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Enhancing the potential exploitation of food waste: Extraction, purification, and characterization of renewable specialty chemicals from blackcurrants (*Ribes nigrum* L.)

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ABSTRACT

Natural colorants were extracted from renewable botanical sources, specifically waste epicarp from the blackcurrant fruit pressing industry. A process was developed which used acidified water extraction followed by a solid-phase extraction (SPE) purification stage which allowed the production of an anthocyanin-rich extract in good yields (*ca.* 2% *w*/*w* based on dry weight of raw material). The components in the extracts were extensively characterized by HPLC, mass spectrometry, IR, NMR and UV-Vis spectroscopy. HPLC confirmed presence of four anthocyanins: delphinidin-3-*O*-rutinoside (45%), cyanidin-3-*O*-rutinoside (31%) and the corresponding glucosides at 16% and 8%, respectively. On sequential liquid-liquid aqueous-organic partitioning of the post-SPE sample, monomeric anthocyanins (54.7%) and polymeric anthocyanins (18%) were found in the aqueous layer with 3-*O*-rutinosides of myricetin (3.1%) and quercetin (3.2%), whilst isopropylacetate achieved selective extraction of caffeic acid (3%), *p*-coumaric acid (5%), and myricetin (2.5%) and quercetin (3.2%) aglycons. 3-*O*-Glucosides of myricetin (3.1%) and quercetin (2%), along with nigrumin-*p*-coumarate (1%) and nigrumin ferulate (0.5%) were selectively extracted from the remaining aqueous fraction using ethylacetate. This allowed for near total quantification of the blackcurrant extract composition.

Keywords: Anthocyanin; polyphenol; fruit waste; dyes; quantification; characterization.

Introduction

The use of renewable materials as sources of interesting and potentially valuable specialty (or effect) chemicals represents a major opportunity on the pathway to a truly sustainable society.[1] Currently, most organic chemicals can be traced back to petrochemical sources, however, the potential for renewable crop-derived products is substantial. Biomass sources of most interest are those that do not compete significantly with food production (and/or the product complements food production) and their carbon footprints are substantially reduced compared to synthetic materials. The potential of the approach is greatly enhanced if the biomass source is an unavoidable waste material, produced on scale as a consistent resource that would otherwise need to be disposed of with negligible return, or indeed, at a cost to the producer.[1] A particularly good example of this is blackcurrant (*Ribes nigrum* L.), which is grown in the UK and used in the manufacture of blackcurrant cordial, most commonly sold under the commercial brand *Ribena*.[2] For this, the berries are pressed and the juice is used to make the cordial, the seeds are also removed and their oils extracted. The residue is a dry pomace consisting mainly of the epicarp (the skin or outermost layer of the fruit) and some small residual twigs from the harvesting process. This represents a substantial volume of a consistent, well-defined food-grade waste material that could potentially be a sustainable source of specialty chemicals.

It is well known that blackcurrants and other berries (*e.g.* strawberries, blackberries, elderberries, black raspberries, chokeberries, blueberries, Concord grapes, black goji berries[3]) are rich sources of colorants and other metabolites, and there is mounting evidence of the potential health benefits of these compounds, with particular focus on anthocyanins.[4-8] Anthocyanins (**1**; **Table 1**) are the largest group of polyphenolic pigments in the plant kingdom. They are non-toxic,[9] water-soluble phenolic compounds responsible for the red, purple and blue coloration of fruits, vegetables and flowers. Their colors are determined by the number of hydroxyl groups (and degree of methylation) and the nature, number and position of sugar moieties including associated aliphatic or aromatic acids attached to the sugar.[10-12] More

than 20 different anthocyanidins (aglycons) have been identified in nature, all based on the flavan nucleus, but the six different aglycons shown in Table 1 are the most common components found in foods, leading to many anthocyanins through diversity of glycosylation.[10-12] Anthocyanins exhibit a remarkable framework of reactions with varying pH. Extensive detailed studies have determined the equilibrium forms[10,13] of the core pyrylium cation, which is vitally important for understanding the physical and chemical properties of anthocyanins.[14,15] In aqueous solution of pH <3, the anthocyanin flavan nucleus exists mainly as the stable flavylium cation (**Table 1**).[10,13] Above this pH more complex equilibria operate, and stability is reduced, so extraction and storage is usually preferred at low pH.

There is a desire to replace synthetic dyes with natural renewable colorants and anthocyanins are widely permitted as natural food/beverage colorants within Europe (E163), Japan, and many other countries.[11,16] In the US, anthocyanin-based colorants are widely used in foods under very specific regulations. Grape extract has been used as a colorant for more than 100 years, first being applied to enhance wine colour.[17] The Code of Federal Regulations[18] allows for the use of two different anthocyanin-based colors from grape: “Grape-color extract” and “Grape skin color extract”. These two extracts are the only anthocyanin-based extracts allowed as food colorants in the US. “Grape-color extract” is obtained as a by-product in processing Concord grapes (*Vitus labrusca* L.), but its application is limited by the FDA to non-beverage food use.

Numerous studies have reported the isolation of anthocyanins, although typically in very small quantities in a highly purified form for characterization, which would be impractical for any commercial application.[10,13,19-21]

Unrefined simple extracts or tinctures, although often colored, contain only low levels of anthocyanins and are of limited use, however approaches for the preparation of extracts containing relatively high levels of anthocyanins for large scale applications have been reported.[22-24]

Certain co-extracted components may also affect the performance of the extract. For example, anthocyanins can undergo co-pigmentation with other components, which significantly affects their stability and light absorption.[25-29] It is therefore particularly important that the full profile of the extract is available, to understand its properties and potentially optimize performance.

