Effect of dehydration on phenolic compounds and antioxidant activity of blackcurrant (Ribes nigrum L.) pomace

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Effect of dehydration on phenolic compounds and antioxidant activity of blackcurrant (*Ribes nigrum* L.) pomace

Running title: Effect of drying on blackcurrant phenolics

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Abstract

This study examined the effect of dehydration on the phenolic compounds and antioxidant activity of blackcurrant (*Ribes nigrum* L.) pomaces (DBP) subjected to hot air oven drying (HOD), industrial rotary drying (IRD) and freeze drying (FD). Temperature and residence time were evaluated for HOD, whereas air-on and air-off temperature, ratio of drum rotor speed to air speed and particle size were evaluated for IRD. The highest total anthocyanins (ATC) and flavonols (FLV) were obtained in particle size of > 5.0 mm using IRD at 475°C/97°C (air-on/air-off) and higher ratio of drum rotor speed to air speed. Smaller size particles were found susceptible to degradation due to high temperature and retention time applied in IRD, resulting in loss of phenolic compounds in DBP, thus HOD was deemed more suitable. Overall, drying method selection and parameters of operation are key in preserving the concentrations of individual HCA and FLV in DBP.

Keywords: Blackcurrant pomaces; phenolic compounds; antioxidant activity; rotary drying; particle size distribution

INTRODUCTION

Waste management is an essential aspect given the extensive production of plant-based by-products that are often marketed as animal feed (Ajila et al., 2012). Improper management of by-products contributes to high amount of waste and pollutants, which negatively affect the environment (Dubey, 2020). The potential of by-products valorisation as value-added alternatives have recently gained growing attention (O'Shea et al., 2012).

In 2017 alone, 11,000 tonnes of blackcurrants were produced in the UK (IBA, 2018). Blackcurrant skins, which are typically treated as residues from blackcurrant juice processing, are rich in polyphenols and anthocyanins (ATC) (250 mg/100 g of berries) (Vagiri, 2011) as well as flavonols (FLV) and phenolic acids that are linked to high antioxidant activity (Szajdek & Borowska, 2008). The content of ATC in blackcurrant skins is higher than in blackcurrant flesh and seeds. Both FLV and phenolic acids are particularly valuable as dietary supplements or food additives (Lapornik et al., 2005). The extraction of bioactive components from dried mass is more effective than the extraction of these components from fresh mass (Karam et al., 2016), due to phytochemical degradation process occurring more rapidly in high water activity environment. Moisture content of by-products within the range of 6% to 11% (w/w) (Yang et al., 2013) is suggested for higher stability of phytochemicals in pigments, restrained microbial growth, and minimal browning reactions of enzymatic and non-enzymatic origin.

Unlike microwave drying methods and combined convective microwave drying methods, the use of convective drying method has been reported to contribute to the linear degradation of ATC, FLV, hydroxycinnamic acids (HCA) (e.g. chlorogenic acids), and antioxidant capacity in blackcurrant pomace (Michalska et al., 2017b). Convective drying can lead to higher amounts of polyphenols and radical scavenging activity than

conventional drying (Bustos et al. 2018). However, studies on the effect of industrial rotary drying (IRD) on phenolic compounds and antioxidant activity of blackcurrant pomaces remain scarce.

This study examined the effect of hot air oven drying (HOD), IRD, and freeze drying (FD) on phenolic compounds, specifically ATC, HCA and FLV, and antioxidant activity of dried blackcurrant (*Ribes nigrum* L.) pomace (DBP). HOD and IRD represented hot air-drying methods, whereas FD served as control. The temperature and residence time were evaluated for HOD, whereas air-on and air-off temperature, ratio of drum rotor speed to air speed, and particle size were evaluated for IRD.

MATERIALS AND METHODS

Chemicals and solvents

Methanol (99.9%) and hydrochloric acid (HCl, 37%), used in the extraction process, were of analytical grade. Methanol was acquired from Sigma-Aldrich (UK) whereas HCl was acquired from Fisher Scientific (Loughborough, UK). Folin-Ciocalteu reagent, sodium carbonate, and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were similarly acquired from Sigma-Aldrich (UK). A stock solution of 2 mM DPPH in methanol was prepared.

