Effect of acidified water on phenolic profile and antioxidant activity of dried blackcurrant (Ribes nigrum L.) pomace extracts

LWT - Food Science and Technology 154 (2022) 112733

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of acidified water on phenolic profile and antioxidant activity of dried blackcurrant (*Ribes nigrum* L.) pomace extracts

Ezzat Mohamad Azman [a,b], Nurfarhana Diana Mohd Nor [c], Dimitris Charalampopoulos [a], Afroditi Chatzifragkou [a,*]

[a] Department of Food and Nutritional Sciences, University of Reading, Whiteknights RG6 6AP, UK
[b] Department of Food Technology, Faculty Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Malaysia
[c] Faculty of Human Development, Sultan Idris Education University, 35900, Tanjong Malim, Perak, Malaysia

ARTICLE INFO

Keywords:
Acidified water
Anthocyanins
Hydroxycinnamic acids
Flavonols
Dried blackcurrant pomace

ABSTRACT

This study investigated the effect of acidified water at different pH values and extraction time on the phenolic profile (anthocyanins, hydroxycinnamic acids and flavonols), antioxidant activity and colour characteristics of dried blackcurrant pomaces (DBP) extracts. Extractions were carried out using acetic acid in water (pH 1.5, 2.0, 2.5 and 3.0) for 2 and 6 h at 30 °C. Phenolics concentration in DBP extracts were influenced by the pH of acidified water ($p < 0.05$), while extraction pH, extraction time and their combination had significant impact on total phenolics, antioxidant activity and colour of DBP extracts ($p < 0.05$). Regardless of the extraction time, very low pH (1.5) was positively associated with high amounts of anthocyanins, hydroxycinnamic acids and flavonols in DPB extracts. Also, extracts obtained at pH 1.5 had the highest amount of total phenolics and antioxidant activity. Overall, acetic acid in water as extraction medium may influence greatly the phenolic profile and colour of DBP extracts, which could be utilised as alternative to synthetic food colourants.

1. Introduction

Blackcurrant (*Ribes nigrum* L.) is the second-largest berry crop in Europe (Laaksonen et al., 2014). United Kingdom is the third-largest blackcurrant producer, with around 11,000–13,000 tonnes of blackcurrants produced per year between 2014 and 2019 (IBA, 2018). Blackcurrant pomace is the main by-product during the juice pressing process, the prominent industrial processing of blackcurrant. Due to its excellent content in phenolic compounds, primarily anthocyanins, blackcurrant pomace can be alternatively utilised as natural ingredient to replace synthetic colourants in food (Sun-Waterhouse et al., 2013; Toscano Martinez et al., 2021) and beverages (Raikos et al., 2019), that can render health-promoting effects in humans (Basegmez et al., 2017; Cyboran et al., 2014). Recent findings on the application of natural colourant from blackcurrant pomaces on fabrics (Tidder et al., 2018), and hair dyes (Rose et al., 2018) have also been reported.

Industrially, blackcurrant pomace is subjected to rotary drying process, where air-on and air-off temperature, the ratio of drum rotor speed and particle size were reported as key parameters to preserve the concentration of thermally sensitive phenolic compounds in moisture content (7.6–8.8%) (Azman et al., 2021). The latter is of importance in order to inhibit browning reactions of enzymatic and non-enzymatic origin and restrain microbial contamination (Michalska et al., 2017; Yang et al., 2013, pp. 332–374).

Due to the polarity and solubility of anthocyanins, polar organic solvents such as ethanol, methanol and acetone have been investigated as extraction medium (Kähkönen et al., 2001; Lapornik et al., 2005). Moreover, acidified aqueous solvents with hydrochloric and formic acid have been employed to disrupt the plant vacuoles and release the phenolic compounds (Amr & Al-Tamimi, 2007; Rodriguez-Soana & Wrolstad, 2001). Examples of extractable phenolic compounds are proanthocyanidins, hydrolysable tannins, hydroxycinnamic acids, and flavonoids such as anthocyanins and flavonols (Durazzo, 2018; Saura-Calixto, 2012). Hot water extraction has gained research attention as a green extraction strategy; for instance, the application of pressurised hot water extraction on the unfermented grape pomace at 100 °C for 5 min efficiently extracted higher anthocyanins than 30 min (Vergara-Salinas et al., 2013). However, lower recovery of anthocyanins from blackcurrant pomace has been reported using hot water compared to organic solvent and acidified water at extractions at lower temperature

* Corresponding author.
E-mail address: a.chatzifragkou@reading.ac.uk (A. Chatzifragkou).

https://doi.org/10.1016/j.lwt.2021.112733
Received 8 June 2021; Received in revised form 27 October 2021; Accepted 28 October 2021
Available online 1 November 2021

(50 °C) (Azman et al., 2020).

According to the mass transfer principles by Spigno et al. (2007), water-containing solvents, especially with high ratio of solvent to water, are efficient for anthocyanins extraction due to their polarity and solubility. As quantified by Azman et al. (2020), 70% (v/v) methanol extracted around 1250 mg/100 g of anthocyanins, 254 mg/100g of hydroxycinnamic acids and 39 mg/100 g of flavonols from dried blackcurrant pomace (DBP), which were linked to high antioxidant activity (up to 45% of DPPH radical scavenging). Even though methanol is claimed as an efficient solvent for anthocyanins extraction, it is less preferred due to its toxicity that might interfere in pharmaceutical and food applications (Oancea et al., 2012). Although numerous studies have reported the extraction of phenolics from blackcurrant by-products using acidified and polar organic solvents, very few are focusing on the profiling of anthocyanins, hydroxycinnamic acids and flavonols in extracts.

Fruit and vegetable generate significant mass losses in the production (post-harvest, processing and distribution) and consumption chains, which accounts for up to 45% of the total product (Fidelis et al., 2019). This is equivalent to 1.3 billion tons of food that is worth around $2.6 trillion, including the social and economic costs. Driven by food safety awareness and development of circular economy, it is necessary to find environmentally friendly solutions to develop and scale up the production of high value-added ingredients from fruit. Therefore, the utilisation of environmentally friendly media to replace organic solvents during extraction of bioactive compounds from inexpensive agricultural by-products has become increasingly popular. Focusing on that, this study aimed at investigating the effect of pH of acidified water and extraction time on the concentration and profile of anthocyanins, hydroxycinnamic acids and flavonols, total phenols, antioxidant activity and colour characteristics of dried blackcurrant pomace (DBP) extracts. In addition, Pearson correlation was also carried out to evaluate the correlations between the obtained total phenolic compounds, total phenols, antioxidant activity and colour characteristics of DBP extracts.

2. Material and methods

2.1. Chemicals

Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetate buffer and iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) were acquired from Sigma-Aldrich (UK). Trolox and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Acros Organics (Geel, Belgium). Purified water obtained from a Purite reserve osmosis system (Oxon, UK). Other chemicals and solvents used were of analytical grade bought from Sigma-Aldrich (UK).

Standards of cyanidin-3-*O*-glucoside (96%), cyanidin-3-*O*-rutinoside (96%), delphinidin-3-*O*-glucoside (95%), delphinidin-3-*O*-rutinoside (95%), kaempferol-3-*O*-glucoside (99%), kaempferol-3-*O*-rutinoside (98%), quercetin-3-*O*-rutinoside (99%) and myricetin-3-*O*-glucoside (99%) were purchased from ExtraSynthese Ltd. (Genay, France). In addition, quercetin (95%), myricetin (98%), kaempferol (99%), caffeic acid (98%), *p*-coumaric (98%), ferulic acid (99%) and quercetin-3-*O*-glucoside (98%) were acquired from Sigma-Aldrich (UK).