Practical sources of anthocyanins are limited by overall economic considerations and availability of suitable raw material, which would not otherwise be suitable for food use. Blackcurrant epicarp is available in substantial, consistent quantities[30] as a potential commercially viable source of anthocyanins for use in areas such as hair[14] and food[15] coloration, depending on regulatory aspects around auxiliaries and processing methods used. This approach provides a potential biodegradable, safe alternative to current coloration methods, from a renewable waste product, using methods designed to minimize environmental impact. Although this paper focuses on blackcurrants, there are many other sources of anthocyanins, all of which have their own characteristic anthocyanin profile, and may have similar potential applications.[31-38]

Materials and methods

Materials

Blackcurrant pomace was obtained from GlaxoSmithKline, UK and more recently from A&R House Ltd., UK. The raw fruit grown in the UK had been pressed in production of blackcurrant cordial (*Ribena*).[2] The crude waste is referred to as pomace, which comprises the fruit epicarp (*ca*. 50 *wt*. %), seeds (ca. 45 *wt*. %) and extraneous matter (*e.g*. berry stalks, *ca*. 5 *wt*. %). Seeds are separated from this pomace and unwanted stalks removed; the subsequent material received was predominantly dried blackcurrant fruit epicarp and used without any further modification. Amberlite XAD7HP was obtained from Rohm & Haas Ltd., Staines, UK. General purpose chemicals were obtained from Sigma-Aldrich. Delphinidin-3-*O*-glucoside was purchased from Polyphenol AS, Sandnes, Norway.

Extraction and semi-purification of polyphenols

Dried blackcurrant epicarp (30 g) was immersed in 600 mL water acidified with 0.01% *v*/*v* conc. HCl and stirred gently by magnetic follower at room temperature for 2 hours. The plant material was filtered off and the resulting aqueous extract loaded onto an Amberlite XAD-7HP resin (60 g) until the eluent was almost colorless. The resin was then washed with acidified water (0.01% *v*/*v* conc. HCl, 1L) before eluting the polyphenols with acidified ethanol (0.01% *v*/*v* conc. HCl). The collected ethanol fractions were combined and concentrated under vacuum on a rotary evaporator, and then subjected to high vacuum to remove trace solvent, yielding a dark violet amorphous solid (660 mg, yield 2.2%), which could be powdered by grinding. ^{1}H NMR (**Table 2** and **SI**) and HPLC (**Figure 1**) analyses confirmed the presence of four anthocyanins and other polyphenols in the extract. The dried blackcurrant extract (500 mg) was then dissolved in acidified water (50 mL, 0.1% *v*/*v* conc. HCl) and partitioned against isopropylacetate (1 × 70 mL) and ethylacetate (3 × 50 mL) in sequential manner. The organic layers were dried under reduced pressure to give isopropylacetate extract (yellow amorphous solid, 68.5 mg) and ethyl acetate extract (yellow amorphous solid, 33 mg), whereas aqueous layer was freeze-dried to afford a red amorphous solid (399 mg).

Analytical HPLC

The extracts were analyzed by HPLC at every stage of the extraction and purification. The analytical HPLC system (Agilent 1290 infinity series) was equipped with diode-array detector (DAD), binary pump system connected with online degasser and Zorbax Eclipse XDB C18, 150 x 4.6 mm, 5 µm. The flow rate was 1ml/min and the injection volume was 10 µl. The chromatograms were recorded by scanning the absorption at 190-600 nm. The anthocyanins were monitored at 520 nm, flavonoids at 350 and hydroxycinnamates at 325 nm. For aqueous extract (anthocyanin analysis), the binary solvent system

consisted of solvent A: water (0.5% TFA) and solvent B: acetonitrile (0.5% TFA). The elution profile consisted of linear gradient from 5% B to 20% B in the first 20 min, then linear increase to 100% B at 20-23 min followed by isocratic elution (100% B) at 23-24 minutes, and then linear decrease to 5% B at 24-25 min followed by 5% B isocratic elution at 25-30 minutes. For ethylacetate and isopropylacetate extracts: the binary solvent system consisted of solvent A: water (0.1% TFA) and solvent B: acetonitrile (0.1% TFA). The elution profile consisted of a linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes.

Preparative HPLC

The aqueous extract after liquid-liquid partitioning experiments was dried and 20 mg was re-dissolved in H_2O/EtOH (9:1, 2 ml, acidified with 0.1% *v/v* HCl). It was then purified on semi-preparative HPLC to give anthocyanins **2-5**. The HPLC system (Agilent 1200 infinity series) was equipped with diode-array detector (DAD), binary pump system connected with online degasser. For anthocyanins: the extract was loaded on to a XBridge™ Prep C18, 10 × 50, 5 µm in 300 µl injections and eluted using gradient solvent system. The binary solvent system consisted of solvent A: water (0.5% TFA) and solvent B: acetonitrile (0.5% TFA). The elution profile consisted of linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes. The flow rate was 5 ml/min and five peaks were collected at 520 nm to give dp-3-rut **4** (4.5 mg), cy-3-rut **5** (4.1 mg), dp-3-glu **2** (1.6 mg) and cy-3-glu **3** (0.8mg) and polymeric anthocyanins (4.5 mg).

For flavonoids and hydroxycinnamates, the isopropylacetate extract (15 mg) and ethylacetate extract (10 mg) were both dissolved in methanol (2 ml) and purified on a semi-preparative column. The peaks were

monitored at 325 for isopropyl acetate and 350 for ethylacetate extracts. The extracts were loaded on to XBridge™ Prep C18, 10 × 50, 5 µm in 300 µl injections and eluted at the flow rate of 5 ml/min using binary solvent system. The binary solvent system consisted of solvent A: water (0.1 % formic acid) and solvent B: acetonitrile (0.1% formic acid). The elution profile consisted of linear gradient from 5% B to 20% B in the first 30 min, then linear increase to 100% B at 30-33 min followed by isocratic elution (100% B) at 33-34 minutes, and then linear decrease to 5% B at 34-35 min followed by 5% B isocratic elution at 35-40 minutes. Caffeic acid **10** (3.3 mg) , *p*-coumaric acid **11** (5.5 mg), myricetin **12** (2.7 mg) and quercetin **13** (3.5 mg) were purified from the isopropylacetate extract whereas glucosides of myricetin **6** (4.7 mg) and quercetin **7** (3.0 mg) alongside nigrumin-*p*-coumarate **14** (1.5 mg) and nigrumin ferulate **15** (0.7 mg) were isolated from the ethylacetate extract (10 mg). Myricetin-3-*β*-rutinoside **8** (0.8 mg) and quercetin-3-*β*-rutinoside **9** (0.8 mg) were isolated from the aqueous extract (20 mg, monitored at 350 nm) also using this method. The isolated compounds were characterised using NMR, IR, UV/Vis spectroscopy and accurate mass spectrometry (See **SI**).

