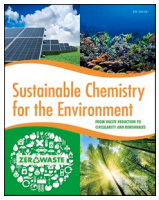
Sustainable Chemistry for the Environment 9 (2025) 100208

Contents lists available at ScienceDirect

Sustainable Chemistry for the Environment

journal homepage: www.editorialmanager.com/scenv



Extraction of anthocyanins and other phenolics from dried blackcurrant (*Ribes nigrum* L.) pomace via ultrasonication

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ARTICLE INFO

Keywords:
Blackcurrant
Anthocyanins
Ultrasonication
Water bath
Non-thermal Extraction
Conventional Extraction
Antioxidant Properties

ABSTRACT

Blackcurrant (*Ribes nigrum* L.) pomaces are rich in phenolic compounds, particularly anthocyanins yet often discarded as waste during juice processing. The heat-sensitive phenolic compounds such as anthocyanins, unavoidably, degrade due to higher temperatures and longer times during conventional extraction methods. Typically, organic solvents such as ethanol, methanol, and acetone were used to extract the phenolic compounds from dried blackcurrant pomaces (DBP). The necessity for employing substantial amounts of solvents in conventional extraction methods, such as water baths, resulted in health hazards and environmental pollution. Hence, this study aimed to investigate the effect of ultrasonication (US) and water bath (WB) extraction time on the anthocyanins composition, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of DBP extracts. The DBP powders were mixed with 50 % ethanol in the beaker (1:40, w/v) and extracted for 5 − 30 min using ultrasonication at 50 % amplitude and a water bath at 180 rpm. The extracts were further analyzed using the HPLC analysis, Folin-Ciocalteu method, flavonoid content, DPPH, and FRAP assays. As a result, ultrasonication only required 10 min to produce ~27 % higher total anthocyanins (37.15 ± 0.71 mg/g) than the water bath at 20 min (26.97 ± 0.18 mg/g), while the highest TPC (38.02 ± 0.24 mg GAE/g) and TFC (38.83 ± 1.41 mg CE/g) were recorded at 20 min. Also, a significantly higher ($p < 0.05$) DPPH inhibition (52.76 ± 0.90 %) and reducing power (352.60 ± 7.64 μmol TE/g) were detected after 25 min of ultrasonication. Overall, ultrasonication is suitably used as an alternative to conventional extraction, where the natural colorant obtained from DBP extracts could be potentially utilized in commercial food applications.

1. Introduction

Blackcurrant (*Ribes nigrum* L.) is one type of berry species with small dark purple fruits produced by moderate-sized arboreous shrubs and extensively grown in temperate regions including the northern region of Asia and northern region of Europe [1]. Blackcurrant pomace consisted of skins, seeds, and stems often generated as industrial waste during the juice pressing process. They are rich in phenolic compounds mainly anthocyanins that are linked to a high antioxidant activity and are potentially utilized as a food colorant in food, beverages, and pharmaceutical industries. Consumers are showing a growing preference for natural food products over synthetic ones due to increased health awareness, despite their higher costs and shorter life [2]. This preference has increased demand for sustainable and health-promoting alternatives within the food industry. Anthocyanins, which act as natural substitutes for synthetic colorants, are one such alternative. They can be efficiently extracted from dried blackcurrant pomace (DBP) using an eco-friendly green extraction technique [3].

Traditionally, phenolic compounds such as anthocyanins can be extracted through the conventional thermal extraction method. The drawbacks of this method are it is susceptible to a high degradation rate of heat-sensitive bioactive compounds, hence high cooling energy is required [4]. For example, anthocyanins are heat-sensitive and prone to degradation upon exposure to high temperatures during extraction [5]. Therefore, non-conventional extraction methods such as pulsed electric fields (PEF), ultrasonication, microwave, and high hydrostatic pressure are developed to achieve better extraction results [6]. [7] evaluated the effect of different extraction methods on the anthocyanins. The authors found that compared to conventional methods (89.59 mg/100 g), microwave (104.9 mg/100 g), ultrasonication (96.07 mg/100 g), and pressurized-liquid extraction (71.64 mg/100 g) showed better sustainable extraction for blackcurrant.

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https://doi.org/10.1016/j.scenv.2025.100208
Received 2 September 2024; Received in revised form 31 December 2024; Accepted 8 January 2025
Available online 9 January 2025

Among non-conventional extraction methods, ultrasonication can be used to facilitate the extraction of anthocyanins, which has been proven to be effective and/or cost-effective [8]. Ultrasonication creates cavitation bubbles from ultrasonic waves during sonication, allowing the extraction solvent to penetrate deeper into the plant cell wall than traditional techniques, thus releasing the plant's intracellular components [9]. Therefore, the current study was carried out to compare the effect of ultrasonication and water bath on the extraction of anthocyanins composition, total phenolics content, and antioxidant activity of the DBP. In addition, Pearson correlation, general linear model (GLM), and principal component analysis (PCA) were conducted to gain a deeper insight into the relationships among the examined parameters.

2. Materials and methods

2.1. Chemicals

Anthocyanin standards such as cyanidin-3-*O*-glucoside (C3G) (96 %), cyanidin-3-*O*-rutinoside (C3R) (96 %), delphinidin-3-*O*-glucoside (D3G) (95 %), and delphinidin-3-*O*-rutinoside (D3R) (95 %) were purchased from ExtraSynthese Ltd (Genay, France). All other chemicals and solvents utilized were of analytical grade and were procured from Fisher Scientific (Leicestershire, UK).

2.2. Sample preparation of plant materials

A&R House (BCL) Ltd (Bleadon, Weston-super-Mare, UK) graciously provided dried blackcurrant pressed by-products obtained from a juice-making operation. The dried blackcurrant pomace (DBP) was ground using a grinder and sieved using a 0.841 mm sieve to separate from the seeds. Then, the samples were kept in a plastic bag and stored under −20°C until further analysis.

