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Effects of four drying methods on the quality, antioxidant activity and anthocyanin components of blueberry pomace

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Abstract

The effects of drying on the quality, antioxidant activity and anthocyanin components of blueberry pomace were investigated using hot air drying (HAD), freeze-drying (FD), microwave vacuum drying (MVD) and microwave freeze vacuum drying (MFD). The quality, antioxidant activity, and individual anthocyanin compositions of blueberry pomace were evaluated and compared with the non-dried control. MVD produced the highest levels of total phenols, total anthocyanins, total sugars and color values in blueberry pomace while HAD gave the lowest. The ABTS⁺ and DPPH radical scavenging abilities of blueberry pomace were significantly high when MVD was used. Moreover, FD, MVD, and MFD resulted in the retention of significantly more anthocyanin species than HAD as determined by HPLC coupled to the mass spectrometer. Overall, MVD proved to be the best technique for preservation of antioxidant capacity and natural color. Therefore, MVD of blueberry pomace can improve efficiency and productivity of the blueberry fruit processing industry while reducing the environmental burden.

Keywords Blueberry pomace, Drying methods, Antioxidant capacity, Total phenolic, Individual anthocyanin

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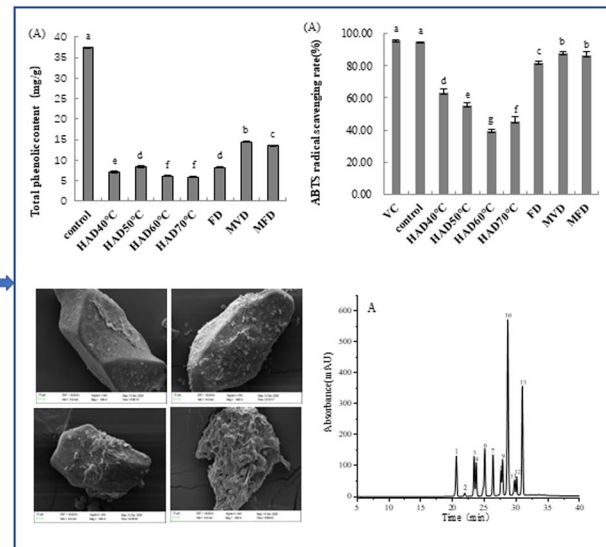


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Graphical Abstract



- Hot air drying (HAD)
- Freeze frying (FD)
- Microwave vacuum drying (MVD)
- Microwave freeze vacuum drying (MFD)



Introduction

Blueberries (*Vaccinium* spp.) are part of the small berry fruit tree of the azalea family, which belongs to a perennial shrub of the genus *Vaccinium*. Blueberries contain a variety of biologically active compounds including anthocyanins, flavonols, and phenolic acids. The main pigment of blueberry is malvidin, an anthocyanin (Cutler et al. 2017). Studies have shown that phenolic compounds and anthocyanins have various biological activities such as preventing brain nerve aging, reducing blood sugar, reducing blood lipid, anti-oxidation, anti-cancer (Johnson et al. 2011) and enhancing immunity (Bornsek et al. 2012). Consumption of foods rich in these compounds may be associated with a reduced risk of cancer as well as improved immune function (Johnson et al. 2011). Therefore, blueberry fresh fruit and its main processed products, mainly blueberry juice and fruit wine are popular among consumers. Inevitably, volumes of blueberry by-products, such as blueberry pomace are yielded in the production process. Blueberry pomace is often discarded or used as animal feed resulting in increased production costs and most likely, a negative environmental impact. However, blueberry pomace is rich in anthocyanins, dietary fiber, and other substances. Compared with synthetic antioxidants, anthocyanins in blueberry pomace have been reported to have higher antioxidant activity and lower production cost (Šarić et al. 2016). Drying blueberry pomace to prepare functional ingredients can greatly improve its application and economic value. Blueberry pomace powder can

be consumed alone or added to other food products as a supplement to improve the color, properties, and nutritional quality of the product, making it more versatile.