2.2. Sample material

Dried samples of pressed blackcurrant pomaces were kindly supplied by A&R House (BCL) Ltd (Bleadon, Weston-super-Mare, UK). Dried blackcurrants were separated from the seeds by grinding in a coffee blender for 30 s and passing through a 0.841 mm (20 mesh) sieve; this constituted the DBP sample (Michalska et al., 2017). Samples were segregated in polyethylene bags and stored at −20 °C until further analysis.

2.3. Preparation of acidified water

Different pH values of acidified water were prepared by adding acetic acid into purified water. Concentrations of 30.0, 7.4, 1.2 and 0.3 mL/100 mL of acetic acid were added to prepare pH 1.5, 2.0, 2.5 and 3.0 of acidified water, respectively. The pH values of the solutions were confirmed using a pH meter (Mettler Toledo Seven Easy, China).

2.4. Preparation of dried blackcurrant pomaces (DBP) extracts

Preliminary studies were carried out to determine the conditions that favour extraction of anthocyanins. The DBP extracts were obtained according to the method previously described by Azman et al. (2020). Phenolic compounds were extracted by adding 25 mL of acidified water (pH 1.5, 2.0, 2.5 or 3.0) or water (control) into 2.5 g of ground DBP. Subsequently, the mixture was shaken in a water bath at 180 rpm for 0.5, 1, 2, 4 and 6 h at 30 °C, respectively. The coloured liquid was vacuum filtered through Whatman No. 1 filter paper (Whatman, Buckinghamshire, UK) using a Buchner funnel to remove any solid residues. Supernatants were then kept at −20 °C for further analysis.

2.5. Determination of total monomeric anthocyanins

In preliminary studies, total monomeric anthocyanins were determined by the pH differential method (Lee et al., 2005). The samples were prepared according to Azman et al. (2020). The results were expressed as mg cyanidin-3-*O*-glucoside equivalents per g of DBP. Each sample was analysed in duplicate.

2.6. HPLC analysis of phenolic compounds

HPLC analysis of phenolic compounds was based on a method by Azman et al. (2021) with slight modifications (in the concentration of formic acid). Briefly, the mobile phase contained formic acid at 2% (v/v) (Solvent A) and 100% (v/v) methanol (solvent B) and the gradient elution system was as follows: 15% (B) at 0 min, 35% (B) at 15 min, 60% (B) at; 30 min and end at 80% (B) at 40 min. A Purospher STAR RP18 end-capped column (250 mm × 4.6 mm i.d., particle size of 5 μm, Merck, Darmstadt, Germany) in a Waters 2695 Alliance HPLC system (Waters Corp., Milford, MA, USA), equipped with a Waters 2478 two-channel UV detector, two Waters 515 HPLC pumps, an auto-sampler, a column oven and an online degasser. The flow rate was set at 1.0 mL/min, with the temperature of the column was 30 °C. The injection volume was fixed at 20 μL and the period of analysis was 45 min. Three different wavelengths of 520, 320 and 360 nm were used to detect the anthocyanins, hydroxycinnamic acids and flavonols, respectively.

Quantification of phenolics was carried out by preparing calibration curves of external standards with the following concentrations range: 10–100 mg/L (anthocyanins) and 1–25 mg/L (hydroxycinnamic acids or flavonols). Table 1 shows the determination coefficient (R^2), the limit of detection (LOD) and limit of quantification (LOQ), which have been calculated as:

$$LOD = \frac{3S_a}{b} \text{ and } LOQ = \frac{10S_a}{b} \quad (1)$$

where *Sa* is the standard deviation of the response and *b* is the slope of the calibration curve (Shrivastava & Gupta, 2011).

2.7. Determination of total phenolics

Total phenolics were determined by the Folin-Ciocalteu method (Kupina et al., 2018) with slight modifications. The samples for total phenolics measurement were prepared according to Azman et al. (2020). Gallic acid (100 mg/L) was dissolved in water and sonicated in an ultrasonic bath and was used as a stock standard solution for the

Table 1
Phenolic compounds quantified by HPLC with determination coefficient (R^2) and limits of detection (LOD) and quantification (LOQ).

Phenolic compounds	R^2	LOD (mg/L)	LOQ (mg/L)
Anthocyanins			
D3G	0.9988	2.34	7.09
D3R	0.9979	3.01	9.13
C3G	0.9985	2.58	7.81
C3R	0.9938	5.23	15.84
Hydroxycinnamic acids			
CAFF	0.9990	0.50	1.51
COU	0.9919	1.44	4.36
FER	0.9991	0.49	1.49
Flavonols			
M3G	0.9997	0.19	0.57
Q3G	0.9994	0.26	0.79
Q3R	0.9998	0.15	0.45
MY	0.9991	0.31	0.94
K3G	0.9998	0.16	0.47
K3R	1.0000	0.05	0.15
QUER	0.9998	0.14	0.42
KA	0.9991	0.31	0.95

*D3G: Delphinidin-3-*O*-glucoside; D3R: Delphinidin-3-*O*-rutinoside; C3G: Cyanidin-3-*O*-glucoside; C3R: Cyanidin-3-*O*-rutinoside; COU: *p*-Coumaric acid; CAFF: Caffeic acid; FER: Ferulic acid; M3G: Myricetin-3-*O*-glucoside; Q3G: Quercetin-3-*O*-glucoside; Q3R: Quercetin-3-*O*-rutinoside; MY: Myricetin; K3G: Kaempferol-3-*O*-glucoside; K3R: Kaempferol-3-*O*-rutinoside; QUER: Quercetin; KA: Kaempferol.

calibration curve (0–100 mg/L) (Kupina et al., 2018). The results were expressed as milligrams of gallic acid equivalents per 100 g of dried weight of extracts (mg GAE/100 g of DBP). Duplicate measurements were taken, and mean values were calculated.

2.8. Determination of DPPH radical scavenging activity

Determination of the antioxidant activity using the DPPH radical scavenging activity and the sample preparations of DBP extracts were carried out according to Azman et al. (2020). A stock solution of 2 mM DPPH was prepared in methanol. The antioxidant activity was expressed as the percentage of DPPH scavenging. Duplicate measurements were taken, and mean values were calculated.

2.9. Determination of ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) was analysed according to Benzie and Strain (1996) with some modifications, whereby the incubation time was increased to 30 min and the extracts were diluted prior to mixing with FRAP reagent. The FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L of TPTZ and 20 mmol/L $FeCl_3{\cdot}6H_2O$ with a ratio of 10:1:1 at 37 °C. A total of 100 μL of 15-fold diluted extracts or Trolox solution (0–2000 μmol/L) were mixed with 300 μL of distilled water and 3 mL of freshly prepared FRAP reagent. Then, the mixture was vortexed for 10 s and the absorbance was measured at 593 nm after 30 min of incubation in the dark. Duplicate measurements were conducted, and antioxidant power was expressed as μmol of Trolox equivalents per gram of the sample (μmol TE/g).

