyer, J. R. (Eds.), *Methods in Plant Biochemistry, Lipids, Membranes and Aspects of Photobiology*, Vol. *4*, Academic Press, London 1990, Ch. 5, pp. 105–158.

[27] Kunst, L., Samuels, A. L., Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid Res.* 2003, *42*, 51–80.

[28] Varady, K. A., Wang, Y., Jones, P. J. H., Role of policosanols in the prevention and treatment of cardiovascular disease. *Nutr. Revs.* 2003, *61*, 376–383.

[29] Irmak, S., Dunford, N. T., Milligan, J., Policosanol contents of beeswax, sugar cane and wheat extracts. *Food Chem.* 2006, *95*, 312–318.

[30] Cherif, A. O., Messaouda, M. B., Kaabi, B., Boukhchina, S. et al., Comparison of the concentrations of long-chain alcohols (policosanol) in three Tunisian peanut varieties (*Arachis hypogaea* L.). *J. Agric. Food Chem.* 2010, *58*, 12143–12148.

[31] Harrabi, S., Boukhchina, S., Mayer, P. M., Kallel, H., Policosanol distribution and accumulation in developing corn kernels. *Food Chem.* 2009, *115*, 918–923.

[32] Irmak, S., Dunford, N. T., Policosanol contents and compositions of wheat varieties. *J. Agric. Food Chem.* 2005, *53*, 5583–5586.

[33] Lin, Y., Rudrum, M., van der Wielen, R. P. J., Trautwein, E. A. et al., Wheat germ policosanol failed to lower plasma cholesterol in subjects with normal to mildly elevated cholesterol concentrations. *Metabolism* 2004, *53*, 1309–1314.

[34] Taylor, J. C., Rapport, L., Lockwood, G. B., Octacosanol in human health. *Nutrition* 2003, *19*, 192–195.

[35] Heinz, E., in: Christie, W. W. (Ed.) *Advances in Lipid Methodology – Three*, Oily Press, Dundee, UK 1996, pp. 211–332.

[36] Holloway, P. J., in: Cutler, D. F., Alvin, K. L., Price, C. E. (Eds.), *The Plant Cuticle*, Academic Press, New York 1982, pp. 45–85.

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