The methods used in drying and processing berries mainly include hot air drying (HAD), vacuum freeze-drying (FD), microwave vacuum drying (MVD), and microwave vacuum freeze-drying (MFD). Hot air drying (HAD) plays a pivotal role in the fruit and vegetable drying industry. However, HAD affects the original color, aroma, taste, and nutritional components of the product. The degree of rehydration is poor following HAD which greatly reduces the potential commercial value of the product. However, there is almost no thermal degradation of active ingredients during the FD process due to the low temperature and absence of liquid water resulting in better retention of the bioactive substances in fruits and vegetables. However, this method has a lengthy drying time and high energy consumption.

At present, active investigations are underway which seek to combine HAD, MVD, FD and other technologies in the drying process of berries. Zia and Alibas (2021) studied the effects of MVD, convection drying, and microwave-convection combined drying on blueberry fruit color, anthocyanins, phenolic compounds, and antioxidant activity. The results showed that MVD was far superior to hot air drying in terms of fruit quality. Michalczyk et al. (2009) found that bilberries processed using FD maintained higher polyphenol content, anthocyanin content, and higher antioxidant activity than HAD. The effects of microwave and hot air-combined

drying on the quality of cabbage was studied (Xu et al. 2020). The product quality was found similar to that of vacuum freeze-dried products. Currently, research on berry dehydration mainly focuses on the effects of HAD, MVD, and microwave-assisted drying methods on their physicochemical properties (Yemmireddy et al. 2013). However, research on microwave vacuum freeze drying of blueberry pomace has not been yet reported and the retention of individual anthocyanin species is still to be understood.

The aim of this study was to investigate the effect of drying blueberry pomace using four techniques on the total phenol, total anthocyanin, total sugar content, color index, moisture levels, antioxidant activity and monomeric anthocyanins. The influence of different drying methods on monomeric anthocyanins in blueberry pomace was analyzed using HPLC–ESI–MS to screen the best drying method. This work would contribute to the original findings on blueberry pomace anthocyanins obtained from microwave freeze vacuum drying (MFD) as well in order to provide a reference for the processing of blueberry fruit powder.

Materials and methods

Materials

Fresh blueberries were obtained from Jurong Fengnian Blueberry Planting Base, Jurong City, Jiangsu in China. Folin-Ciocalteu reagent (FCR), 2,2'-azino-bis(3-ethylbenzo-thia-zoline-6-sulfonic acid) (ABTS⁺), 1,1-diphenyl-2-picrylhydrazyl (DPPH), pectinase (EC number 3.2.1.15) enzyme activity of 1.0×10^5 – 6.0×10^5 U/g and cyanidin-3-O-glucoside were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Phenol, vitamin C (Vc), and salicylic acid were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of blueberry pomace

The fresh blueberries was washed, crushed, mixed thoroughly with 0.1% pectinase, and incubated at 50 °C in a water bath for enzymatic hydrolysis for 1 h. The mixture was then cooled to room temperature and centrifuged (TGL-16B centrifuge, Shanghai Anting Scientific Instrument Factory) at 4500 rpm for 20 min. The resulting precipitate of blueberry pomace was collected, stored at 4 °C and served as the raw material for the subsequent experiments (Zhang et al. 2020a, b).

Drying methods of blueberry pomace

For hot air drying (HAD), the pre-treated blueberry pomace was spread uniformly on the metal trays of an oven dryer (DHG-9146A, Shanghai Jinghong Co., Ltd., Shanghai, China) with a thickness less than 1 cm, and drying

time was set at 24 h. The set air temperatures were 40 °C, 50 °C, 60 °C, and 70 °C, respectively. The set air velocity was 0.1 m/s. The end of the drying period was marked by a moisture content of 5% (dry weight) (Yemmireddy et al. 2013; Zia & Alibas 2021).

For freeze frying (FD), the blueberry pomace was dried using the freeze dryer (D-ZD-F12, SANYO Co., Ltd., Japan). The samples were first pre-frozen for 24 h before drying and then placed on trays of the freeze dryer at -49 °C with an absolute pressure of 14 bar for 36 h.