2.10. Colour measurement

The colour of the extracts was determined using a Hunter-Lab colourimeter (Hunter Lab, ColorQuest, Hunter Associates Laboratory, Reston, VA, USA) based on three colour coordinates, L^*, a^*, and b^* at D65 standard illuminant and 10° standard observer angle. The instrument was calibrated using a black card. The colour was measured by the Hunter Lab units, L^* (Lightness/darkness; 0 to 100), a^* (positive = redness/negative = greenness), and b^* (positive = yellowness/negative = blueness). Total colour difference (ΔE) of each sample was calculated according to the following formula:

$$\Delta E = [(L^*\text{-}L_o)^2 + (a^*\text{-}a_o)^2 + (b^*\text{-}b_o)^2]^{1/2} \quad (2)$$

where L_o, a_o, b_o = blank value of each sample extracted at 0.5 h.

Chroma (C) is the quantitative attribute of colour intensity, and hue (h°) gives a qualitative attribute of colours which are defined as reddish, greenish, yellowish, and bluish. The chroma and hue angle can be calculated using the a^* and b^* values according to the equations below (Wrolstad & Smith, 2017):

$$(C) = [(a^*)^2 + (b^*)^2]^{1/2} \quad (3)$$

$$(h^\circ) = \text{ArcTan}\ (b^*/a^*) \quad (4)$$

2.11. Statistical analysis

Minitab V.19 (Minitab Inc., State College, Pennsylvania, USA) was used for data analysis. The data obtained were checked for the normality (Anderson-Darling test) and homoscedasticity (Levene's test) before analysis. The significance of main independent variables (pH of acidified water and extraction time) and their interactions were determined using general linear model (GLM) procedure. The differences between extracts were further analysed using Tukey's pairwise comparison in analysis of variance (ANOVA) at 0.05 confidence interval. In addition, Pearson correlation was used to measure the correlation between concentrations and profile of anthocyanins, hydroxycinnamic acids and flavonols, total phenolics, antioxidant activity and colour characteristics of DBP extracts.

3. Results and discussion

According to the normality test, non-normal distributions ($p < 0.05$) were detected for the anthocyanins, hydroxycinnamic acids, flavonols, total phenolics, antioxidant activity and colour characteristics results. However, the objective of this study was to investigate the significance of main independent variables (pH of acidified water and extraction time) and their interactions, therefore parametric tests such as ANOVA were carried out. Pearson (1931) found that ANOVA was robust for highly skewed non-normal distributions and sample sizes of 4, 5 and 10. This is supported by Norman (2010), who claimed that parametric statistics can be used for small sample size (greater than 5) with unequal variances and non-normal distributions and will yield nearly correct answers as asymmetric distributions like exponentials.

3.1. Anthocyanins

According to the results of preliminary studies (Supplementary Table 1), no significant difference was observed in the extraction of total monomeric anthocyanins within the first 6 h in all acidified water extracts (pH 1.5–3.0). However, anthocyanins concentration in water extracts (control) started to increase at 2 and 6 h, thus were chosen to be investigated as extraction time. Many studies have reported that anthocyanins exist in abundance in blackcurrant pomaces (Basegmez et al., 2017; Michalska et al., 2017). According to HPLC analysis in this study, delphinidin-3-*O*-rutinoside appeared as the predominant anthocyanin in DBP extracts (delphinidin-3-*O*-rutinoside; ~52.8% > cyanidin-3-*O*-rutinoside; ~33.6% > delphinidin-3-*O*-glucoside; ~19.4% > cyanidin-3-*O*-glucoside; ~8.4%) (Table 2). This finding is in agreement with previous data reported by Paunović et al. (2017) in the extraction of blackcurrant fruits using 96% ethanol (v/v) in an ultrasonic bath for 1 h. The main factor that influences the anthocyanin composition is the blackcurrant cultivars, as confirmed by Paunović et al. (2017).

Table 2
Anthocyanin concentrations in DBP extracts (mg/100 mg of DBP) using different pH of acidified water for 2 and 6 h as quantified using HPLC.

Acidified water -extraction time (h)	Anthocyanins (mg/100 g)				
	D3G	D3R	C3G	C3R	Total
Control - 2	23.1 (1.2) ^{d}	53.6 (1.9) ^{e}	7.9 (0.5) ^{d}	35.5 (2.3) ^{e}	120 (5.9) ^{f}
Control - 6	24.2 (0.3) ^{d}	54.6 (0.6) ^{e}	9.5 (0.1) ^{cd}	31.7 (0.5) ^{e}	119 (1.5) ^{f}
pH 1.5–2	251 (2.9) ^{a}	629 (8.7) ^{a}	114 (1.5) ^{a}	373 (5.3) ^{a}	1368 (18.4) ^{a}
pH 1.5–6	239 (6.2) ^{a}	604 (14.7) ^{a}	110 (2.5) ^{a}	356 (7.2) ^{a}	1310 (30.7) ^{a}
pH 2.0–2	183 (15.4) ^{b}	453 (1.0) ^{b}	72.9 (8.8) ^{b}	273 (29.1) ^{b}	983 (50.1) ^{b}
pH 2.0–6	197 (5.1) ^{b}	454 (39.7) ^{b}	79.8 (1.6) ^{b}	286 (2.0) ^{b}	1017 (31.1) ^{b}
pH 2.5–2	48.5 (3.4) ^{c}	194 (14.0) ^{c}	21.0 (1.1) ^{c}	123 (8.8) ^{c}	387 (27.2) ^{c}
pH 2.5–6	43.4 (0.7) ^{cd}	174 (1.9) ^{cd}	18.8 (0.3) ^{cd}	110 (0.8) ^{cd}	346 (3.7) ^{cd}
pH 3.0–2	22.4 (0.6) ^{d}	128 (2.3) ^{d}	10.9 (0.5) ^{cd}	81.2 (1.2) ^{d}	242 (4.5) ^{e}
pH 3.0–6	24.9 (0.1) ^{d}	142 (0.1) ^{cd}	13.1 (0.1) ^{cd}	91.4 (0.2) ^{cd}	272 (0.1) ^{de}

Control: water; D3G: Delphinidin-3-*O*-glucoside; D3R: Delphinidin-3-*O*-rutinoside; C3G: Cyanidin-3-*O*-glucoside; C3R: Cyanidin-3-*O*-rutinoside. Figures in parentheses indicate the standard deviation (n = 2). Values with the same letter $^{a, b}$ in each row are not significantly different ($p > 0.05$).

Worth mentioning is that the pH of extracts upon extraction completion at pH 1.5, 2.0, 2.5 and 3.0 of acidified water were slightly increased to 1.92, 2.38, 2.82 and 3.16, respectively, while the pH of water extract (control) decreased to 3.16, indicating that the pH of acidified water was more stable compared to water. In previous studies, it has been shown that the presence of organic acids such as citric and malic acid (Milivojević et al., 2009) in DBP increased the concentration of hydrogen ions (acidity), thus decreased the pH of water. Statistical analysis verified that only the pH of acidified water influenced the anthocyanins concentration ($p < 0.05$), while extraction time and their interaction had no impact ($p > 0.05$). Based on the HPLC results, total anthocyanins concentration was highest ($p < 0.05$) at pH 1.5, up to 1368 mg/100 g pH 1.5 extract had approximately 1.3-, 3.5-, 5.0- and 11.4-fold higher levels of anthocyanins compared to pH 2.0, 2.5, 3.0, and water, respectively. This is similar to results reported by Lapornik et al. (2005), where ~1360 mg/100 g of anthocyanins were extracted from 70% methanol for 24 h at room temperature. Compared to our previous study (Azman et al., 2020), extraction of DBP using pH 1.5 acidified water at 30 °C for 2 h produced higher concentrations of anthocyanins compared to 50% methanol (~1135 mg/100 g), 70% methanol (~1249 mg/100 g) and 100% methanol (~1164 mg/100 g). In contrast, higher extraction temperatures (50 °C) seemed to favour more the extraction of anthocyanins from DBP (Azman et al., 2020).