Quantitative HPLC of extracts

The anthocyanins in the post-SPE blackcurrant extract were quantified using calibration graphs (obtained using Agilent Chem Software) for delphinidin-3-*O*-glucoside (Dp3glc) from samples purified in this work and obtained commercially. Delphinidin-3-*O*-glucoside was purchased from Polyphenol AS. The isolated as well as commercial samples of Dp3glc were dissolved in buffer pH 1.0 to give 1 mg/ mL stock solutions and then several dilutions were prepared. UV/Vis absorption spectra were recorded on-line during HPLC analysis using a photodiode array detector and the external calibration graphs were obtained. Using these calibration graphs and Agilent Chem Software the absolute amount of delphinidin-3-*O*-glucoside and the relative amounts of rest of the anthocyanins were calculated. The relative ratios of the anthocyanins given by HPLC chromatograms and ^{1}H NMR were in good agreement. The amounts

of neutral polyphenols is based on their isolated yield. The amounts of individual polyphenols were consistent with the relative peak area of each compound in the ^{1}H NMR of the post-SPE extract (**S2**).

Other methods

High resolution mass spectra (HRMS) were recorded on a Dionex Ultimate 3000 spectrometer using electron spray ionization (ESI). All masses quoted are correct to four decimal places. Agilent Carry Series UV/Vis spectrophotometer was used for uv/vis measurements. Infrared (IR) spectra were recorded using a Perkin Elmer Spectrum One FT-IR spectrophotometer or Bruker Alpha Platinum AR FTIR. Vibrational frequencies are reported in wavenumbers (cm^{-1}).

The NMR experiments were done at 500 and 125 MHz for ^{1}H and ^{13}C respectively on Bruker DRX 500 spectrometer equipped with a multinuclear inverse probe for one-dimensional ^{1}H and two-dimensional heteronuclear single quantum coherence (^{1}H-^{13}C HSQC), heteronuclear multiple bond correlation (^{1}H-^{13}C HMBC), and double quantum filtered correlation (^{1}H-^{1}H COSY). The samples were either dissolved in CD_3OD or CD_3OD-CF_3COOD (95:5) depending on nature of the compound. Chemical shifts (δ) are quoted in ppm downfield of tetramethylsilane or residual solvent peaks (3.31 and 49.0 ppm for CD_3OD in ^{1}H and ^{13}C respectively; 110 and 160 ppm for CF_3COOD). The coupling constants (*J*) are quoted in Hz.

Results and discussion

Extraction and purification of anthocyanins

The main goal of this work was to develop a potentially scalable extraction procedure that gave anthocyanins in a reasonably concentrated form and in the absence of any co-extractants (e.g. free sugars) that may have a deleterious effect on coloration performance. The polar character of anthocyanins affords solubility in polar solvents such as methanol, ethanol, acetone and water.[39] The use of water as an

198 extraction solvent was of particular interest to us, as we wished to keep methods as simple and scalable
199 as possible, and to ensure the extract (as well as the residual material) was free from potentially hazardous
200 solvent residues which may otherwise limit potential commercial applications.[40]

201 It was necessary to carry out initial small scale studies for reference and optimization. Dried blackcurrant
202 epicarp was investigated for extraction efficiency and anthocyanin profile, using an acidic aqueous
203 system. Acidification during extraction was necessary for two main reasons. The primary function was
204 to maintain a low pH (<3.0) in order to ensure stability and structural consistency (hence, consistent
205 chemical and physical properties) of the anthocyanins (**Table 1**). The secondary functions are to disrupt
206 the cell walls and increase accessibility of polyphenolic compounds, and solvent transport, and to inhibit
207 enzymes that may catalyze polyphenol decomposition (e.g. polyphenol oxidase).

208 The optimized procedure required stirring the dried blackcurrant fruit epicarp for 2 h in acidified water
209 (0.01% conc. HCl *v/v*) at ambient temperature (*ca*. 22 °C). HPLC analysis (520 nm) of the fresh extracts
210 confirmed the presence of four anthocyanins, in agreement with literature,[30,41-44] with Dp3rut (**4**; 48%)
211 and Cy3rut (**5**; 33%) being the predominant anthocyanins in the extract. Dp3glc (**2**) and Cy3glc (**3**)
212 constituted about 13% and 6%, respectively, of the total anthocyanins present. The crude aqueous extract
213 was subsequently purified by solid-phase extraction (SPE) in order to remove free sugars and other
214 particularly polar molecules.[45]

215 Initial SPE trials on a small lab scale were conducted using a C-18 reverse phase silica SPE column
216 (Phenomenex Strata-E), as is common in literature.[36,37] However, C-18 reverse phase silica is relatively
217 expensive for practical purification on a large industrial scale, and has very low particle size (50 μm),
218 which would require much higher pressure of flow for loading than potential replacements of larger
219 particle size. Several alternative resins were trialled with consideration of key bulk properties (cost,
220 performance, particle size), resulting in Amberlite XAD-7HP, an aliphatic non-ionic acrylic ester
221 polymer of moderate polarity, being chosen for further extraction studies as it was cost effective and

showed excellent purification performance and high net yield of anthocyanins, compared to other resins trialled. Extraction of anthocyanins from *Aronia melanocarpa* L. using acidified water, followed by purification by solid-phase extraction was reported to remove undesired water-soluble organic and inorganic compounds with minimal reported loss of color (≤5%), a feature which was a priority for us.[45] Hence this was our initial method of choice, although other extraction procedures are also reported.[31-38,41-49]

The SPE step involved loading the aqueous extract onto the polymeric resin (XAD-7HP) and washing it with acidified water (0.01% conc. HCl *v/v*) to remove unwanted sugars, followed by acidified ethanol (0.01% conc. HCl *v/v*) which provided an ethanolic solution rich in phenolics, readily concentrated *in vacuo* to give the blackcurrant extract as a purple amorphous powder (*ca*. 2% yield *w/w*). HPLC analysis (**Figure 1A**) of the fresh post-SPE ethanolic extract showed an anthocyanin profile almost identical to the crude extract. This simple method has been scaled up to >50 kg blackcurrant epicarp, using a conceptually similar procedure.