For microwave vacuum drying (MVD), the blueberry pomace was spread on the oven tray which was inserted inside the vacuum microwave dryer (RD-MZK10S, Anhui Ruida Microwave Application Technology Co., Ltd., Anhui, China). The drying conditions were set as follows: temperature of 60 °C, power of 7.5 W/g, vacuum degree of -0.09 Pa, and a drying time of 1 h (Lachowicz et al. (2019); Mitra & Meda. 2009).

For, microwave freeze vacuum drying (MFD), the blueberry pomace was evenly spread on the metal trays, pre-frozen for 24 h, and then placed inside the microwave vacuum freeze dryer (ZY-108HM, Guangzhou Zhiya microwave equipment Co., Ltd., Guangzhou, China). The drying conditions were set as follows: power of 400 W, vacuum degree of -0.08 kPa, the temperature of -49 °C, and a drying time of 4 h.

The samples which did not undergo drying heat treatment served as the control.

Determination of total phenolic content

The samples (1.00 g) were accurately weighed, transferred into the 100-mL conical flask, and mixed with 100 mL distilled water. The flask was placed in a water bath at 35 °C for 2 h. The supernatant of blueberry pomace solution (BPS) was obtained by centrifugation (centrifuge model and manufacturer?) at 10,000 rpm/min for 10 min. The BPS was used for the determinations that followed below.

The total phenolic content was determined by Folin-Ciocalteu colorimetric method (Amoussa et al. 2021) with minor modifications using gallic acid as the standard. One mL aliquot of BPS were transferred into test tubes to which 1.0 mL of 0.2 M FCR and 5 mL of 7% Na₂CO₃ solution were added, respectively. The reaction proceeded under protection from light for 10 min and the absorbance was measured at 765 nm using a spectrophotometer detector (P5 UV–Vis Spectrophotometer, Shanghai Mapada Instruments Co. Ltd., China). The total phenolic content was calculated using the standard curve generated from the formula $y = 0.0194x + 0.0512$ ($R^2 = 0.9995$), where x is the concentration of gallic acid and y is the absorbance value.

The results were expressed as milligrams of gallic acid equivalents per gram of blueberry pomace (mg/g).

Determination of total anthocyanin content

Total anthocyanins content of BPS was determined based on a pH differential method as previously described by Tchabo et al. (2015) and Zhang et al. (2020a, b) using two buffers, namely pH 1.0 buffer (0.2 mol/L KCl:0.2 mol/L HCl=25:67 (v/v)) and pH 4.5 buffer (1 mol/L NaAc:1 mol/L HCl: H₂O=100:60:90 (v/v/v)). The pH was adjusted using acetic acid. A 0.1 mL aliquot of the BPS was transferred to each of the 10 mL volumetric flask and the volume made up with either pH 1 or pH 4.5 buffers respectively. Each of these flasks was stored away from light for 2 h at 25 °C. The absorbance of the test BPS were then detected at 520 nm (λ_{max}) and 700 nm using the UV-vis spectrophotometer detector. Total anthocyanins were calculated as cyanidin-3-O-glucoside according to the following equation:

$$\text{Total anthocyanin content (mg/g)} = \frac{A \times MW \times DF \times V}{\epsilon \times L \times m} \quad (1)$$

where $A = (A_{520} - A_{700})_{pH 1.0} - (A_{520} - A_{700})_{pH 4.5}$; MW is the molecular weight of cyanidin-3-O-glucoside, 449.2 g/mol; V is the volume of solution, mL; DF is the dilution factor; ϵ is the molar extinction coefficient of cyanidin-3-O-glucoside, 26,900 L/mol*cm; L is the optical path, 1 cm, and m is the mass of dry blueberry pomace. The final results were expressed in mg/g.

Determination of total sugar content

Phenol-sulfuric acid was used to determine the total sugar content following a slightly modified method described by (Byung-Taek et al. 2017). A 1.0 mL aliquot of BPS suitably diluted with deionized water was transferred into test tubes where 1.0 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid were added in turn followed by thorough mixing. The mixture was placed inside a constant temperature water bath at 70 °C for 15 min before cooling to room temperature. Anhydrous glucose was used as the standard. The absorbance was measured at 490 nm. The total sugar content was calculated by substituting the standard curve into the formula $y = 0.0336x + 0.0229$ ($R^2 = 0.9964$), where x is the concentration of glucose, and y is the absorbance value.