According to Fernandes et al. (2017), anthocyanins are located in the vacuoles encapsulated by the skin cell wall. The combination of acid and water facilitates the disruption of the cell wall membrane and release of water-soluble pigments, including anthocyanins, as suggested by Amr and Al-Tamimi (2007). As the pH of acidified water increased, the percentage yield of anthocyanins glucosides such as delphinidin-3-*O*--glucoside (~19.4%) and cyanidin-3-*O*-glucoside (~8.4%) were decreased to ~9.1% and ~4.5%, respectively. On the other hand, increasing trends were observed in the percentage yield of anthocyanin rutinosides such as delphinidin-3-*O*-rutinoside (~44.6%–~52.8%) and cyanidin-3-*O*-rutinoside (~27.2%–~33.6%) as the extraction pH decreased. This suggested that the application of stronger acid is required to extract the anthocyanin glucosides from the vacuole in the cell membrane, compared to anthocyanin rutinosides. In addition, the concentration of cyanidin-3-*O*-glucoside was the lowest detected in all DBP extracts. According to Azman et al. (2020), cyanidin-3-*O*-glucoside may still be bound to the vacuole in cell wall membrane and requires acid hydrolysis at high temperatures (90 °C) to be released from DBP residues.

3.2. Hydroxycinnamic acids

Other than anthocyanins, hydroxycinnamic acids are another type of phenolic compounds that also can be recovered from the DBP cell wall. Caffeic acid, *p*-coumaric and ferulic acid are among the common hydroxycinnamic acids found in blackcurrant (Hui et al., 2021). In this study, similar to anthocyanins, the pH of acidified water appeared as a significant parameter ($p < 0.05$) while extraction time had no effect ($p > 0.05$) on the yield of *p*-coumaric, caffeic and ferulic acids. The interaction between these two parameters was only significant ($p \leq 0.05$) on the recovery *p*-coumaric and total hydroxycinnamic acids. Based on HPLC analysis, *p*-coumaric acid was the dominant hydroxycinnamic acid (~59.8%) in all extracts, followed by caffeic acid (~40.3%), and ferulic acid (~18.7%) was the lowest (Table 3). The compositions of hydroxycinnamic acids obtained are similar to the findings by Azman et al. (2021) in the extraction of DBP using 1% (v/v) HCl in methanol 30 °C for 24 h. As claimed by Clifford (2000), these three aforementioned hydroxycinnamic acids act as commoner acylating agents that bear anthocyanin glycosidic sugars in extracts.

Furthermore, there was no significant difference in the total hydroxycinnamic acids between pH 1.5 and 2.0 extracts. pH 1.5 and 2.0 demonstrated approximately 1.6-, 2.6- and 3.1-fold higher total hydroxycinnamic acid than pH 2.5, pH 3.0 and water, respectively. Highest *p*-coumaric acid (~126.6 mg/100 g) and ferulic acid (~32.5 mg/100 g) concentrations were detected at pH 1.5 ($p < 0.05$). On the other hand, pH 2.0 was found to be the richest in caffeic acid (~84.5 mg/100 g) ($p < 0.05$). However, no trend was observed in the concentrations of caffeic, *p*-coumaric and ferulic acid of the extracts.

3.3. Flavonols

Other than anthocyanins, flavonols are also another subgroup of flavonoids commonly found in the plant vacuoles. According to Panche et al. (2016), the most commonly studied flavonols are quercetin,

Table 3
Hydroxycinnamic acid concentrations in DBP extracts (mg/100 g of DBP) using different pH of acidified water for 2 and 6 h as quantified using HPLC.

Acidified water -extraction time (h)	Hydroxycinnamic acids (mg/100 g)			
	CAFF	COU	FER	Total
Control - 2	26.1 (1.3) ^{e}	29.3 (1.1) ^{d}	12.2 (1.2) ^{e}	67.6 (3.5) ^{e}
Control - 6	27.2 (1.3) ^{e}	30.3 (1.6) ^{d}	13.3 (1.4) ^{d}	70.8 (4.2) ^{de}
pH 1.5–2	55.1 (0.5) ^{b}	120 (1.5) ^{a}	30.4 (0.3) ^{a}	206 (2.3) ^{b}
pH 1.5–6	52.5 (0.4) ^{bc}	126 (1.2) ^{a}	32.5 (0.1) ^{a}	211 (1.7) ^{ab}
pH 2.0–2	84.0 (0.7) ^{a}	101 (0.1) ^{b}	22.5 (0.1) ^{b}	208 (0.8) ^{ab}
pH 2.0–6	84.5 (5.2) ^{a}	110 (5.1) ^{b}	22.5 (0.1) ^{b}	217 (0.1) ^{a}
pH 2.5–2	45.8 (1.8) ^{cd}	73.2 (3.9) ^{c}	18.5 (0.3) ^{c}	137 (6.0) ^{c}
pH 2.5–6	42.9 (0.1) ^{d}	69.6 (0.1) ^{c}	18.2 (0.1) ^{c}	130 (0.2) ^{c}
pH 3.0–2	31.5 (0.5) ^{e}	35.9 (1.8) ^{d}	14.8 (0.3) ^{de}	82.2 (2.5) ^{de}
pH 3.0–6	31.3 (1.5) ^{e}	34.5 (0.8) ^{d}	15.1 (0.9) ^{d}	80.9 (1.6) ^{d}

Control: water; COU: *p*-Coumaric acid; CAFF: Caffeic acid; FER: Ferulic acid. Figures in parentheses indicate the standard deviation (n = 2). Values with the same letter $^{a, b}$ in each row are not significantly different ($p > 0.05$).

myricetin, kaempferol and fisetin. Other than the first three mentioned flavonols, myricetin-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside are among the flavonols discovered in blackcurrant juices (Laaksonen et al., 2013, 2014) and pomaces (Azman et al., 2021; Kapasakalidis et al., 2006).

Similar to anthocyanins and hydroxycinnamic acids, only the pH of acidified water impacted the flavonols concentration ($p < 0.05$), while extraction time and interaction between these two parameters had no influence ($p > 0.05$). As shown in Table 4, pH 1.5 had higher ($p < 0.05$) values of total flavonols with 1.4-, 2.6-, 2.9- and 3.4-fold higher than those extracted at pH 2.0, 2.5, 3.0 and water, respectively. The total concentration of flavonols decreased significantly ($p < 0.05$) as the pH increased from pH 1.5 (~47.0 mg/100 g) to pH 3.0 (~13.8 mg/100 g). HPLC profiling indicated that myricetin-3-*O*-glucoside (~46.8%) appeared as the predominant flavonol, followed by quercetin-3-*O*-glucoside (~30.5%). The rest of flavonols found in DBP extracts were as follows: quercetin-3-*O*-rutinoside (~23.5%) > kaempferol-3-*O*-rutinoside (~5.3%) > kaempferol-3-*O*-glucoside (~3.8%). Notably, myricetin (~19.6%) and quercetin (~9.6%) could only be quantified when using the strongest acidified water (pH 1.5). However, none of the acidified water used could extract kaempferol. These finding are in agreement with Azman et al. (2020), whereas these three types of flavonols can only be obtained when using an organic solvent or strong acid (pH 1.5) especially at higher extraction temperature (50 °C).