2.3. Extraction of DBP samples

2.3.1. Preliminary test: determination of ultrasonication amplitude

Ultrasonication extraction was carried out according to the method previously described by [10] with slight modifications using a 50 W sonic dismembrator (FB 50, Thermo Fisher Scientific Inc., Pennsylvania, USA) operating at a fixed frequency (20 kHz) with a probe 3.175 mm i.d. In the 250 mL Duran laboratory bottle, 2.5 g of DBP was extracted in 100 mL of 50 % ethanol. The probe was positioned 1 cm from the bottom of the bottle. For preliminary studies, ultrasonication amplitudes were set at 10, 20, 30, 40, 50, 60, 70, and 80 % for 20 min. The temperature of the extraction solvent was controlled at 30 ± 3°C by placing an ice water bath surrounding the bottle.

2.3.2. Ultrasonication and water bath-assisted extraction

To compare with the conventional extraction method, a similar amount of DBP and solvent was applied for the water bath (WITEG, Labortechnik, Wertheim, Germany) extraction which was set at 180 rpm and 30 ± 1°C. Ultrasonication amplitude was set at the best amplitude obtained from the preliminary test. Both extraction methods were evaluated at 5, 10, 15, 20, 25, and 30 min in continuous mode. Then, the separation of the residues and extracts was carried out by using vacuum filtration through Whatman No. 1 filter paper (Whatman, Maidstone, UK). The extracts were kept at −20°C for subsequence analysis.

2.4. Determination of total monomeric anthocyanins content (TMAC)

In the preliminary studies, the total monomeric anthocyanin content was determined using the modified pH-differential method by [11]. The sample preparation followed the procedure outlined by [5] with some adjustments. Specifically, 0.05 mL of 80-fold diluted DBP extract solution was mixed with 3.95 mL potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate buffer ($CH_3COONa.3H_2O$, 0.4 M, pH 4.5), respectively. The mixture was left to equilibrate in the dark for 30 min. Subsequently, the absorbance was measured at 520 nm and 700 nm for each sample using a spectrophotometer (Biomate 3, Thermo Fisher Scientific, Waltham, MA, USA). Each sample was analyzed in duplicate.

$$Anthocyanins\ content\left(\frac{mg}{g}\right) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \times \frac{extract(L)}{sample(g)} \quad (1)$$

where, MW = molecular weight of cyanidin-3-*O*-glucoside (MW = 449.2 g/mol); DF = dilution factor; and ε = molar absorptivity constant of cyanidin-3-*O*-glucoside (26,900) were used.

2.5. HPLC analysis of anthocyanins

The HPLC analysis of anthocyanin compounds was conducted following a method by [12] with slight modifications. To quantify the anthocyanins, calibration curves were prepared using external standards over a concentration range of 0.1 − 0.02 mg/mL, as detailed in Table 1. The anthocyanins profile of DBP extracts was characterized using HPLC, employing a Purosphere STAR RP18 end-capped column (250 mm × 4.6 mm i.d., particle size of 5 μm, Merck, Darmstadt, Germany). The eluents consisted of 2 % (v/v) formic acid in water (A) and 100 % (v/v) HPLC-grade methanol (B). The chromatographic conditions were 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. The column temperature was maintained at 30°C, and the flow rate was set to 1.0 mL/min. Detection was carried out using a Perkin Elmer Series 200 HPLC system equipped with a Perkin Elmer Series 200 UV/Vis detector. The analysis duration was 45 min, and a wavelength of 520 nm was employed to detect the anthocyanins, with a fixed injection volume of 20 μL.

2.6. Determination of total phenolic content (TPC)

Folin-Ciocalteu method by [13] was used with slight modifications to assess the total phenolic content (TPC). The absorbance was measured at 765 nm using a spectrophotometer as described above. The calibration curve used gallic acid (100 − 0 mg/mL) as the standard. The results were expressed as milligrams of gallic acid equivalents per g of dried weight of extracts (mg GAE/g of DBP).

2.7. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using the method by [14]. The catechin solution (300 − 0 mg/L) was used to plot a standard curve and the absorbance was measured at 510 nm using a spectrophotometer as described above. The results were expressed as milligrams of catechin equivalent per gram (mg CE/g DBP) of the sample.

2.8. Determination of DPPH radical scavenging activity

Determination of the antioxidant activity using the DPPH radical

Table 1
The determination coefficients (R^2), limits of detection (LOD), and limits of quantification (LOQ) for each individual anthocyanin as determined through HPLC.

Anthocyanins	R^2	LOD (mg/mL)	LOQ (mg/mL)
D3R	0.9862	0.03	0.09
D3G	0.9787	0.07	0.21
C3R	0.9991	0.01	0.03
C3G	0.9993	0.01	0.02

D3R: Delphinidin-3-*O*-rutinoside, D3G: Delphinidin-3-*O*-glucoside, C3R: Cyanidin-3-*O*-rutinoside, C3G: Cyanidin-3-*O*-glucoside.

scavenging activity and the sample preparations of DBP extracts were performed by the following [12] with slight modifications. A stock solution of 0.15 mM DPPH was prepared in methanol. The absorbance was assessed after 30 min at 517 nm using a spectrophotometer as described above after the samples were kept in the dark at 30°C.

$$DPPH\ Inhibition(\%) = \frac{A_o - A_e}{A_o} \times 100 \quad (2)$$

where, A_o = Absorbance of the control; A_e = Absorbance of the sample.

2.9. Determination of ferric reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power (FRAP) followed the protocol by [15] with slight modifications, involved extending the incubation time to 30 min and diluting the extracts before mixing them with the FRAP reagent. After 30 min of incubation in the dark, the spectrophotometer was set at 593 nm as described above, to measure absorbance. Duplicate measurement was conducted, and antioxidant power was expressed as μmol of Trolox equivalents per g of the sample (μmol TE/g DBP).