Total sugar content was calculated using the following equation:

$$\text{Total sugar content (mg/g)} = \frac{c \times v \times N \times 100}{m} \quad (2)$$

where c is the total sugar concentration (mg/mL), v is the volume of solution in the test tube (mL), N is the dilution

multiple, and m is the weight of blueberry pomace (g). Each sample was measured in triplicate.

Color measurement

The color change of blueberry pomace was determined using a chromaticity analyzer (WSC-S chromaticity analyzer, Shanghai Precision Scientific Instrument Co. Ltd., China) (Wang et al. 2015). The larger the L value, the closer the sample is to white. The a value represents red and green values with positive numbers indicating red and negative numerals representing green shades of color. The b value represents yellow and blue values, with positive and negative numerals illustrating yellow and blue shades of color. ΔE refers to the difference between the L , a , and b values of the sample and the standard plate. ΔE was calculated as follows:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (3)$$

where the L value was the brightness coordinate, the a value was the greenness coordinate, and the b value was the yellowness coordinate.

ABTS⁺ radical scavenging activity assay

ABTS⁺ radical scavenging activity of BPS was measured according to a modified method (Amoussa et al. 2021). The ABTS⁺ working solution made by mixing 7.4 mM of ABTS stock solution with 2.6 mM of potassium persulfate (K₂S₂O₈) solution in equal amounts was stored at 4 °C for 12–16 h under light-proof conditions. Then The ABTS⁺ working solution was then diluted with phosphate buffered saline (PBS, 0.1 M, pH 7.4) to an absorbance of 0.70 at 734 nm. A 2 mL of diluted ABTS⁺ working solution was mixed with 2 mL of BPS. The ABTS⁺ radical scavenging effect was calculated as follows:

$$\text{ABTS radical scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (4)$$

where A_0 is absorbance of distilled water and ABTS solution, A_1 is absorbance of BPS with ABTS solution, and A_2 is absorbance of BPS without ABTS solution.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity of BPS was measured according to a reported method (Hu et al. 2020) with some modifications. Briefly, 2.0 mL of ethanol solution of DPPH (0.1 mM) was added to 1.0 mL of the BPS. The mixture was immediately vortexed and stored in the dark for 30 min. The absorbance of the resulting solution was recorded at 517 nm. The DPPH radical scavenging activity was evaluated as follows:

$$\text{DPPH free radical scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (5)$$

where A_0 is absorbance of distilled water and DPPH solution, A_1 is absorbance of BPS with DPPH solution and A_2 is absorbance of BPS without DPPH solution.

Scanning electron microscopy (SEM)

The microstructures of blueberry pomace were observed using scanning electron microscope (EVO-LS10 Scanning electron microscope, Carl Zeiss AG., Germany). The blueberry pomace powder was glued onto the plate with carbon conductive adhesive. The gold was sprayed on the cross section of the sample by ion sputtering apparatus and the microstructure of the cross section of blueberry pomace was observed (Noormets & Olson 2006).

HPLC analysis and quantification of anthocyanins

The monomeric anthocyanins of blueberry was detected by HPLC according to a method reported previously (Zhang et al. 2012). Analysis was carried out using an HPLC apparatus (1260 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump (G1311A) and a diode array detector (DAD, G1315D), and a 250 mm × 4.6 mm, 5 μm particle size, end-capped reverse-phase Eclipse XDB-C18 column (Agilent Technologies, USA), according to our previous report.

Briefly, BPS (what volume?) were mixed with 5% formic acid methanol and centrifuged (centrifuge model and manufacturer?) at 10,000 rpm for 15 min. The supernatant was filtered through a 0.45 μm membrane before analysis. The injection volume was 20 μL and the column temperature was 25 °C. The absorbance at 520 nm was used to quantify anthocyanin. The mobile phases A and B were 1% phosphoric acid and 100% acetonitrile, respectively. The flow rate was 0.6 mL/min. The elution gradient was as follows: 5% B (0–3 min), 10% B (3–10 min), 12% B (10–14 min), 15% B (14–20 min), 18% B (20–24 min), 25% B (24–32 min), 30% B (32–40 min). A calibration curve obtained using cyanidin-3-O-glucoside at 520 nm, was applied for quantification with the equation $y = 9.6285x + 69.654$ ($R^2 = 0.9976$), where y represents the peak area, and x represents concentration of cyanidin-3-O-glucoside (20, 40, 60, 80, and 100 μg/mL). The results were expressed as a microgram of cyanidin-3-O-glucose per one gram of the dried blueberry pomace (μg/g DW).