Also, similar to anthocyanin glucosides, a decreasing trend of flavonol glucosides such as myricetin-3-*O*-glucoside (~46.8%–~42.5%), quercetin-3-*O*-glucoside (~30.5%–~25.2%) and kaempferol-3-*O*-glucoside (~3.8%–~3.0%) were observed as the pH of acidified water increased from pH 1.5 to 3.0. In contrast, increase of the pH in DBP extracts resulted in the increasing trend of quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, ~15.9%–~23.7% and ~3.4%–~5.3%, respectively.

3.4. Total phenolics and antioxidant activity

Determination of total phenolics, scavenging activity and reducing power were carried out using Folin-Ciocalteu method and DPPH and FRAP assays, respectively. DPPH and FRAP assays efficiently assessed the antioxidant activity in DBP extract and strongly correlated to each other with $R = 0.927$ ($p < 0.05$). In this study, pH of acidified water, extraction time and their interaction significantly ($p < 0.05$) influenced total phenolics, scavenging activity and reducing power in DBP extracts. Extremely strong pH and longer time were more efficient towards extracting total phenolics that also correlated to high antioxidant activity of the obtained extracts. A significantly ($p < 0.05$) higher total phenolics (2099.4 ± 42.0 mg GAE/100 g), scavenging activity (48.2 ± 0.1% of DPPH inhibition) and reducing power (174.7 μmol TE/g) were detected in pH 1.5 extract after 6 h of extraction (Table 5). This was also proved by the Pearson Correlation that indicated a strong correlation ($p < 0.05$) between total phenolics and scavenging activity ($r = 0.996$), and reducing power ($r = 0.919$), respectively. Known as excellent antioxidanta, phenolic compounds, and particularly anthocyanins can prevent the radical formation in cells by donating hydrogen to highly reactive radicals.

All phenolics including total anthocyanins ($r = 0.994$), total hydroxycinnamic acids ($r = 0.924$) and total flavonols ($r = 0.990$) indicated strong correlation ($p < 0.05$) with total phenolics. Total anthocyanins exhibited the highest correlation ($p < 0.05$) with scavenging activity and reducing power ($r = 0.993$ and $r = 0.929$, resp.), followed by total flavonols ($r = 0.989$ and $r = 0.892$, respectively); total hydroxycinnamic acids exhibited the weakest correlation ($r = 0.918$ and $r = 0.831$, respectively). This suggested that anthocyanins were the main contributor to the antioxidant activity in DBP extracts. Viljanen et al. (2005) claimed that higher content of delphinidin and cyanidin glycosides and their beneficial combination act as potent antioxidants toward lipid and protein oxidation. Apart from anthocyanins, hydroxycinnamic acids and flavonols, it can be assumed that strong acid also extracted proanthocyanins such as procyanidins and prodelphinidins, which were not identified or quantified in this study. However, these proanthocyanidins are not strongly reactive with Folin and DPPH reagents (Wu et al., 2004). Some other extracted compounds from DBP such as organic acids, reducing sugars, proteins and vitamins, can also significantly react with Folin and antioxidant assays (Everette et al., 2010).

Table 5
Total phenolics, scavenging activity and reducing power of DBP extracts extracted using different pH of acidified water for 2 and 6 h.

Acidified water -extraction time (h)	Total phenolics (mg GAE/100 g)	Scavenging activity (% DPPH scavenging)	Reducing power (μmol TE/g)
Control - 2	208 (2.1) ^{f}	13.3 (0.1) ^{h}	58.1 (0.0) ^{e}
Control - 6	223 (2.8) ^{ef}	15.1 (0.3) ^{h}	63.1 (1.2) ^{e}
pH 1.5–2	1997 (73.6) ^{a}	45.2 (0.4) ^{b}	154 (4.0) ^{a}
pH 1.5–6	2094 (42.0) ^{a}	48.2 (0.1) ^{a}	174 (3.3) ^{b}
pH 2.0–2	1271 (154.2) ^{c}	32.4 (0.7) ^{d}	83.7 (1.3) ^{c}
pH 2.0–6	1595 (8.5) ^{b}	38.4 (1.1) ^{c}	92.6 (2.1) ^{d}
pH 2.5–2	474 (98.1) ^{de}	20.4 (0.4) ^{e}	59.4 (0.1) ^{e}
pH 2.5–6	527 (4.8) ^{d}	18.5 (0.1) ^{ef}	65.0 (0.9) ^{e}
pH 3.0–2	308 (31.5) ^{def}	15.9 (0.3) ^{gh}	58.1 (0.0) ^{e}
pH 3.0–6	315 (20.7) ^{def}	17.9 (0.8) ^{fg}	63.1 (2.0) ^{e}

Control: water; TE: Trolox equivalents. Figures in parentheses indicate the standard deviation ($n = 2$). Values with the same letter $^{a, b}$ in each row are not significantly different ($p > 0.05$).

Table 4
Flavonol concentrations in DBP extracts (mg/100 g of DBP) using different pH of acidified water for 2 and 6 h as quantified using HPLC.

Acidified water -extraction time (h)	Flavonols (mg/100 g)								
	M3G	Q3G	Q3R	MY	K3G	K3R	QUER	KA	Total
Control - 2	5.8 (0.2) ^{d}	3.5 (0.1) ^{e}	3.3 (0.2) ^{f}	ND	0.4 (0.1) ^{f}	0.7 (0.1) ^{c}	ND	ND	13.8 (0.7) ^{d}
Control - 6	6.1 (0.1) ^{d}	3.6 (0.2) ^{e}	3.4 (0.2) ^{f}	ND	0.4 (0.1) ^{ef}	0.8 (0.1) ^{c}	ND	ND	14.3 (0.1) ^{d}
pH 1.5–2	10.4 (0.1) ^{b}	13.1 (0.1) ^{a}	6.5 (0.1) ^{a}	8.6 (0.1) ^{b}	1.5 (0.0) ^{ab}	1.4 (0.0) ^{a}	4.3 (0.1) ^{b}	<LOQ	45.8 (0.5) ^{a}
pH 1.5–6	10.9 (0.2) ^{b}	13.6 (0.1) ^{a}	6.1 (0.1) ^{ab}	9.2 (0.2) ^{a}	1.5 (0.0) ^{ab}	1.2 (0.0) ^{b}	4.5 (0.2) ^{a}	<LOQ	47.0 (0.1) ^{a}
pH 2.0–2	16.0 (1.3) ^{a}	10.3 (0.7) ^{b}	5.4 (0.3) ^{c}	<LOQ	1.3 (0.1) ^{c}	1.1 (0.1) ^{b}	ND	ND	34.2 (2.4) ^{b}
pH 2.0–6	16.4 (0.1) ^{a}	10.7 (0.1) ^{b}	5.6 (0.0) ^{bc}	<LOQ	1.3 (0.0) ^{bc}	1.2 (0.0) ^{b}	ND	ND	35.2 (0.1) ^{b}
pH 2.5–2	8.3 (0.1) ^{c}	5.2 (0.3) ^{c}	4.1 (0.1) ^{d}	<LOQ	0.7 (0.0) ^{d}	0.8 (0.0) ^{c}	ND	ND	19.0 (0.4) ^{c}
pH 2.5–6	7.8 (0.1) ^{c}	4.7 (0.0) ^{cd}	4.0 (0.0) ^{de}	<LOQ	0.6 (0.0) ^{d}	0.8 (0.0) ^{c}	ND	ND	17.9 (0.0) ^{c}
pH 3.0–2	7.4 (0.1) ^{cd}	4.2 (0.0) ^{e}	3.5 (0.0) ^{ef}	ND	0.6 (0.0) ^{d}	0.8 (0.0) ^{c}	ND	ND	16.4 (0.1) ^{cd}
pH 3.0–6	7.3 (0.1) ^{cd}	4.2 (0.1) ^{e}	3.5 (0.0) ^{ef}	ND	0.6 (0.0) ^{de}	0.8 (0.0) ^{c}	ND	ND	16.4 (0.1) ^{cd}

Control: water; M3G: Myricetin-3-*O*-glucoside; Q3G: Quercetin-3-*O*-glucoside; Q3R: Quercetin-3-*O*-rutinoside; MY: Myricetin; K3G: Kaempferol-3-*O*-glucoside; K3R: Kaempferol-3-*O*-rutinoside; QUER: Quercetin; KA: Kaempferol; <LOQ: Below limit of quantification; ND: Not detected. Figures in parentheses indicate the standard deviation ($n = 2$). Values with the same letter $^{a, b}$ in each row are not significantly different ($p > 0.05$).