2.10. Statistical analysis

One-way analysis of variance (ANOVA) was conducted for all treatment groups, applying a significance level of 95 % ($p < 0.05$) in Tukey's multiple-range tests. To assess the significance of the main dependent variables, which include extraction methods and times, as well as their interactions, the General Linear Model (GLM) was employed. Additionally, Principal Component Analysis (PCA) was utilized to examine the relationships between the measured variables. All statistical analyses were carried out using the Minitab V.19 software (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Preliminary test

During the preliminary test, the DBP was ultrasonicated at different amplitudes of 10, 20, 30, 40, 50, 60, 70, and 80 %. The time and temperature were fixed at 20 min and 30°C, respectively. Preliminary studies were performed to determine the best ultrasonication amplitude that can produce the highest TMAC as measured using the pH differential method. As shown in Fig. 1, there was no significant difference in TMAC (~15.5 mg/g to ~15.39 mg/g) between 50, 60, and 80 % of amplitudes. [16] stated that the yield of anthocyanins during ultrasonication extraction is more significantly affected by time than amplitudes. In their study using Korean black soybeans (*Glycine max* [L.] Merr. Cheongja4ho) at different amplitudes (20 − 100 %), anthocyanins reached the highest content at 60 % of amplitude. Ultrasonication can effectively extract targeted compounds by inducing cavitation, a process where small bubbles undergo rapid compression and expansion and collapse on the surface of plant material, generating pressure that disrupts plant tissues and releases the desired compounds [17]. The selection of 50 % amplitude as the ideal condition for anthocyanin extraction reflects a balance between extraction efficiency, energy utilization, and the findings of this study. Previous research [16], implies that increased amplitudes could result in anthocyanin loss caused by excessive cavitation. In contrast, this study identified a positive relationship between amplitude and TMAC up to a specific threshold. As shown in Fig. 1, TMAC increases significantly with amplitude up to 50 %, beyond which the increase becomes marginal, and no degradation was observed within the experimental range.

Choosing 50 % amplitude achieves a compromise between maximizing TMAC yield and minimizing energy consumption. While higher amplitudes, such as 60 % or 80 %, could result in slightly higher yields, they demand much larger energy requirements with only slight efficiency improvements. Additionally, higher amplitudes heighten the risk of cavitation-induced degradation and reduced energy transmission due to excessive bubble formation and collisions, as noted by [18]. Thus, selecting 50 % amplitude aligns with optimizing energy efficiency and process effectiveness while minimizing risks of degradation or diminishing returns.

This is directly influenced by the amplitude of the waves where higher amplitudes generate more cavities, maximizing extraction yield by enhancing mass transfer rates. Following the result by [19] the study on sonicated strawberry juice found that anthocyanin content started to decrease as the amplitude increased. However, the yield of ultrasonication extraction increases with the increase in power up to a peak value, above which it eventually decreases or reaches a plateau. However, power intensity contributing through amplitude above the peak value may decrease or not affect the extraction yield, since the excessive number of cavitation bubbles can increase the inter-bubble collision, reducing the impacts of bubble implosion. In addition, these bubbles can surround the probe and hinder energy transmission [18]. Following that, the amplitude that favors the anthocyanins extraction was decided at 50 %, due to the utilization of less energy compared to 60 and 80 %.

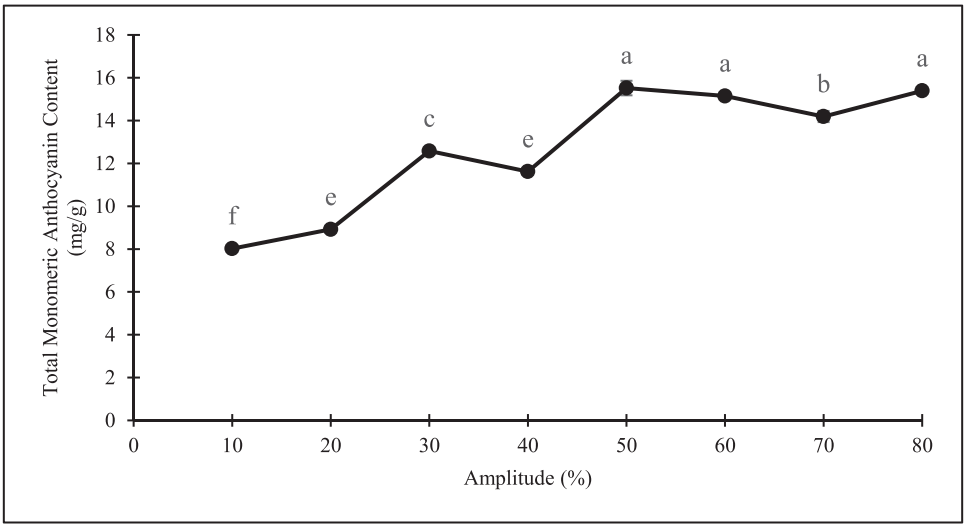


Fig. 1. Effect of different ultrasonication amplitude (%) on the total monomeric anthocyanins content in DBP extract at the constant extraction time (20 min) and temperature (≤30°C), as measured by spectrophotometer.

3.2. Ultrasonication and water bath-assisted extraction

Fig. 2 shows the typical HPLC profile of anthocyanins in DBP extract. In this study, four individual anthocyanins were identified namely D3G, D3R, C3G, and C3R. This is in agreement with previous studies [5, 20–22] where the four types of individual anthocyanins represented up to 90 % of total anthocyanins in blackcurrants.