Quantitative HPLC (Q-HPLC) is the most reliable method for quantification of compounds in a sample.[50] In this case, we used it to determine the quantity of anthocyanins in the extract as a whole, rather than focusing simply on the anthocyanin content alone. Dp3glc isolated in a pure form from our blackcurrant extract (*vide infra*) was used as standard for quantitative HPLC analysis of all anthocyanins, and compared with a commercial sample. This method allows estimation of the other anthocyanins without requiring data on all the anthocyanins present, however it does not take into account the potentially different extinction coefficients for all the anthocyanins in a sample. As seen in **Table 2**, we found that there was a very good agreement between relative ratios of Dp3glc and individual anthocyanin peaks in the HPLC chromatogram and ^{1}H NMR (characteristic peaks at 8.8-9.2 ppm); for example, the relative ratio of Dp3rut and Dp3glc given by HPLC and ^{1}H NMR was 2.8 and 2.7, respectively. The amount of Dp3glc (7.7%) in the extract was calculated using external calibration graphs and Agilent Chem software

(from our isolate as well as a commercial sample) and then the relative ratio of the other anthocyanins used to calculate the amount of Dp3rut (22.6%), Cy3glc (4.0%) and Cy3rut (20.4%). On this basis the total anthocyanin content of the post-SPE blackcurrant extract was estimated to be *ca*. 55%.

Isolation of other polyphenols from the blackcurrant extract.

The presence and ratio of anthocyanins within an extract is often the limit of analysis for many publications. Analysis by HPLC at 520 nm gives deceptively simple chromatograms, typically showing anthocyanin peaks and relatively little else. Initial HPLC (**Figure 1A**) and ^{1}H NMR (**Table 2** and **SI**) analysis of the post-SPE blackcurrant extract indicated the presence of the four main anthocyanins. However, the sample also clearly showed other polyphenolic compounds as evidenced by peaks in the HPLC chromatogram at 350 nm (**Figure 1B**) and additional aromatic peaks at 6-8 ppm in the ^{1}H NMR spectra. It is clear that whilst anthocyanins are present, many other UV active molecules are also present in substantial amounts. Given that our potential applications required a full understanding of the components present, we embarked on an extensive analysis of this partially refined extract.

In order to identify all components and isolate individual samples, further separation was carried out. An acidified aqueous solution of the post-SPE sample was prepared, and partitioned against isopropylacetate, then ethylacetate in a sequential manner to afford three fractions. These fractions were distinct in the composition of their polyphenols, which was expected based on their polarity and solubility in the respective solvents. The highly polar, water soluble anthocyanins (**2-5)** were found in the aqueous layer (**Figure 2A** at 520 nm), alongside My3rut (**8**) and Qu3rut (**9**) (**Figure 2B** at 350 nm). Polymeric anthocyanins (**PA**) were also present in this layer. Isopropylacetate achieved selective extraction of CA (**10**), *p*CA (**11**), My (**12**) and Qu (**13**) (**Figure 2C**). My3glc (**6**), Qu3glc (**7**), NCA (**14**) and NF (**15**) were extracted from the remaining aqueous fraction using ethylacetate (**Figure 2D**).

Hence, these solubility differences allowed the preparation of three distinctly different polyphenol fractions. The first is a highly colored aqueous fraction dominated by anthocyanins alongside rutinoside of neutral polyphenols. The combination of a cationic anthocyanin and a monosaccharide, would appear to confer a similar degree of aqueous solubility to the presence of the rutinoside disaccharide on a neutral polyphenol. The isopropylacetate extract was a relatively non-polar fraction containing neutral polyphenols and phenolic acids as their aglycons, whereas the ethylacetate extract gave an intermediate polarity fraction containing various monosaccharides of neutral polyphenols. Hence, from the single SPE refined extract, three distinct potentially useful fractions can be readily obtained which have significantly different well defined chemical constituents and properties and hence potential applications (*e.g.* as colorants or anti-oxidants).

Characterization of isolated polyphenolic components.

From the extracts prepared, fourteen compounds were isolated using preparative HPLC and characterized using ^{1}H NMR, ^{1}H–^{1}H COSY, HRMS, UV/Vis, IR and ^{13}C, DEPT135, ^{1}H–^{13}C HMBC, and ^{1}H–^{13}C HSQC spectroscopy where possible. Compound **4** was isolated from the aqueous layer and identified to be Dp3rut (**Figure 1**) using ^{1}H NMR spectra best obtained in CD_3OD containing 5% deuterated trifluoroacetic acid.[51] Under such conditions, anthocyanins are in the cationic flavylium form, and the proton in position 4 (**see Figure 1 for numbering**) has a particularly diagnostic chemical shift (8.7–9.2 ppm) for each anthocyanin. NMR data is summarized in **Tables 2** and **3**, and full assignments of all the anthocyanins and flavonoid glucosides are provided (see **SI material**). NMR data for flavonoids My3glc (**6**), My3rut (**8**), Qu3glc (**7**) and Qu3rut (**9**; rutin) alongside their aglycons, My (**12**) and Qu (**13**) is given in **Table 3**, and in the **SI material**. Hydroxycinnamic acids (**10-11**) and esters (**14-15**) were also characterized and compared with the literature[52] when possible (see **SI material**). NCA (**14**) and NF (**15**)

have been previously identified in blackcurrant seeds,[53] and pressed juice,[53,54] but not specifically in epicarp extracts. However the possibility of some carry over during the processing cannot be excluded.

Total quantification of blackcurrant extract composition.

Anthocyanins were quantified using HPLC, whereas the flavonoids and hydroxycinnamic acids were based on their isolated yields which was also reflective of their ^{1}H NMR quantification (relative to anthocyanins) in the extract. The chemical composition of the blackcurrant extract is summarized in detail in **Figure 3** and by chemical class in **Figure 4**. Anthocyanins constituted the largest class of polyphenols in the extract (54.7%) followed by neutral flavonoids (17.1%) and hydroxycinnamates (9.5%). The percentage of individual anthocyanins in this blackcurrant extract was found to be: Dp3rut (22.6%) > Cy3rut (20.4%) > Dp3glc (7.7%) > Cy3glc (4%). Also isolated were polymeric anthocyanins (PA, 18%) which gave broad ^{1}H NMR spectra consistent with the general structure. *p*CA (**11**) was the predominant (5%) neutral polyphenol, and CA (**10**) was also found (3%), whereas the diglycoside, glycosidic and aglycon forms of myricetin and quercetin were found in similar amounts (2-3% each). Nigrumin-*p*-coumarate (1%) and nigrumin ferulate (0.5%) were also present in small amounts.