ATC standards of cyanidin-3-O-glucoside (C3G, 96%), cyanidin-3-O-rutinoside (C3R, 96%), delphinidin-3-O-glucoside (D3G, 95%), delphinidin-3-O-rutinoside (D3R, 95%), kaempferol-3-O-glucoside (K3G, 99%), kaempferol-3-O-rutinoside (K3R, 98%), myricetin-3-O-glucoside (MY3G, 99%), and quercetin-3-O-rutinoside (QU3R, 99%) were acquired from ExtraSynthese Ltd (Genay, France). Caffeic acid (98%), ferulic acid (99%), kaempferol (KA, 99%), myricetin (MYR, 98%), *p*-coumaric acid (98%), quercetin (QU, 95%), and quercetin-3-O-glucoside (QU3G, 98%) were acquired from

Sigma-Aldrich (UK). Purified water acquired using a Purite reverse osmosis system (Oxon, UK), was utilised in sample preparation.

Sample preparation

A&R House (BCL) Ltd (Bleadon, Weston-super-Mare, UK) kindly supplied both fresh and dried samples of pressed blackcurrants pomaces from the processing of blackcurrant juice for the use of this study.

Freeze drying (FD- control). 40.0 g fresh blackcurrant pomace were lyophilised (Virtis SP Scientific Model 2KBTES, Stone Ridge, New York) at -45 ± 2°C for 48 h and was used as the control.

Hot air oven drying (HOD). 40 g of fresh blackcurrant pomace were placed on a tray (23 cm × 33 cm) and dried (SalvisLab Thermocenter TC-40T, Rotkreuz, Switzerland) at various temperatures (70°C to 120°C) and two separate residence times, (15 min- short and 30 min-long) and moisture content of samples was recorded with a Halogen Moisture Analyser (HE73, Mettler Toledo, Greifensee, Switzerland).

Industrial rotary drying (IRD). Dried samples of blackcurrant pomaces subjected to different drying parameters of IRD were received from A&R House (BCL) Ltd (Bleadon, Weston-super-Mare, UK) (**Table S1**). Sample A was sieved to different particle sizes (< 0.8 mm, < 5.0 mm, and > 5.0 mm) and a mixture of all particle sizes at a ratio of 1:1:1 (w/w/w) (Mix).

For the preparation of DBP samples, the seeds of blackcurrant samples were removed using a coffee blender by grinding for 30 s to pass through a 0.841 mm (20 mesh) sieve (Michalska et al. 2017a). All samples were separated into polyethylene bags and stored at -20°C for further analysis.

Extraction of phenolic compounds

ATC, HCA and FLV were extracted based on Bao et al. (2005) with sling modifications, in a sample to solvent ratio of 1:10 (w/v). 2.0 g of ground DBP were added into 20 mL of 1% (v/v) HCl in methanol before the mixture was shaken at 180 rpm and 30°C for 24 h. The coloured liquid was vacuum filtered through Whatman No. 1 filter paper (Whatman, Buckinghamshire, UK) using a Buchner funnel to separate the supernatants and residues and 20 mL of fresh solvent was added to the solids for another 24 h. Supernatants were pooled together and kept at -20 ℃ for further analysis. The flow diagram of different drying methods followed by extraction procedures of blackcurrant pomaces is shown in **Figure S1**.

Identification of phenolic compounds by liquid chromatography-mass spectrometry (LC-MS)

The ATC, HCA and FLV profile of freeze dried DBP was obtained (**Table S2**) using a Thermo Scientific Accela HPLC system with a photo diode array (PDA) detector interfaced to a Thermo Scientific LTQ Orbitrap XL mass spectrometer and electrospray ionisation (ESI). Chromatography was carried out on a Zorbax C18 column (250 × 4.6 mm i.d., particle size 5 µm, Agilent) at 25°C. A binary mobile phase was composed of eluent A (acetonitrile/water/formic acid; 5: 92: 3; v/v/v) and eluent B (0.1% formic acid in acetonitrile), in a flowrate of 1.0 mL/min, with 10 10 µL injection volume, on the following gradient elution: 0−20 min, 5 to 25% B; 20−26 min, 25 to 35% B; 26−28.5 min, 35 to 55% B; 28.5−32 min, 55 to 95% B; 32−42 min, 95 to 5% B. The detections were carried out at 520 nm (ATC), 360 nm (FLV) and 320 nm (HCA). Approximately 75% of the analysed sample was diverted to waste using a post PDA splitter. Another 25% was directed into the MS which was operated using an Orbitrap detector in positive and negative ion modes scanning from m/z 85 to m/z 2000, at a scan resolution of

100,000. To obtain the conformation data, the samples were also directly infused into the same MS using similar acquisition settings, and the ions of interest were subjected to MS^2. The MS analysis Qual Browser of Xcalibur software (Thermo Scientific, USA) was used to analyse the acquired data.