3.5. Colour characteristics

Anthocyanin-rich fruits are widely utilised as an alternative ingredient to replace the synthetic food colourants such as FD&C Red 40 and carmine in the jellies, yoghurts, confectioneries, etc. (Chigurupati et al., 2002; de Souza et al., 2015; Kırca et al., 2006; Mourtzinos et al., 2018). The structure of anthocyanins has been shown to be dependent on the pH of the media. There are four structures that exist in equilibrium: red flavylium cation at pH 1–3, colourless carbinol pseudobase at pH 4–5 and blue-purple quinoidal base at pH 7–8, which could change to a chalcone form (Lapidot et al., 1999).

Other than total phenolics and antioxidant activity, the colour characteristics of extracts were also affected by pH of acidified water ($p < 0.05$), extraction time and their interaction. As shown in Table 6, water extracts (control) showed significantly ($p < 0.05$) higher L^*, a^* and b^* values, indicating the higher lightness, redness and yellowness, followed by pH 3.0 of acidified water extracts. Subsequently, these higher values of L^*, a^* and b^* were also contributed to significantly ($p < 0.05$) higher chroma and hue angle values. The L^* values of extract increased as the pH increased ($p < 0.05$), suggesting that extracts became less dark over time at higher pH. The redness (a^*), yellowness (b^*), chroma and hue angle of the extracts were also increased ($p < 0.05$) as the pH increased but decreased at longer extraction time (6 h). In comparison to another pH, pH 1.5 had significantly ($p < 0.05$) high total colour difference up to 10.2 ± 0.1, while water extract had the lowest colour changes (3.7 ± 0.1) after 6 h of extraction.

Chroma or intensity indicates the purity of the colour. Water extracts (control) with higher intensity were brighter in colour. Due to higher anthocyanins concentration, acidified water extracts, particularly pH 1.5 and 2.0, had lower purity and darker colour compared to water. Hue is described as a shade of colour that distinguishes one colour from another such as green, red, blue and yellow. In this study, the combination of high values of a^* and b^* in water extract contributed to the highest hue angle value (~13.5°), the closest to the synthetic colourant FD&C Red # 40 (25°) as measured by Wrolstad (2004). Overall, pH influenced the colour shades of DBP extracts to become very dark pink (pH 1.5 and 2.0), dark pink (pH 2.5) and dark red (pH 3.0 and control). The visualisations of anthocyanins colour from DBP extracts were converted from L^*, a^*, and b^* values to RGB (Red, Green, Blue) colour model as shown in Table 6.

From an application point of view, acetic acid might negatively interfere with the sensory properties of extracts due to the distinctive sour taste and pungent smell, thus it needs to be removed. Generally, there are various processes that can be applied for the preparation of

Table 6
Colour characteristics of DBP extracts obtained using different pH of acidified water for 2 and 6 h.

Acidified water -extraction time (h)	L^*	a^*	b^*	C	h°	ΔE	Visualisation & description
Control - 2	29.8	46.0	10.9	47.3	13.4	2.2	
	(0.3) ^{a}	(0.4) ^{a}	(0.4) ^{a}	(0.5) ^{a}	(0.3) ^{ab}	(0.4) ^{de}	Dark Red
Control - 6	30.0	46.2	11.1	47.5	13.5	1.9	
	(0.7) ^{a}	(0.1) ^{a}	(0.1) ^{a}	(0.1) ^{a}	(0.1) ^{a}	(0.3) ^{e}	Dark Red
pH 1.5–2	19.1	27.2	2.6	27.3	5.5	7.5	
	(0.1) ^{f}	(0.8) ^{d}	(0.2) ^{e}	(0.8) ^{d}	(0.2) ^{e}	(0.4) ^{b}	Very Dark Pink
pH 1.5–6	20.4	26.8	1.9	26.8	4.0	10.2	
	(0.1) ^{e}	(0.2) ^{d}	(0.1) ^{f}	(0.2) ^{d}	(0.1) ^{f}	(0.1) ^{a}	Very Dark Pink
pH 2.0–2	19.0	26.0	2.1	26.5	4.6	2.3	
	(0.2) ^{f}	(0.4) ^{d}	(0.1) ^{ef}	(0.4) ^{d}	(0.1) ^{g}	(0.6) ^{de}	Very Dark Pink
pH 2.0–6	18.5	23.5	1.1	26.0	2.7	3.7	
	(0.1) ^{f}	(0.1) ^{e}	(0.1) ^{g}	(0.1) ^{d}	(0.1) ^{f}	(0.1) ^{d}	Very Dark Pink
pH 2.5–2	24.4	36.6	6.1	37.2	9.5	5.6	
	(0.1) ^{c}	(0.2) ^{c}	(0.1) ^{d}	(0.2) ^{c}	(0.1) ^{d}	(0.2) ^{c}	Dark Pink
pH 2.5–6	22.8	35.0	5.9	35.5	9.6	7.7	
	(0.1) ^{d}	(0.2) ^{c}	(0.1) ^{d}	(0.2) ^{c}	(0.1) ^{d}	(0.2) ^{b}	Dark Pink
pH 3.0–2	28.0	43.5	9.8	44.6	12.7	5.3	
	(0.1) ^{b}	(0.1) ^{b}	(0.1) ^{b}	(0.1) ^{b}	(0.1) ^{b}	(0.1) ^{c}	Dark Red
pH 3.0–6	27.2	43.4	9.1	44.3	11.8	6.2	
	(0.5) ^{b}	(0.9) ^{b}	(0.1) ^{c}	(0.9) ^{b}	(0.4) ^{c}	(0.9) ^{bc}	Dark Red

Visualisation: Conversion of L^*, a^*, and b^* values to RGB (Red, Green, Blue) colour model (http://www.easyrgb.com/en/). Colour description: ColorHexa (https://www.colorhexa.com/). Control: water; C: Chroma; h°: Hue angle; ΔE: Total colour difference. Figures in parentheses indicate the standard deviation (n = 2). Values with the same letter $^{a, b}$ in each row are not significantly different ($p > 0.05$).

acetic acid extracts prior to their incorporation into food matrices; these include azeotropic distillation, simple distillation, vacuum evaporation, or adsorption. Xu et al. (1999) reported an efficient process using catalytic distillation, where over 50% (v/v) of acetic acid can be removed from the feed containing 2.5–9.9% (v/v) of acetic acid in water. Then, water extract needs to undergo a purification process such as solid-phase anthocyanin extraction (SPE) method using mixed mode cation-exchange chromatography (He & Giusti, 2011), aiming at the removal of undesirable aroma and non-anthocyanin phenolics before incorporation into food as natural colourant.