The results for individual and total anthocyanins in DBP extracts obtained by US and WB extraction are presented in Fig. 3. D3R (Fig. 3 **(b)**) indicated the most dominant anthocyanins in DBP extracts in US (D3R; ~47.8 % > D3G; ~44.5 % > C3R; ~5.6 % > C3G; ~2.1 %) and WB extraction (D3R; ~49.4 % > D3G; ~43.4 % > C3R; ~5.2 % > C3G; ~2.1 %). The statistical analysis confirmed the significance ($p < 0.05$) of both the extraction methods and times, as well as the interaction between them, on the total anthocyanins content in the DBP extracts. Throughout the ultrasonication process, no significant difference was found between individual anthocyanins, where 10 min being the optimum time to extract the highest total anthocyanins (~37.2 mg/g) in DBP (Fig. 3**(e)**). However, in water bath extraction, 20 min showed the highest for individuals and total anthocyanins (~27.0 mg/g). It should be noted that exposure to longer extraction time can lead to the degradation of anthocyanins. Ultrasonication efficiently reduced the extraction time yet produced ~27 % higher total anthocyanins than water bath. In both methods, the extraction yield was time-dependent as the yield increased over 20 min but started to degrade after 25 min of extraction. Thus, 30 min extraction time was considered as the maximum value to extract DBP.

[23] stated that ultrasonication is associated with chemical and thermal degradation following various variables during the process such as processing time, ultrasonication power as well as temperature. Due to the acoustic energy applied, the exposure of plant material to thermal and mechanical stress contributes to the severe degradation of anthocyanins. While prolonged extraction time and greater amplitudes might degrade anthocyanins due to the generation of hydrogen peroxide (H_2O_2) and radical hydroxyl (OH*) inside cavitation bubbles, both amplitude and time could prevent anthocyanins degradation [24,25]. However, [26] reported their study on the ultrasonication from jaboticaba peel at 50 W/L did not cause degradation on anthocyanins extraction yield after 40 min. Maintaining the temperature below 50°C might prevent the degradation of anthocyanins caused by heat produced during the extraction.

3.3. Total phenolic content (TPC) and total flavonoid content (TFC)

DBP was a major source of phenolic compounds and flavonoids [27]. In this study, higher TPC values were observed in the US compared to WB extraction at different times. Based on Fig. 4**(a)**after 20 min, a significant ($p < 0.05$) higher TPC (~38.0 mg GAE/g) was observed in the US, while only ~29.1 mg GAE/g was detected in WB extraction. These findings were in agreement with the study by [28] on blueberry (*Vaccinium ashei*) wine pomace, where ultrasonication increased TPC by 3.2-fold at 30 min with 16.41 mg GAE/g compared to the conventional method (5.08 mg GAE/g).

Meanwhile, a similar TPC value (~32.8 mg GAE/g) was obtained from DBP after 10 min of WB and 5 min of US, indicating that there is no significant difference within the first 10 min of extraction. In a shorter time, US gives more advantages by producing higher yield efficiency at low heating compared to WB extraction. Correlation showed a significantly strong correlation between total anthocyanins and TPC ($r = 0.799$, $p < 0.05$) in both US and WB extraction, suggesting that higher TPC contributed to total anthocyanins. As stated by [29], US extraction is the best for extracting a higher yield of phenolic compounds compared to conventional methods. Similar findings were reported by [21] in the extraction of blackcurrant pomace where the TPC obtained ranged from 9.4 to 73.0 mg GAE/g. The efficiency of TPC extraction increases with time through acoustic cavitations, enabling the diffusion of solvent to dissolve the TPC [30].

Both phenolic and flavonoids contributed to high antioxidant activity in DBP. A strong positive correlation ($r = 0.703$, $p < 0.05$) was detected between TFC and TPC in both extraction methods, emphasizing that both compounds are released through similar mechanisms. Additionally, based on the GLM analysis, it was determined that extraction methods and times, and the interaction between extraction methods and times had a significant ($p < 0.05$) influence on both TPC and TFC. In this study, TFC values were compared between US and WB at different extraction times **(**Fig. 4**(b))**. After 10 min, TFC values were recorded significantly ($p < 0.05$) higher in the US (~36.0 mg CE/g) compared to WB extraction (~29.7 mg CE/g). Employing US extraction for a shorter duration resulted in a higher yield of TFC [31]. US extraction was developed to replace conventional extraction such as WB performed hours to extract plant materials and large quantities of solvents [32]. [33] reported that a longer extraction time (44 min) can increase the TFC yield (5.84 mg RTE/g) in jujube. However, degradation might happen as the extraction rate decreases due to the diffusion of other substances in plant tissue that are attached to the sample surface, which reduces the extraction efficiency.

US extraction demonstrates high efficiency within a short time, as evidenced in this study. For example, TPC and TFC were significantly higher in US-treated samples compared to WB extraction after 10 min. This efficiency arises from the mechanism of acoustic cavitation, which produces micro-jets and shock waves that disrupt plant cell walls, facilitating the release of phenolics and flavonoids. However, prolonged cavitation can generate excessive heat and free radicals, which may oxidize or degrade sensitive bioactive compounds, thereby reducing

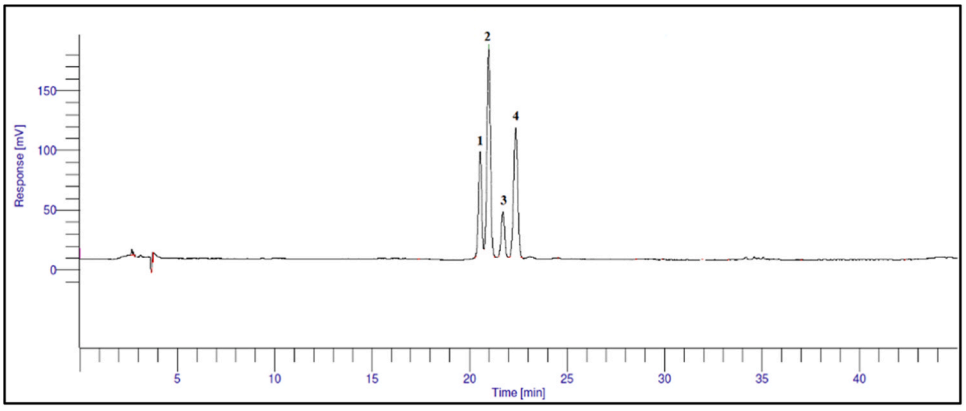


Fig. 2. Typical HPLC chromatograms showing anthocyanins in DBP extracts detected at 520 nm after ultrasonication and water bath-assisted extraction. (1) D3G: Delphinidin-3-*O*-glucoside, (2) D3R: Delphinidin-3-*O*-rutinoside, (3) C3G: Cyanidin-3-*O*-glucoside, and (4) C3R: Cyanidin-3-*O*-rutinoside.