Quantification of phenolic compounds by high performance liquid chromatography (HPLC)

A Zorbax C18 column (250 mm × 4.6 mm i.d., particle size of 5 µm, Agilent) in an 1260 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a diode-array detector (DAD), was used to measure the concentration of phenolic compounds in the extracts at 30°C. The mobile phase contained 5% (v/v) formic acid in Milli-Q system (Millipore, Billerica, MA, USA) (solvent A) and 100% (v/v) methanol (solvent B) whereas the gradient elution system involved 15% (B) at 0 min and followed by 35% (B) at 15 min, 60% (B) at 30 min, and finally, 80% (B) at 40 min. The flow rate was set at 1.0 mL/min, while injection volume was fixed at 20 µL. The period of analysis was 50 min. The ATC, FLV and HCA were concurrently detected at varying wavelengths (520, 360, and 320 nm, respectively) (**Figure S2**). The quantification of individual phenolic compounds was carried out using external standard calibration curves.

Total phenols

Folin-Ciocalteu method (Waterman & Mole, 1994) was slightly modified for this study to determine total phenols. 20 µL of appropriately diluted extracts, 1.58 mL of distilled water, and 100 µL of Folin–Ciocalteu reagent were mixed and left for 8 min before 300 µL of sodium carbonate (75 g/L) was added. After 2 h of incubation at 25°C, the absorbance of the samples was measured at 765 nm against a blank sample (water sample) to obtain the average values in terms of milligram of gallic acid equivalent per 1 g of

dried weight (mg GAE/g DW). Gallic acid (0–100 mg/L) served as the standard of calibration curve.

Total antioxidant activity

Total antioxidant activity of DBP extracts was measured according to Blois (1958), with slight modifications. 200 µL of 50-fold diluted extracts and 2 mL of 2 mM methanolic solution of DPPH were mixed and kept in the dark at 30°C for 30 min before the absorbance of the samples was measured at 517 nm. The inhibition (in percentage) was determined based on the following equation:

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

where *Ao* denotes absorbance of the control and *Ae* denotes absorbance of the sample.

Statistical analysis

Minitab V.16 (Minitab Inc., State College, Pennsylvania, USA) was used for data analysis. Apart from one-way analysis of variance (ANOVA), Tukey's multiple range tests were also performed at 0.05 level. In addition, Pearson correlation was conducted to examine the correlations of phenolic compounds (total ATC, FLV and HCA), total phenols, and antioxidant activity of DBP.

RESULTS AND DISCUSSION

Hot air oven drying (HOD)

The initial moisture content of fresh blackcurrant pomaces was 59.82% (w/w). Moisture contents in DBP were varied from 0.78% to 32.65% (w/w) after HOD (**Figure S3**). However, only DBP samples with moisture content less than 10% (w/w) were considered

for further analysis, as suitable to avoid microbial contamination and quality deterioration (Michalska et al. 2017a). ATC was the main phenolic compound (≈66%, R = 0.660) found in DBP. D3R was found to be highest, followed by C3R, D3G, and C3G ($p<0.05$) **(Figure 1a)**. When the temperature exceeded 100°C for 30 min, the ATC content significantly declined (1.0–1.3-fold) ($p < 0.05$) due to their thermal sensitivity (Patras et al., 2010). Moreover, higher drying temperature leads to ATC degradation in shorter time (Bustos et al. 2018). Likewise, Sadilova et al. (2006) revealed no correlation between moisture content and total ATC. In other words, temperature and residence time during HOD substantially affect the yield of ATC, regardless of the sample's moisture content. On the other hand, relatively higher ATC content in freeze-dried DBP suggests that minor modifications during lyophilisation process can prevent the degradation of thermally sensitive pigments such as ATC (Sablani et al., 2011).

DBP samples dried at 110°C for 15 min had moisture content of 8.23% (w/w) and a relatively higher total HCA **(Figure 1b)**. *p*-Coumaric acid was dominant ($p < 0.05$), followed by caffeic and ferulic acid. Between these three compounds, *p*-coumaric and caffeic acid were found higher at 110°C–15 min, whereas ferulic acid was highest in FD sample. Ferulic acid is heat-sensitive and suspectable to oxidation during conventional heating methods (Li et al., 2009). In the current study, dehydration of blackcurrant pomaces in FD prevented the deterioration of ferulic acid. The moisture content and total HCA were found to be moderately correlated ($R = 0.560$, $p < 0.05$). This implies that drying at > 100°C overheated DBP which resulted in HCA deterioration. This is in line with Bustos et al. (2018), who stated that drying berries at 50°C–48 h, 65°C–20 h and 130°C–2 h reduced the moisture contents to same levels, but temperatures of 50°C and 130°C resulted in the degradation of phenolic compounds due to long processing time and high drying temperature, respectively.