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Notes

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Supporting Information. Full characterization details for compounds **2** to **15**. Calibration graphs for extinction coefficient calculations. ^{1}H NMR spectra for compounds **2** to **15**.

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453 **Figure Captions**

454

455 **Figure 1.** HPLC chromatograms of post-SPE blackcurrant extract: **(A)** post-SPE blackcurrant extract
456 monitored at 520 nm; **(B)** post SPE-blackcurrant extract monitored at 350 nm. Structures of predominant
457 anthocyanins (**2-5**) isolated from blackcurrant epicarp are shown below and correspond to peak numbers
458 in HPLC chromatograms above.

459

460 **Figure 2.** HPLC chromatograms of all the fractions after sequential solvent-solvent extractions: **(A)**
461 aqueous fraction at 520 nm; **(B)** aqueous fraction at 350 nm; **(C)** isopropylacetate fraction at 325 nm;
462 **(D)** ethylacetate fraction at 350 nm. Structures of neutral polyphenols (**6-15**) isolated from blackcurrant
463 epicarp are shown below and correspond to peak numbers in HPLC chromatograms. For structures of
464 anthocyanins (**2-5**) see Figure 1. **PA** denotes polymeric anthocyanins.

465

466 **Figure 3.** Summary of the chemical composition of the blackcurrant epicarp SPE extract. Abbreviations
467 are as follows: Dp3rut, delphinidin-3-*O*-rutinoside; Dp3glc, delphinidin-3-*O*-glucoside; Cy3rut,
468 cyanidin-3-*O*-rutinoside; Cy3glc, cyanidin-3-*O*-glucoside; PA, polymeric anthocyanins; My3rut,
469 myricetin-3-*O*-rutinoside; My3glc, myricetin-3-*O*-glucoside; My, myricetin; Qu3rut, quercetin-3-*O*-
470 rutinoside; Qu3glc, quercetin-3-*O*-glucoside; Qu, quercetin; p-CA, *p*-coumaric acid; CA, caffeic acid;
471 NCA, nigrumin-*p*-coumarate; NF, nigrumin ferulate.

472

473 **Figure 4.** Chemical composition of blackcurrant epicarp extract by compound class.

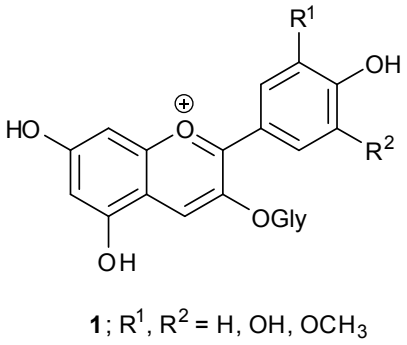
474

475

476 **Tables**

477

478 **Table 1.** Structures and absorption maxima for common anthocyanins.[10,11]



1; R^1, R^2 = H, OH, OCH_3

Anthocyanin	**R^1**	**R^2**	**$\lambda_{max\text{-}vis}$***
pelargonidin	H	H	503
cyanidin	OH	H	517
peonidin	OCH_3	H	517
delphinidin	OH	OH	526
petunidin	OCH_3	OH	526
malvidin	OCH_3	OCH_3	529

479 *$\lambda_{max\text{-}vis}$ values shown are for corresponding 3-*O*-glucoside in water at pH 3.

480 **Table 2.** ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for delphinidin-3-*O*-glucopyranoside (**2**),

481 cyanidin-3-*O*-glucopyranoside (**3**), delphinidin-3-*O*-rutinoside (**4**), and cyanidin-3-*O*-rutinoside (**5**).

No.	**2**		**4**		**3**	**5**	
	δ_H (ppm), *J* (Hz)	**δ_c (ppm)**	**δ_H (ppm), *J* (Hz)**	**δ_c (ppm)**	**δ_H (ppm), *J* (Hz)**	**δ_H (ppm), *J* (Hz)**	**δ_c (ppm)**
2		160.2		164.2			163.0
3		145.9		145.8			144.3
4	8.98 s	136.3	8.90 s	135.4	9.04 s	8.85 s	134.9
4a		115.8		112.8			111.9
5		159.2		159.1			162.0
6	6.66 d (1.5)	103.3	6.68 d (2.0)	103.4	6.69 d (2.0)	6.69 d (1.50)	102.8
7		177.5		170.7			169.1
8	6.88 d (1.5)	95.0	6.88 d (2.0)	95.1	6.91 d (2.0)	6.91 d (1.5)	93.9
8a		158.6		157.6			154.5
1′		117.6		120.0			119.9
2′	7.79 s	116.2	7.78 s	112.7	8.06 d (2.5)	8.05 d (2.5)	117.1
3′		147.6		147.6			146.1
4′		148.8		147.8			148.8
5′		147.6		147.6	7.02 d (8.5)	7.04 d (8.5)	116.1
6′	7.79 s	116.2	7.78 s	112.7	8.27 dd (8.5, 2.5)	8.27 dd (8.5, 2.5)	127.1
Glc							
1″	5.32 d (7.5)	103.7	5.30 d (7.5)	103.3	5.31 d (8.0)	5.29 d (7.5)	102.1
2″	3.72 dd (9.0, 7.5)	74.8	3.71 dd (9.0, 7.5)	74.7	3.71 dd (9.0, 7.0)	3.67 dd (9.0, 7.5)	73.4
3″	3.56 t (9.1)	78.1	3.55 t (9.0)	77.5	3.56 t (9.0)	3.54 t (9.0)	76.7
4″	3.47 dd (9.0, 9.1)	71.1	3.43 t (9.0)	71.2	3.44 t (9.0)	3.42 t (9.0)	69.9
5″	3.54 dd (9.0, 6.0)	78.8	3.73 dd (9.0, 7.2)	78.0	3.55 m	3.72 dd (9.0, 7.0)	76.1
6a″	3.93 dd (12.3, 2.1)	62.3	4.06 dd (11.3, 1.8)	67.8	3.91 dd (12.0, 2.0)	4.06 dd (11.1, 1.5)	
6b″	3.73 dd (12.3, 6.0)	62.3	3.59 dd (11.3, 7.2)	67.8	3.72 dd (12.0, 5.9)	3.59 dd (11.1, 7.0)	