4. Conclusions

The pH of acidified water significantly affected the anthocyanins, hydroxycinnamic acids, flavonols of DBP extracts. Strongly acidified water (pH 1.5) favoured the extraction of these phenolics ($p < 0.05$). On the other hand, pH of acidified water, extraction time and their interaction showed a significant impact on the total phenolics, antioxidant activity and colour of the extracts. Total phenolics and antioxidant activity increased ($p < 0.05$) as the pH decreased and extraction time increased. The redness (a^*), yellowness (b^*), chroma and hue angle of the extracts were also increased ($p < 0.05$) as the pH increased but decreased after longer extraction time. Correlation results revealed that anthocyanins were the highest antioxidant contributor, followed by flavonols whereas hydroxycinnamic acids were the lowest. Also, three different colour shades of DBP extracts (very dark pink, dark pink and dark red) were observed between pH 1.5 to 3.0 and water. Overall, higher amounts of anthocyanins, hydroxycinnamic acids, flavonols, antioxidant activity and total phenolics were found at pH 1.5. DBP extracts obtained in this study had different phenolics concentration and colour shades depending on the pH, which can be potentially used for applications in food matrices as natural colourants after undergoing acetic acid removal and purification processes.

CRediT authorship contribution statement

Ezzat Mohamad Azman: Writing – original draft, conducted the research, interpreted the results, and drafted the manuscript. **Nurfarhana Diana Mohd Nor:** Formal analysis, interpreted the statistical analysis results. **Dimitris Charalampopoulos:** Conceptualization, Writing – review & editing, conceptualized, designed the study, and reviewed-finalized the manuscript. **Afroditi Chatzifragkou:** Conceptualization, Writing – review & editing, conceptualized, designed the study, and reviewed-finalized the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Malaysian Higher Education and Universiti Putra Malaysia (UPM) for the financial support in this research. Also, we acknowledge A & R House (BCL) Ltd (Bleadon, Weston-super-Mare, UK) for the kind supply of blackcurrants pomaces.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112733.

References

Amr, A., & Al-Tamimi, E. (2007). Stability of the crude extracts of *Ranunculus asiaticus* anthocyanins and their use as food colourants. *International Journal of Food Science and Technology, 42*(8), 985–991. https://doi.org/10.1111/j.1365-2621.2006.01334.x

* Azman, E. M., Charalampopoulos, D., & Chatzifragkou, A. (2020). Acetic acid buffer as extraction medium for free and bound phenolics from dried blackcurrant (*Ribes nigrum* L.) skins. *Journal of Food Science, 85*(11), 3745–3755. https://doi.org/10.1111/1750-3841.15466.

* Azman, E. M., House, A., Charalampopoulos, D., & Chatzifragkou, A. (2021). Effect of dehydration on phenolic compounds and antioxidant activity of blackcurrant (*Ribes nigrum* L.) pomace. *International Journal of Food Science and Technology, 56*(2), 600–607. https://doi.org/10.1111/ijfs.14762.

Basegmez, H. I. O., Povilaitis, D., Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Alasalvar, C., & Venskutonis, P. R. (2017). Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO_2, pressurised liquid and enzyme assisted extractions. *The Journal of Supercritical Fluids, 124*, 10–19. https://doi.org/10.1016/j.supflu.2017.01.003

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Chigurupati, N., Saiki, L., Gayser, C., Jr., & Dash, A. K. (2002). Evaluation of red cabbage dye as a potential natural color for pharmaceutical use. *International Journal of Pharmaceutics, 241*(2), 293–299. https://doi.org/10.1016/S0378-5173(02)00246-6

Clifford, M. N. (2000). Anthocyanins–nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 80*(7), 1063–1072. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1063::AID-JSFA605>3.0.CO;2-Q

Cyboran, S., Bonarska-Kujawa, D., Pruchnik, H., Żyłka, R., Oszmiański, J., & Kleszczyńska, H. (2014). Phenolic content and biological activity of extracts of blackcurrant fruit and leaves. *Food Research International, 65*, 47–58. https://doi.org/10.1016/j.foodres.2014.05.037

Durazzo, A. (2018). Extractable and non-extractable polyphenols: An overview. In *Non-extractable polyphenols and carotenoids* (pp. 37–45). London, UK: Royal Society of Chemistry. https://doi.org/10.1039/9781788013208.

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin−Ciocalteu reagent. *Journal of Agricultural and Food Chemistry, 58*(14), 8139–8144. https://doi.org/10.1021/jf1005935

Fernandes, A., Oliveira, J., Teixeira, N., Mateus, N., & De Freitas, V. (2017). A review of the current knowledge of red wine colour. *Oeno One, 51*(1), 1–21.

Fidelis, M., de Moura, C., Kabbas Junior, T., Pap, N., Mattila, P., Mäkinen, S., … Granato, D. (2019). Fruit seeds as sources of bioactive compounds: Sustainable production of high value-added ingredients from by-products within circular economy. *Molecules, 24*(21), 3854. https://doi.org/10.3390/molecules24213854

He, J., & Giusti, M. M. (2011). High-purity isolation of anthocyanins mixtures from fruits and vegetables–A novel solid-phase extraction method using mixed mode cation-exchange chromatography. *Journal of Chromatography A, 1218*(44), 7914–7922. https://doi.org/10.1016/j.chroma.2011.09.005

Hui, X., Wu, G., Han, D., Gong, X., Stipkovits, L., Wu, X., … Brennan, C. S. (2021). Bioactive compounds from blueberry and blackcurrant powder alter the physicochemical and hypoglycaemic properties of oat bran paste. *LWT-Food Science and Technology, 143*, 111167. https://doi.org/10.1016/j.lwt.2021.111167

IBA (International Blackcurrant Association) (2018). http://www.internationalblackcurrantassociation.com. Accessed 13 April 2021.

Kähkönen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*(8), 4076–4082. https://doi.org/10.1021/jf010152t

* Kapasakalidis, P. G., Rastall, R. A., & Gordon, M. H. (2006). Extraction of polyphenols from processed black currant (*Ribes nigrum* L.) residues. *Journal of Agricultural and Food Chemistry, 54*(11), 4016–4021. https://doi.org/10.1021/jf052999l.

Kırca, A., Özkan, M., & Cemeroglu, B. (2006). Stability of black carrot anthocyanins in various fruit juices and nectars. *Food Chemistry, 97*(4), 598–605. https://doi.org/10.1016/j.foodchem.2005.05.036

Kupina, S., Fields, C., Roman, M. C., & Brunelle, S. L. (2018). Determination of total phenolic content using the Folin-C assay: Single-laboratory validation, First action 2017.13. *Journal of AOAC International, 101*(5), 1466–1472. https://doi.org/10.5740/jaoacint.18-0031

* Laaksonen, O. A., Mäkilä, L., Sandell, M. A., Salminen, J. P., Liu, P., Kallio, H. P., & Yang, B. (2014). Chemical-sensory characteristics and consumer responses of blackcurrant juices produced by different industrial processes. *Food and Bioprocess Technology, 7*(10), 2877–2888. https://doi.org/10.1007/s11947-014-1316-8.

* Laaksonen, O., Mäkilä, L., Tahvonen, R., Kallio, H., & Yang, B. (2013). Sensory quality and compositional characteristics of blackcurrant juices produced by different processes. *Food Chemistry, 138*(4), 2421–2429. https://doi.org/10.1016/j.foodchem.2012.12.035.