DBP samples dried in HOD between 80°C and 120°C for 15 min and 30 min had higher FVL content compared to FD. FLV content was the highest in DBP samples dried at 110°C for 15 min (**Figure 1c**). No correlation could be made between total moisture and FLV content, confirming that the drying parameters in HOD affect the content of FLV in DBP, regardless of the moisture content. This study further identified that in HOD, the concentration of individual FLV was as follows: MY3G > QU3R > QU3G. However, no significant difference was recorded between these FLV in freeze-dried DBP. Zhang et al. (2019) reported that MY3G increased and QU3R decreased during drying pre-treatment at 75°C in *Dryopteris erythrosora* leaves, whereas QU3R has better heat stability than QU3G (Rohn et al. 2007).

The drying process affects total ATC and total phenols as well as the antioxidant activity of the processed sample. As shown in **Figure 2a**, both total phenols and antioxidant activity were found similar in all drying conditions despite the low content of ATC, HCA and FLV in freeze-dried DBP samples. FD, unlike hot air-drying methods, preserves better certain thermally sensitive phenolic compounds (Sogi et al. 2013). Although HOD offers homogenous drying temperature, the drying process may overheat smaller particles at a certain point. Furthermore, Spigno et al. (2007) found that FD did not degrade total phenolic compounds and reduce the antioxidant activity in grape marc. Besides that, Argyropoulos et al. (2011) reported lower shrinkage (from 5% to 15%) and insignificant collapse (lower than 10%) for berries during FD.

HOD appeared to degrade ATC or sugar moieties into smaller molecules, such as aldehydes, and monomeric phenolic acids or their corresponding anthocyanidins, respectively (Keppler & Humpf, 2005; Fleschhut et al., 2006). Michalska et al. (2017b) reported an exponential formation of hydroxymethylfurfural (HMF) in blackcurrant pomace after drying at > 80°C. Also, overheating of DBP samples potentially extracts

phenolic compounds and reducing sugars, proteins, and organic acids that can react with the Folin-Ciocalteu reagent, as shown by medium correlations between total ATC and total phenols ($R = 0.660$, $p < 0.05$) and total phenols and antioxidant activity ($R = 0.784$, $p < 0.05$). Nevertheless, total ATC and antioxidant activity were not correlated, implying that HMF, reducing sugars, proteins, and organic acids contribute more to the antioxidant activity, rather than total ATC alone.

Industrial rotary drying (IRD)

Fresh blackcurrant pomaces were subjected to different drying parameters in IRD and hot air was rapidly raised from 25°C to 450°C or 475°C (air-on temperature) and around 97°C (air-off temperature) at the end of the drying process, where blackcurrant pomaces were adequately dried. The moisture content of the samples ranged from 7.62% to 8.77% (w/w). The results in **Figure 3a** demonstrated that the increase in the ratio of drum rotor speed to air speed significantly increased ATC content ($p < 0.05$). However, the slight differences in the air-on and air-off temperature did not exhibit any changes on ATC content. On the other hand, freeze-dried DBP recorded the lowest ATC content. In IRD, the residence time of particles can be reduced due to the increase in the ratio of drum rotor to air speed. With that, overheating of blackcurrant pomaces and, hence, loss of ATC can be prevented. HCA degraded during gradient heating but increased significantly ($p < 0.05$) in freeze-dried DBP (**Figure 3b**). Moreover, the content of *p*-coumaric acid was found high in DBP samples that were dried in IRD ($p < 0.05$), followed by caffeic and ferulic acid. (**Figure 3c**), The hot air in IRD appeared to assist the extraction of FLV, as compared to FD. MYR was the predominant FLV compound found in DBP, followed by QU. Additionally, the concentration of MY3G was significantly higher ($p > 0.05$) than QU3G and QU3R in IRD. The decrease in the ratio of drum rotor speed to air speed caused longer residence time during blackcurrant pomace processing. Consequently,

lower concentrations of QU3G than QU3R were detected due to degradation of quercetin glycosides to their corresponding aglycones, but QU3R was more stable against heat treatment. Rohn et al. (2007) also revealed that roasting temperature, period, and sugar moiety attached to the flavonol aglycone affected the degradation kinetics of onion quercetin glucosides. However, there was no significant differences between MY3G, QU3G and QU3R in the freeze-dried DBP.