Rha							
1‴			4.65 d (1.5)	102.2		4.65 d (1.5)	100.8
2‴			3.80 dd (3.5, 1.5)	71.9		3.80 dd (3.5, 1.5)	70.5
3‴			3.63 dd (9.5, 3.5)	72.5		3.63 dd (9.3, 3.0)	71.1
4‴			3.33 t (9.0)	73.9		3.33 m	72.6
5‴			3.57 m	69.8		3.57 m	68.4
6‴			1.15 d (6.0)	17.9		1.13 d (6.0)	16.5

482

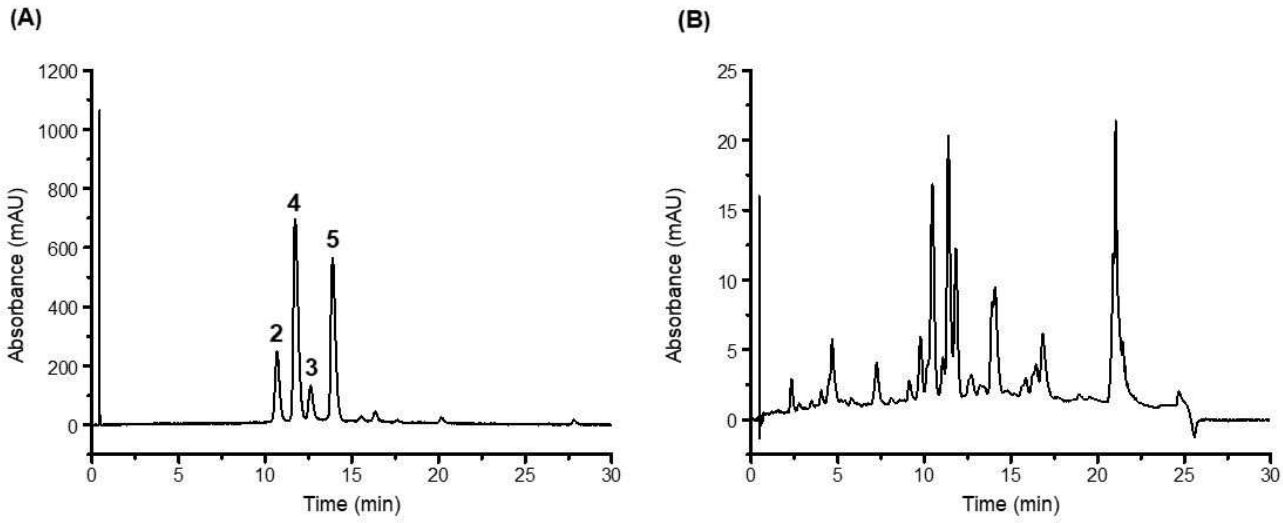
483 **Table 3.** ^{1}H (500 MHz) NMR data for myricetin-3-*O*-glucoside (**6**), myricetin-3-*O*-rutinoside (**8**),

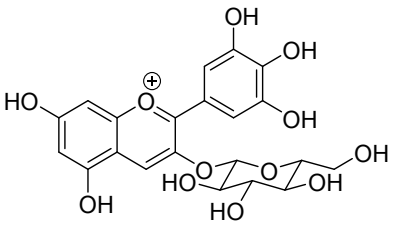
484 quercetin-3-*O*-glucoside (**7**) and quercetin-3-*O*-rutinoside (**9**).

No.	**6**	**8**	**7**	**9**
	δ_H (ppm), *J* (Hz)	**δ_H (ppm), *J* (Hz)**	**δ_H (ppm), *J* (Hz)**	**δ_H (ppm), *J* (Hz)**
6	6.22 d (2.0)	6.22 d (2.5)	6.22 d (2.0)	6.23 d (2.0)
8	6.40 d (2.0)	6.41 d (2.5)	6.41 d (2.0)	6.43 d (2.0)
2′	7.31 s	7.30 s	7.72 d (2.0)	7.68 d (2.2)
5′			6.88 d (8.5)	6.89 d (8.5)
6′	7.31 s	7.30 s	7.59 dd (8.5, 2.0)	7.64 dd (8.5, 2.2)
Glc				
1″	5.23 d (8.0)	5.08 d (8.0)	5.24 d (8.0)	5.11 d (8.0)
2″	3.51 dd (8.9, 8.0)	3.43 m	3.49 dd (9.1, 8.0)	3.46 dd (9.5, 8.0)
3″	3.44 t (8.9)	3.40 t (9.0)	3.43 t (9.1)	3.41 t (9.5)
4″	3.39 t (9.3)	3.29 t (9.1)	3.35 t (9.5)	3.26 d (9.5)
5″	3.24 dd (9.3, 5.0)	3.45 m	3.22 m	3.32 m
6a″	3.73 dd (12.0, 2.3)	3.80 dd (11.5, 1.5)	3.71 dd (12.0, 2.5)	3.80 dd (11.0, 1.5)
6b″	3.62 dd (12.0, 5.0)	3.42 dd (11.5, 5.0)	3.56 dd (12.0, 5.2)	3.39 dd (11.0, 5.5)
Rha				
1‴		4.53 d (1.3)		4.53 d (1.5)
2‴		3.63 dd (3.5, 1.5)		3.63 dd (3.5, 1.5)
3‴		3.55 dd (9.5, 3.5)		3.54 dd (9.5, 3.5)
4‴		3.30 t 9.0		3.28 t (9.5)
5‴		3.50		3.43 m
6‴		1.12		1.13 d (6.5)

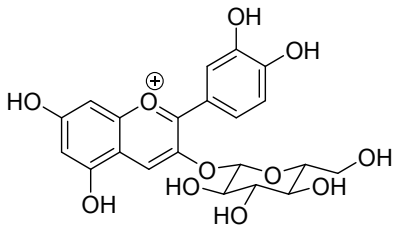
485

Figures

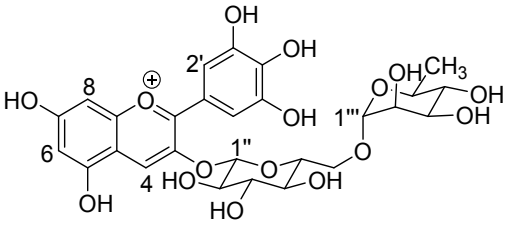




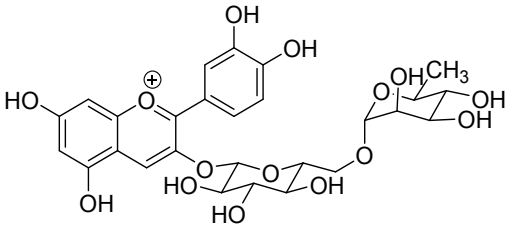
Delphinidin-3-*O*-glucoside (Dp3glc, **2)**