Lapidot, T., Harel, S., Akiri, B., Granit, R., & Kanner, J. (1999). pH-dependent forms of red wine anthocyanins as antioxidants. *Journal of Agricultural and Food Chemistry, 47*(1), 67–70. https://doi.org/10.1021/jf980704g

Lapornik, B., Prošek, M., & Wondra, A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering, 71*(2), 214–222. https://doi.org/10.1016/j.jfoodeng.2004.10.036

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International, 88*(5), 1269–1278. https://doi.org/10.1093/jaoac/88.5.1269

Michalska, A., Wojdyøo, A., Lech, K., èysiak, G. P., & Figiel, A. (2017). Effect of different drying techniques on physical properties, total polyphenols and antioxidant capacity of blackcurrant pomace powders. *LWT- Food Science and Technology, 78*, 114–121. https://doi.org/10.1016/j.lwt.2016.12.008

Milivojević, J., Maksimović, V., & Nikolić, M. (2009). Sugar and organic acids profile in the fruits of black and red currant cultivars. *Journal of Agricultural Sciences, Belgrade, 54*(2), 105–117. https://doi.org/10.2298/JAS0902105M

Mourtzinos, I., Prodromidis, P., Grigorakis, S., Makris, D. P., Biliaderis, C. G., & Moschakis, T. (2018). Natural food colorants derived from onion wastes: Application in a yoghurt product. *Electrophoresis, 39*(15), 1975–1983. https://doi.org/10.1002/elps.201800073

Norman, G. (2010). Likert scales, levels of measurement and the "laws" of statistics. *Advances in Health Sciences Education, 15*(5), 625–632. https://doi.org/10.1007/s10459-010-9222-y

Oancea, S., Stoia, M., & Coman, D. (2012). Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium corymbosum* in the perspective of food applications. *Procedia Engineering, 42*, 489–495. https://doi.org/10.1016/j.proeng.2012.07.440

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutrition Sciences, 5*. https://doi.org/10.1017/jns.2016.41

Paunović, S. M., Mašković, P., Nikolić, M., & Miletić, R. (2017). Bioactive compounds and antimicrobial activity of black currant (*Ribes nigrum* L.) berries and leaves extract obtained by different soil management system. *Scientia Horticulturae, 222*, 69–75. https://doi.org/10.1016/j.scienta.2017.05.015

Pearson, E. S. (1931). The analysis of variance in cases of non-normal variation. *Biometrika*, 114–133. https://doi.org/10.2307/2333631

Raikos, V., Ni, H., Hayes, H., & Ranawana, V. (2019). Antioxidant properties of a yogurt beverage enriched with salal (*Gaultheria shallon*) berries and blackcurrant (*Ribes nigrum*) pomace during cold storage. *Beverages, 5*(1), 2. https://doi.org/10.3390/beverages5010002

Rodriguez-Saona, L. E., & Wrolstad, R. E. (2001). Extraction, isolation, and purification of anthocyanins. *Current Protocols in Food Analytical Chemistry*, (1)https://doi.org/10.1002/0471142913.faf0101s00. F1-F1.

Rose, P. M., Cantrill, V., Benohoud, M., Tidder, A., Rayner, C. M., & Blackburn, R. S. (2018). Application of anthocyanins from blackcurrant (*Ribes nigrum* L.) fruit waste as renewable hair dyes. *Journal of Agricultural and Food Chemistry, 66*(26), 6790–6798. https://doi.org/10.1021/acs.jafc.8b01044

Saura-Calixto, F. (2012). Concept and health-related properties of nonextractable polyphenols: The missing dietary polyphenols. *Journal of Agricultural and Food Chemistry, 60*(45), 11195–11200. https://doi.org/10.1021/jf303758j

Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists, 2*(1), 21–25. https://doi.org/10.4103/2229-5186.79345

de Souza, V. R., Pereira, P. A. P., Teixeira, T. R., Silva, T. L. T. D., Pio, R., & Queiroz, F. (2015). Influence of processing on the antioxidant capacity and bioactive compounds in jellies from different blackberry cultivars. *International Journal of Food Science and Technology, 50*(7), 1658–1665. https://doi.org/10.1111/ijfs.12819

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering, 81*(1), 200–208. https://doi.org/10.1016/j.jfoodeng.2006.10.021

Sun-Waterhouse, D., Zhou, J., & Wadhwa, S. S. (2013). Drinking yoghurts with berry polyphenols added before and after fermentation. *Food Control, 32*(2), 450–460. https://doi.org/10.1016/j.foodcont.2013.01.011

Tidder, A., Benohoud, M., Rayner, C. M., & Blackburn, R. S. (2018). July). Extraction of anthocyanins from blackcurrant (*Ribes nigrum* L.) fruit waste and application as renewable textile dyes. In *91st textile institute world conference book of abstracts* (pp. 18–19). Textile Institute.

Toscano Martinez, H., Gagneten, M., Díaz-Calderón, P., Enrione, J., Salvatori, D., Schebor, C., & Leiva, G. (2021). Natural food colorant from blackcurrant spray-dried powder obtained by enzymatic treatment: Characterisation and acceptability. *Journal of Food Processing and Preservation, 45*(1), Article e15011. https://doi.org/10.1111/jfpp.15011

Vergara-Salinas, J. R., Bulnes, P., Zúñīga, M. C., Pérez-Jiménez, J., Torres, J. L., Mateos-Martín, M. L., … Pérez-Correa, J. R. (2013). Effect of pressurized hot water extraction on antioxidants from grape pomace before and after enological fermentation. *Journal of Agricultural and Food Chemistry, 61*(28), 6929–6936. https://doi.org/10.1021/jf4010143

Viljanen, K., Kylli, P., Hubbermann, E. M., Schwarz, K., & Heinonen, M. (2005). Anthocyanin antioxidant activity and partition behavior in whey protein emulsion. *Journal of Agricultural and Food Chemistry, 53*(6), 2022–2027. https://doi.org/10.1021/jf047975d

Wrolstad, R. E. (2004). Anthocyanin pigments—bioactivity and coloring properties. *Journal of Food Science, 69*(5), C419–C425. https://doi.org/10.1111/j.1365-2621.2004.tb10709.x

Wrolstad, R. E., & Smith, D. E. (2017). Color analysis. In *Food analysis* (pp. 545–555). Cham: Springer. https://doi.org/10.1007/978-3-319-45776-5_31.

Wu, X., Gu, L., Prior, R. L., & McKay, S. (2004). Characterisation of anthocyanins and proanthocyanidins in some cultivars of *Ribes, Aronia*, and *Sambucus* and their antioxidant capacity. *Journal of Agricultural and Food Chemistry, 52*(26), 7846–7856. https://doi.org/10.1021/jf0486850

Xu, Z., Afacan, A., & Chuang, K. T. (1999). Removal of acetic acid from water by catalytic distillation. Part 1: Experimental studies. *Canadian Journal of Chemical Engineering, 77*(4), 676–681. https://doi.org/10.1002/cjce.5450770408

Yang, J., He, X., & Zhao, D. J. (2013). Factors affecting phytochemical stability. Handbook of plant food phytochemicals: Sources, stability and extraction. https://doi.org/10.1002/9781118464717.ch15.

5 Key references: These articles investigated the phenolic compounds including anthocyanins, hydroxycinnamic acids and flavonols from blackcurrant pomaces and juices.