Total ATC was not correlated with the total phenols and antioxidant activity, despite the high content of total ATC in DBP samples that were dried in IRD under varying conditions (**Figure 2b**). Nevertheless, the results of Pearson correlation revealed that total HCA and total phenols in DBP samples were strongly correlated ($R = 0.840$, $p < 0.05$). Meanwhile, the antioxidant activity was found to be strongly correlated with the total HCA ($R = 0.791$, $p < 0.05$) and total phenols ($R = 0.875$, $p < 0.05$). The results clearly demonstrated that the high antioxidant activity is most likely linked to the total HCA in freeze-dried DBP samples.

Different particle sizes of DBP from industrial rotary drying (IRD)

The following drying conditions in the IRD were applied for the DBP samples: (1) air-on temperature of 450°C; (2) air-off temperature of 97°C; (3) decrease in the ratio of drum rotor speed to air speed. The dried DBP samples were then separated into different particle sizes. The moisture content of the samples for each category of particle size was measured: (1) moisture content of 8.34% (w/w) for particle size of > 5.0 mm; (2) moisture content of 8.83% (w/w) for particle size of < 5.0 mm; (3) moisture content of 6.96% (w/w) for particle size of < 0.8 mm; (4) moisture content of 8.06% (w/w) for the mixtures of all particle sizes (Mix). The DBP particle size was presumed to have an effect on ATC and other phenolics content.

The content of ATC in DBP was found significantly ($p < 0.05$) higher for particle size > 5.0 and was the lowest for particle size < 0.8 mm (**Figure 4a**). Lower ratio of drum rotor speed to air speed seems to allow particles of larger size to undergo efficient mass and heat transfer, while particles of smaller size may experience overheating. In this case, the residual temperature of smaller particles potentially exceeds the air-off temperature.

Total HCA in DBP appeared to be the highest ($p < 0.05$) for all particle sizes (**Figure 4b**). *p*-Coumaric acid was the dominant HCA component in DBP ($p < 0.05$), regardless of the particle size. On the other hand, the results for total ATC (**Figure 4a**) and total FLV (**Figure 4c**) in DBP samples for varying particle sizes were similar. Although the particles with size of > 5.0 mm and < 5.0 mm tolerated higher residence time (lower ratio of drum rotor speed to air speed), higher moisture content was recorded, which was reaffirmed by the significant correlation between moisture content and total ATC ($R = 0.645$, $p < 0.05$) and FLV ($R = 0.818$, $p < 0.05$) in DBP. Higher moisture content suggests non-overheated particles and successful preservation of thermally sensitive phenolic compounds, such as ATC and FLV. Samples with particle size of > 5.0 mm showed higher concentration of MY3G than QU, while particle sizes of < 0.8 mm, < 5.0 mm and the mix had higher concentrations of QU than MY3G. Overheating of smaller particle sizes might lead to rapid degradation of QU3G and QU3R and QU aglycone production (Deng et al. 2011). DBP samples exhibited varying amounts of total phenols for different particle sizes, which can be explained by the exposure of surface area to the hot air in the rotary dryer (**Figure 2c**). Despite the above results, particle sizes of > 5.0 mm and < 5.0 mm appeared to be poorly correlated with total ATC, FLV and HCA as well as total phenols. However, the correlation between total phenols and antioxidant activity was intermediate ($R = 0.691$), which may be due the high drying temperature and shorter drying time for DBP. Such drying conditions potentially reduce substances and

nitrogen-containing compounds that can react with the Folin-Ciocalteu reagent, resulting in higher antioxidant activity (Escarpa & González, 2001; Michalska et al., 2016; Bustos et al., 2018).

CONCLUSIONS

This study successfully demonstrated that the application of HOD at lower temperature and longer residence time prevents the degradation of total ATC, whereas higher temperature and shorter residence time (110°C–15 min) prevents the degradation of total HCA and FLV in DBP. Meanwhile, the increase in air-on (475°C) and air-off temperature (97°C) and the ratio of drum rotor speed to air speed were found to directly increase the contents of ATC and FLV. However, the application of IRD was found not appropriate for thermally sensitive HCA. Particles of smaller size are more likely to be damaged by high temperature and retention time in IRD, resulting in the loss of phenolic compounds. The application of FD efficiently retains thermally sensitive phenolic compounds and non-phenolic compounds with high antioxidant activity such as HCA.

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ETHICS APRROVAL STATEMENT

Ethics approval was not required for this research

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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