Cyanidin-3-*O*-glucoside (Cy3glc, **3**)

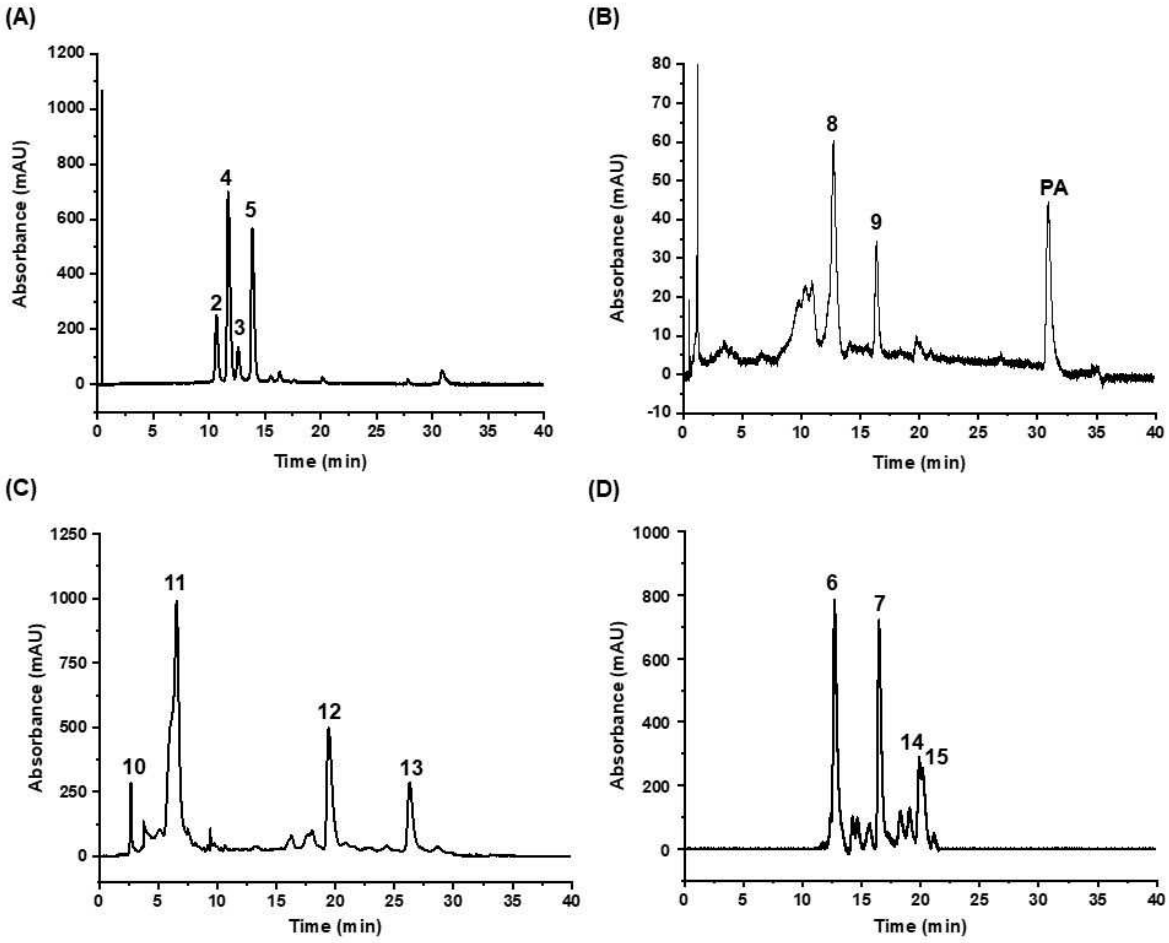


Delphinidin-3-*O*-rutinoside (Dp3rut, **4**)



Cyanidin-3-*O*-rutinoside (Cy3rut, **5**)

Figure 1



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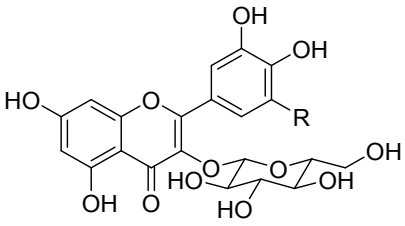
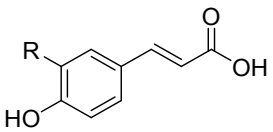
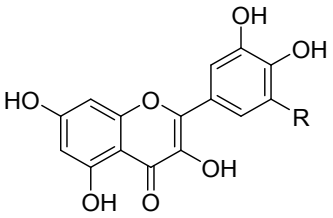


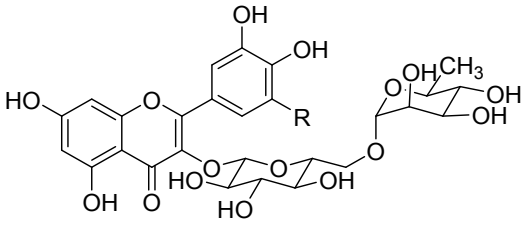
R = OH Myricetin-3-*O*-glucoside, **6**
R = H Quercetin-3-*O*-glucoside, **7**



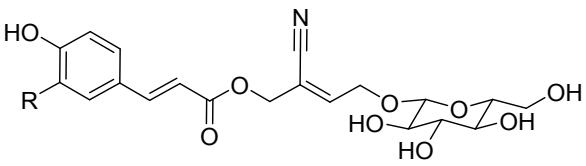
R = OH Caffeic acid, **10**
R = H *p*-Coumaric acid,**11**