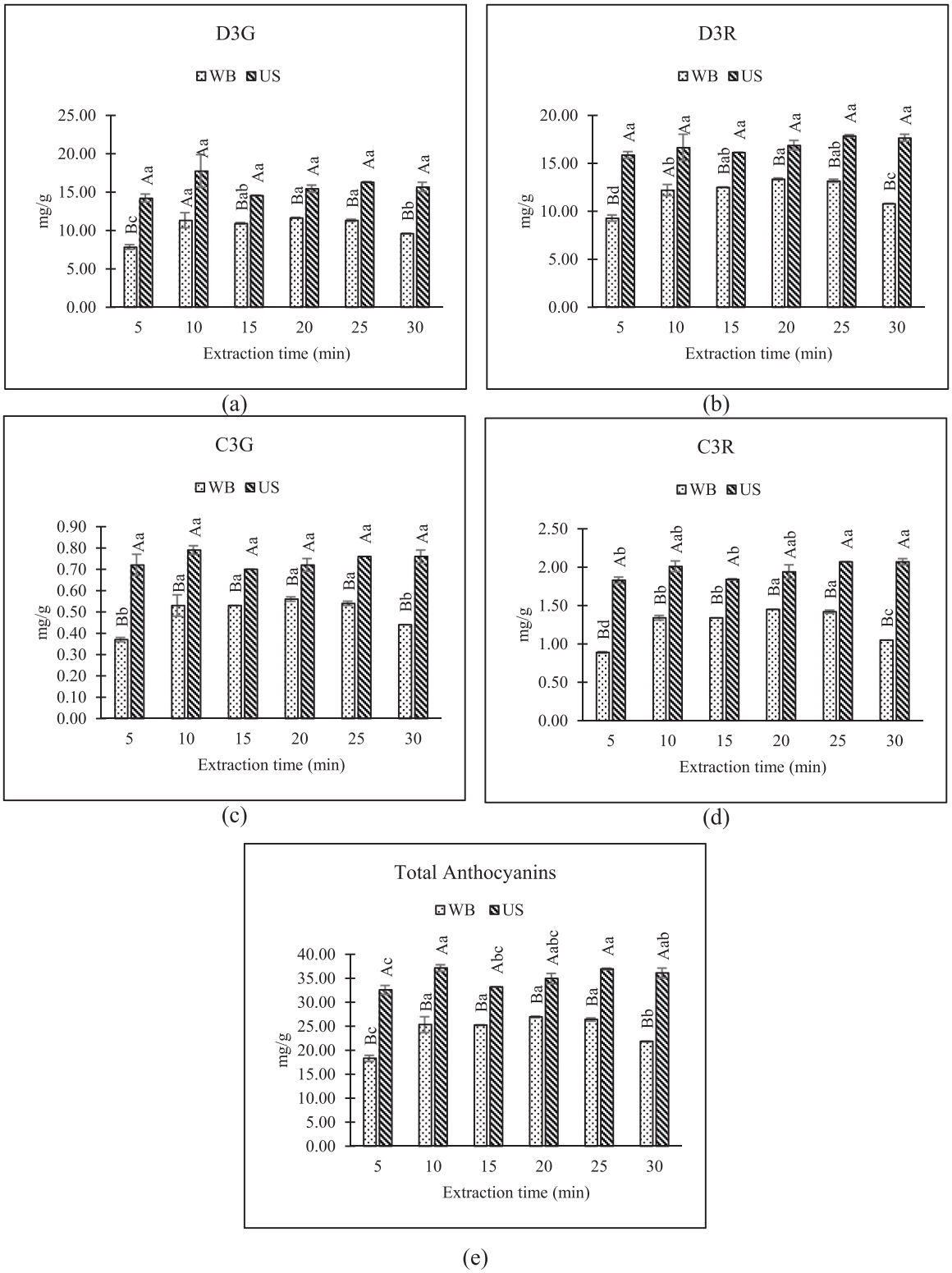


Fig. 3. Anthocyanin content in DBP extract during ultrasonication (US) and water bath (WB) extractions at different times, as measured by HPLC. D3G: Delphinidin-3-*O*-glucoside, D3R: Delphinidin-3-*O*-rutinoside, C3G: Cyanidin-3-*O*-glucoside, and C3R: Cyanidin-3-*O*-rutinoside. Values with different uppercase letters in each extraction time are significantly different ($p < 0.05$). Values with different lowercase letters in each extraction method are significantly different ($p < 0.05$).

their yield. These findings underscore the importance of selecting US extraction parameters to maximize efficiency while minimizing potential degradation of bioactive compounds.