HPLC–ESI–MS analysis

Individual anthocyanins of blueberry pomace were identified according to our method reported earlier (Chai et al. 2021; Hutabarat et al. 2019) using an HPLC system (1100 Series, Agilent Technologies, Santa Clara, CA,

USA) equipped with a UV detector and LCQ ion-trap mass spectrometer (MS) fitted with an ionization interface (ESI) source (Agilent Technologies, Santa Clara, CA, USA). The analytical column was a Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase and elution gradient was the same as described above. The ESI capillary temperature was 350 °C and the voltage was 3.0 kV in the positive ion mode. A nebulizing gas of 1.5 L/min and a drying gas of 10 L/min were applied for ionization using nitrogen. ESI was performed with the scan range between m/z 100 and 1200. The anthocyanins in the samples were identified and quantified through comparison of the retention times and the spectra of the samples with those of authentic standards as well as literature on similar compounds.

Statistical analysis

All analyses were carried out in triplicates and the results were expressed as mean ± standard deviation (SD). The data were analyzed with a statistical program SPSS v.17 (SPSS Inc., Chicago, IL, USA), Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA). Statistical significance was set at $p < 0.05$ level for differences in phenolic content, sugar content, color and antioxidant activity of the samples dried using four techniques.

Results and discussion

Total phenolic content of blueberry pomace

The total phenolic content (TPC) of blueberry pomace under different drying methods is shown in Fig. 1A. MVD resulted in blueberry pomace with the highest TPC (14.49 mg/g), followed by MFD (13.49 mg/g) while HAD-70 °C (5.88 mg/g) had the least. The TPC decreased from the original level of 37.44 mg/g prior to drying. HAD-70 °C and HAD-60 °C resulted in samples with significantly ($p < 0.05$) lower TPC than MVD and MFD. The latter were more effective in retaining the phenol content in blueberry pomace compared to HAD. Low temperature drying treatment could further concentrate the phenolic compounds in blueberry pomace. Some thermosensitive compounds are likely to be susceptible to degradation due to exposure of blueberry pomace to high temperatures. The decrease to 6.12 mg/g in TPC following drying at 60 °C could be due to some phenolics undergoing isomerization (Zia & Alibas 2021). Likewise, phenolic substances are also susceptible to oxidation reactions by the action of polyphenol oxidase (Murtijaya 2007). MVD has the advantages of high product quality and short drying time, therefore, the loss of