R = OH Myricetin, **12**
R = H Quercetin, **13**



R = OH Myricetin-3-*O*-rutinoside, **8**
R = H Quercetin-3-*O*-rutinoside, **9**

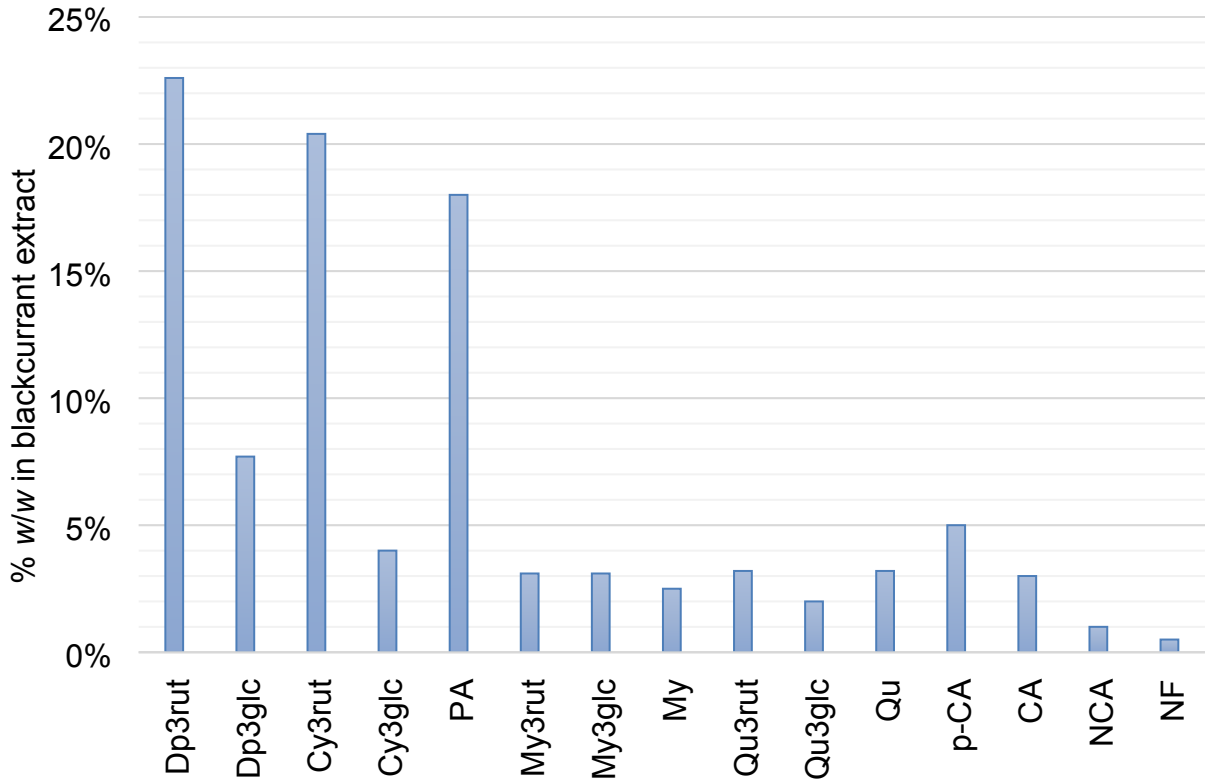


R = H Nigrumin-*p*-coumarate, **14**
R = OMe Nigrumin ferulate **15**

492

493 **Figure 2**

494

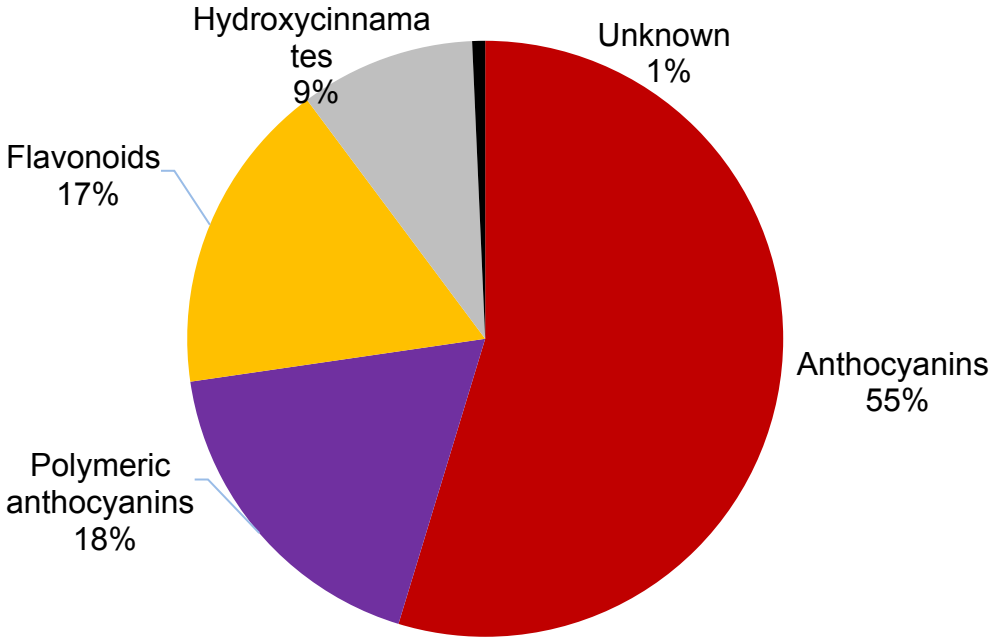


495

496 **Figure 3**

497

498



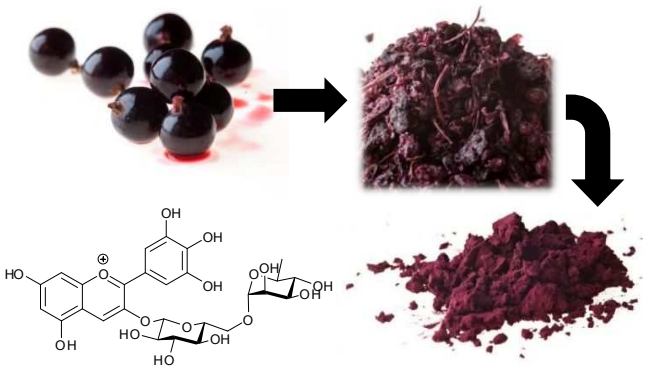
499

500 **Figure 4**

501 **Table of Contents Graphic**

502

503



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