3.4. Antioxidant activities (DPPH and FRAP assays)

Data in Fig. 5 indicated the antioxidant activities that were determined using the DPPH assay. After 20 min, the percentage of DPPH scavenging activity in the US (~45.2 % of DPPH inhibition) was

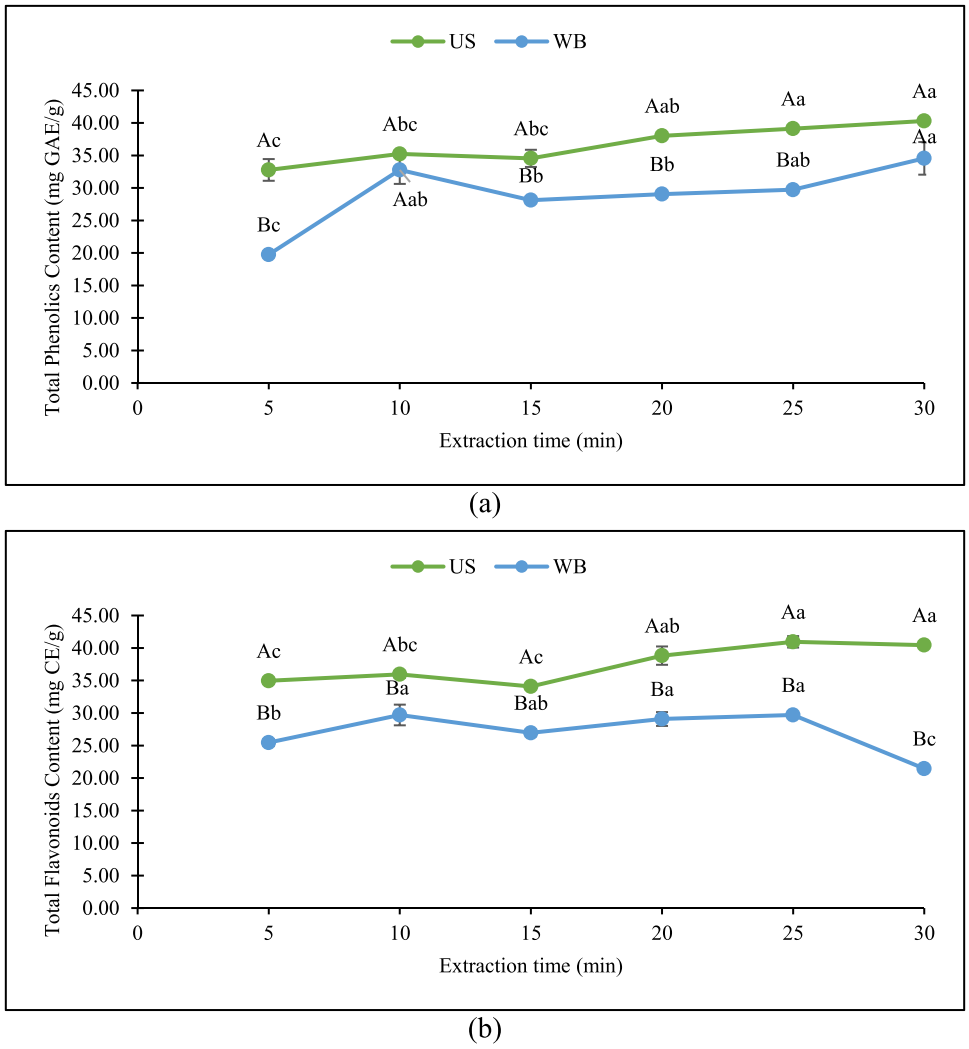


Fig. 4. (a) Total phenolic contents and (b) total flavonoid contents in DBP extracts obtained after ultrasonication (US) and water bath-assisted (WB) extractions at different times, as measured by spectrophotometer. Values with different uppercase letters in each extraction time are significantly different ($p < 0.05$). Values with different lowercase letters in each extraction method are significantly different ($p < 0.05$).

significantly ($p < 0.05$) higher compared to WB extraction (~28.9 % of DPPH inhibition) (Fig. 5**(a)**). Statistical analysis validated that both the extraction methods, times, and their interaction had a notable and statistically significant impact ($p < 0.05$) on the % DPPH inhibition and reducing the power of the DBP extracts. This proved that the US doubled the scavenging activity of DBP extract by 36.1 %, whereas anthocyanins, phenolics, and flavonoids were responsible for antioxidant activity in the blackcurrant. In contrast, [34] reviewed the antioxidant degradation in food products when using ultrasonication including tomato juice [35], watermelon juice [36], and strawberry juice [19]. It should be noted that some parameters might be taken into consideration such as ultrasonication power, temperature, solvent type, solvent-to-ratio, and time that can affect the ultrasonication process.

The values of reducing power determined using FRAP assay were shown in Fig. 5**(b)**. The obtained result showed a significantly ($p < 0.05$) higher reducing power after 10 min through US extraction (~326.0 μmol TE/g) whereas, in the WB, only ~233.0 μmol TE/g of reducing power was recorded. The Pearson correlation showed a strong correlation ($p < 0.05$) between % DPPH inhibition with reducing power ($r = 0.941$), TPC ($r = 0.815$), TFC ($r = 0.894$), and total anthocyanins ($r = 0.916$) indicated that the antioxidant activity of DBP was mainly contributed by phenolics compounds, particularly anthocyanins in both extraction methods.

According to [5], extraction of DBP in the water bath for a longer time (6 h) efficiently extracted total phenolics in DBP extracts that resulted in high antioxidant activity (~48.2 % of DPPH inhibition). However, in this study, a shorter time of extraction using ultrasound efficiently produced extracts with higher antioxidant activity. A previous study by [37] resulted in ultrasonication extraction as a green alternative for grape skin phenolics with a 30 % increase in the yield of phenolic compounds. The strong correlation between TPC and antioxidant activity suggested that other compounds also extracted such as proteins, organic acids, vitamins, and reducing sugars from DBP might also react with antioxidant assays as well as Folin [38].