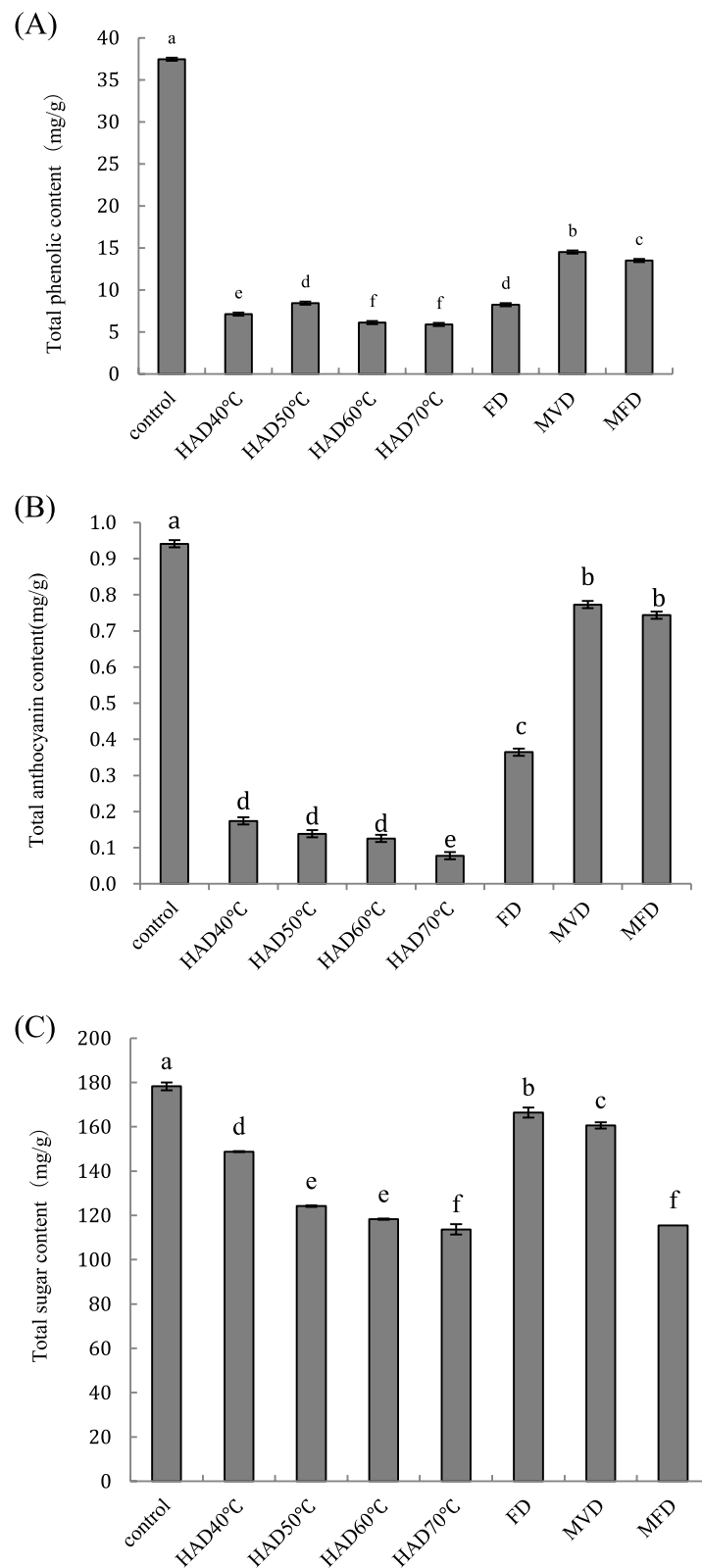


Fig. 1 Effects of different drying methods on total phenolic (A), total anthocyanins (B) and total sugar (C) content of blueberry pomace. Samples that were not dried served as the control. HAD: hot air drying (40, 50, 60, and 70 °C), FD: freeze drying, MVD: microwave vacuum drying, and MFD: microwave freeze vacuum drying

phenolic substances is reduced and the drying efficiency is improved.

Total anthocyanin content of blueberry pomace

The anthocyanin content of blueberry pomace varied under different drying methods (Fig. 1B). Similar to the TPC of blueberry pomace, MVD resulted in the highest anthocyanin content, followed by MFD, while HAD-70 °C had the least. Compared with HAD, MVD was more effective in reducing the degradation of anthocyanins. The anthocyanin contents were significantly ($p < 0.05$) lowered following HAD-70 °C. This may be due to high temperature and hot air causing anthocyanin degradation. Anthocyanins have been shown to be extremely unstable and easily decomposed under the influence of light, temperature, oxygen and other factors (Abdel-Aal & Hucl 2003). The retention of anthocyanins in the pomace was higher because FD and MFD were carried out in a vacuum environment. However, the lengthy drying time, resulted in greater anthocyanin loss with FD than with MFD. MVD uses microwaves to directly heat the sample shortening the drying time and preventing oxidation of anthocyanins to some extent (Shao et al. 2015).