3.5. *Principal component analysis (PCA)*

PCA was performed to summarize the overall effects of the different extraction methods on the individual anthocyanins, total anthocyanins, TPC, TFC, and antioxidants activities (Fig. 6**a**). PC1 and PC2 were retained based on the cumulative percentage of total variation (96.3 %). As shown (Fig. 6**b**), the first two principal components accounted for 92.3 % PC1 and 4.0 % PC2 of the total variance which was presented in the score and loading plots obtained from the PCA. Extractions by ultrasonication were close to each other and located in the positive PC1 direction, whereas the water bath was in the negative PC1 direction,

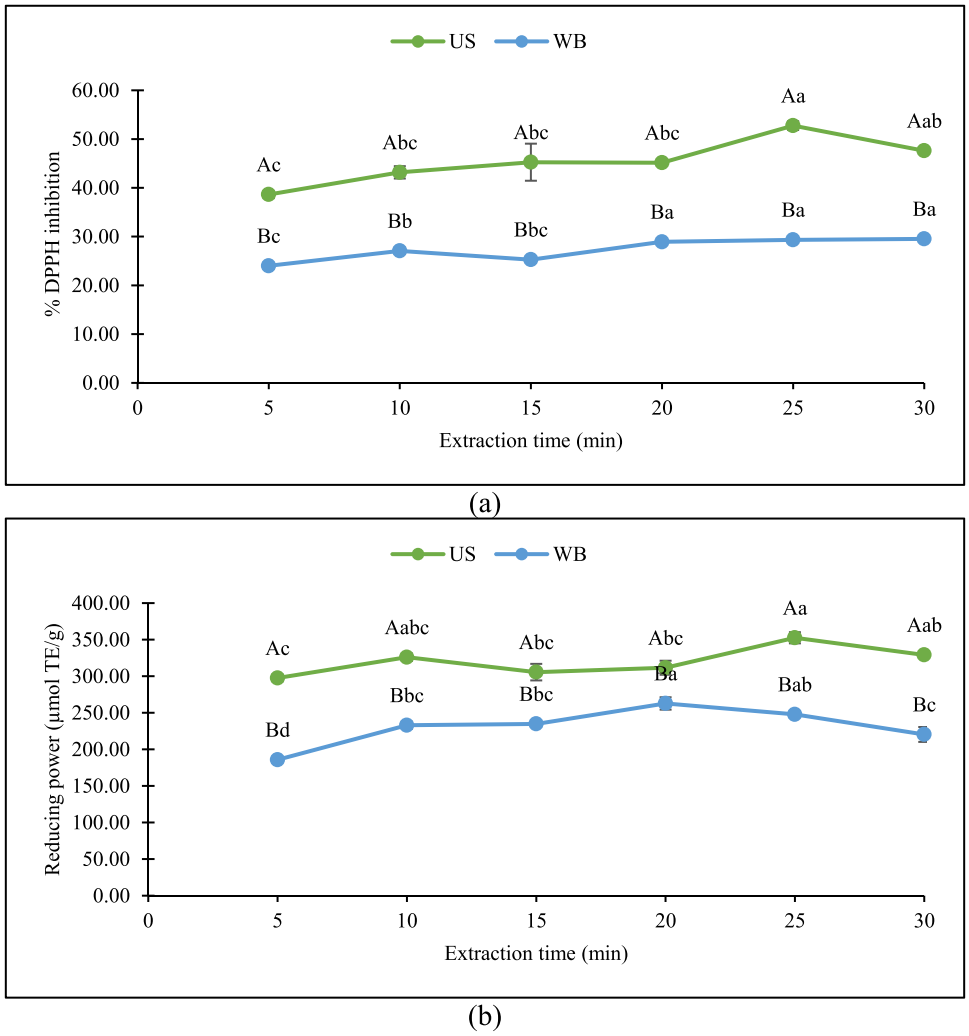


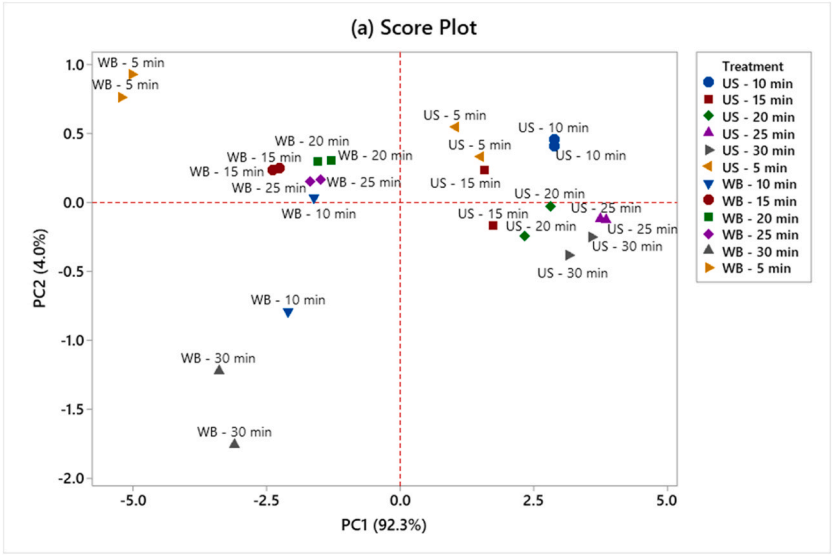
Fig. 5. (a) DPPH radical scavenging activity and (b) reducing power obtained in DBP extracts after ultrasonication and water bath-assisted extractions at different times, as measured by a spectrophotometer. Values with different uppercase letters in each extraction time are significantly different ($p < 0.05$). Values with different lowercase letters in each extraction method are significantly different ($p < 0.05$).

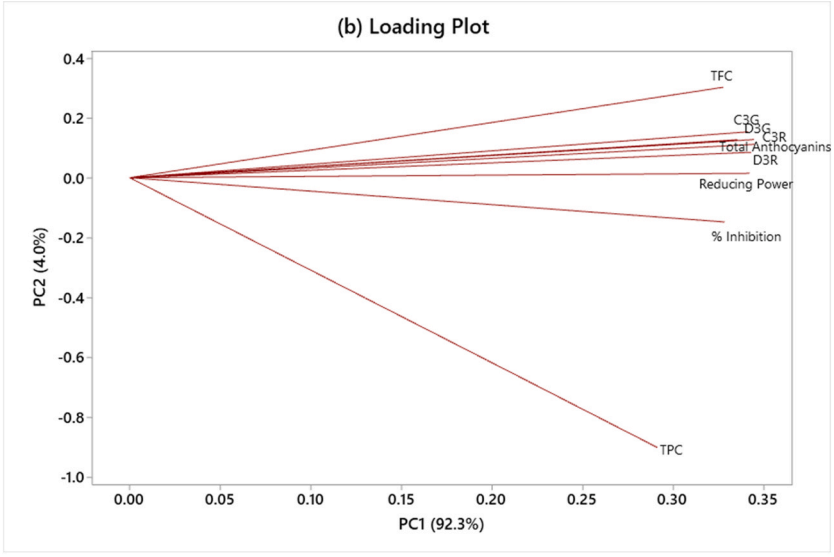
indicating the difference between the extraction methods. Accordingly, the biplot (Fig. 6**c**) showed that extractions using ultrasonication at different times were located on the positive axis of PC1, suggesting favorable results for total anthocyanins, individual anthocyanins, TPC, TFC, and antioxidant activities. This result suggests that ultrasonication extraction provides more effects on the principal components, whereas water baths have significant negative effects on the DBP samples during the extraction process.

Contrary to that, water bath extraction was placed on the opposite side, which showed a weak correlation with the principal components. PCA analysis revealed that ultrasonication has a strong effect on the preservation of phenolic content, and antioxidant activities at different times, mainly at 20 min. This might be related to cavitation effects by ultrasonication that help in the increase of anthocyanins yield, especially anthocyanins that contributed to higher antioxidants in DBP extracts compared to conventional extraction. The heat produced during the ultrasonication effectively helps in the extraction of anthocyanins after 15 min, where the principal components exhibited high positive scores along the PC1 and PC2 axis. Overall, ultrasonication is considered the most efficient method for extraction of DBP that increases the yield of phenolic compounds including anthocyanins and antioxidant activities.

4. Conclusions

Ultrasonication has been regarded as an advanced extraction method that utilizes less amount of energy, time, and solvent, as well as enhancing the extraction yield that serves as an alternative to conventional methods. The yields of anthocyanins and phenolic compounds are affected by the extraction time. The longer extraction time increases the anthocyanins yield asthese thermal-sensitive compounds started to degrade after 25 min in both ultrasonication and water bath extraction. In this study, ultrasonication only required 10 min to extract the highest total anthocyanins, which was ~27 % higher than water bath at 20 min. During the extraction time, ultrasonication exhibited significantly higher antioxidant activities, TPC, and TFC than water baths, indicating that ultrasonication is a suitable alternative for water bath extraction. Overall, DBP extracts obtained from ultrasonication can be potentially used for applications in food matrices as natural colorants after undergoing solvent removal and purification process. This will offer the benefits of efficiency, higher yields, improved quality, reduced solvent usage, and environmental sustainability for both researchers and the food industry. It aligns with the growing consumer demand for natural and high-quality food products while offering opportunities for innovation and cost-effectiveness.





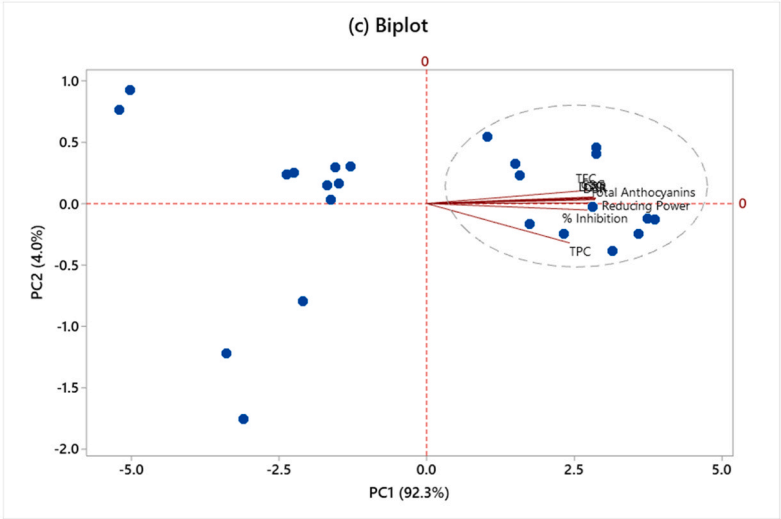


Fig. 6. (a) Score plot, (b) Loading plot, and (c) Biplot from Principal Component Analysis illustrating the effect of different extraction methods and times on DBP extracts. US: Ultrasonication, WB: Water bath, D3G: Delphinidin-3-*O*-glucoside, D3R: Delphinidin-3-*O*-rutinoside, C3R: Cyanidin-3-*O*-rutinoside, C3G: Cyanidin-3-*O*-glucoside, TPC: Total phenolic content, and TFC: Total flavonoid content. Results are expressed as mean ± standard deviation (n = 2).

CRediT authorship contribution statement

Giroon Ijod: Formal analysis. **Nur Izzati Mohamed Nawawi:** Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Ezzat Mohamad Azman:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Nur Allisya Akma Ahmad Khushairi:** Formal analysis.

Acknowledgements

The authors extend their gratitude to the Ministry of Higher Education (MOHE) Malaysia for their financial support of this research through Universiti Putra Malaysia under the Fundamental Research Grant Scheme with Project No. FRGS/1/20202/STG04/UPM/02/6.

Data availability

Data will be made available on request.

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