Total sugar content of blueberry pomace

The effects of different drying methods on the total sugar content of blueberry pomace are shown in Fig. 1C. The total sugar content of blueberry pomace obtained following HAD-70 °C significantly decreased ($P < 0.05$), compared with MVD, FD and MFD which yielded 160.62 mg/g, 166.41 mg/g and 115.48 mg/g, respectively. Increasing temperature and prolonged drying time caused both fructose and glucose to undergo browning reactions, thereby reducing the total sugar content (Wall & Gentry 2007). However, the vacuum and low temperature environment of MVD reduced the browning reaction of sugars resulting in better retention of sugar levels. Both FD and MFD reduced browning reaction of sugars under high-frequency electromagnetic fields in the absence of air. Microwaves dehydrated blueberry pomace in a vacuum state, which resulted in a high internal temperature of pomace leading to a reduced total sugar content (Hou et al. 2021).

The color change of blueberry pomace

Color is one of the most important parameters of a food as directly affects the sensory quality of the product. The color was significantly different ($P < 0.05$) when blue berry pomace was dried using the four techniques (Table 1). The *a* values were positive before and after drying, indicating that the samples were red. The *b*

Table 1 Color values of blueberry pomace under different drying processes ^{A,B,C}

Drying process	<i>L</i>	<i>a</i>	<i>b</i>	ΔE
Control	14.86 ± 0.04 ^h	9.23 ± 0.03 ^e	-0.78 ± 0.03 ^g	
HAD(40 °C)	31.85 ± 0.09 ^e	9.69 ± 0.01 ^d	4.62 ± 0.06 ^e	22.35 ± 0.31 ^e
HAD(50 °C)	34.04 ± 0.08 ^c	9.67 ± 0.02 ^d	4.90 ± 0.02 ^d	22.83 ± 0.38 ^d
HAD(60 °C)	29.72 ± 0.37 ^g	7.97 ± 0.02 ^f	5.12 ± 0.01 ^c	25.08 ± 0.08 ^c
HAD(70 °C)	30.43 ± 0.40 ^f	7.47 ± 0.05 ^g	5.64 ± 0.02 ^b	27.09 ± 0.06 ^b
FD	35.51 ± 0.30 ^a	10.82 ± 0.02 ^c	4.08 ± 0.01 ^f	28.59 ± 0.27 ^a
MVD	32.71 ± 0.42 ^d	16.62 ± 0.12 ^b	5.64 ± 0.09 ^b	17.9 ± 0.10 ^g
MFD	34.67 ± 0.03 ^b	17.33 ± 0.05 ^a	6.32 ± 0.03 ^a	19.25 ± 0.05 ^f

A: Data are mean ± S.D. Mean values within the same column with different superscript letters are significantly different ($P < 0.05$) using ANOVA with Duncan's multiple comparison test, $n = 3$

B: *L*: brightness/darkness; *a*: Greenness/redness; *b*: Yellowness/blueness; ΔE : color change

C: HAD Hot air drying (40, 50, 60, and 70 °C), FD Freeze drying, MVD Microwave vacuum drying, and MFD Microwave freeze vacuum drying

values were negative indicating that no browning had occurred. As drying temperature of HAD increased, the *L* and *a* values decreased significantly ($p < 0.05$), while the *b* and ΔE values increased significantly ($p < 0.05$), indicating that the color of blueberry pomace turned brown. MVD resulted in the lowest ΔE value, indicating that the loss of pigment was minimal and the content of anthocyanin was high, consistent with other literature (Zia & Alibas 2021). Compared MVD and MFD resulted in a significant increase in *L* values, indicating an enhancement in the brightness of the samples likely due to the physical changes of the blueberry pomace following prolonged exposure to microwave radiation. The color change of blueberry pomace was mainly due to the reduction in anthocyanin content and browning reaction (Liu et al. 2021).

Antioxidant activity of blueberry pomace

The ABTS⁺ scavenging method determines the total antioxidant capacity of lipophilic and hydrophilic substances and results on blueberry pomace are shown in Fig. 2A. HAD resulted in the least ABTS⁺ scavenging compared to other techniques. ABTS⁺ scavenging ability was more than 80% when FD, MVD and MFD were used to dry blueberry pomace. Figure 2B shows the DPPH free radical scavenging ability of blueberry pomace. DPPH values obtained following HAD-40 °C and HAD-50 °C were similar but significantly higher ($p < 0.05$), than those of pomace dehydrated using HAD-60 °C and HAD-70 °C. In contrast, MVD resulted in pomace with the strongest ability to scavenge DPPH

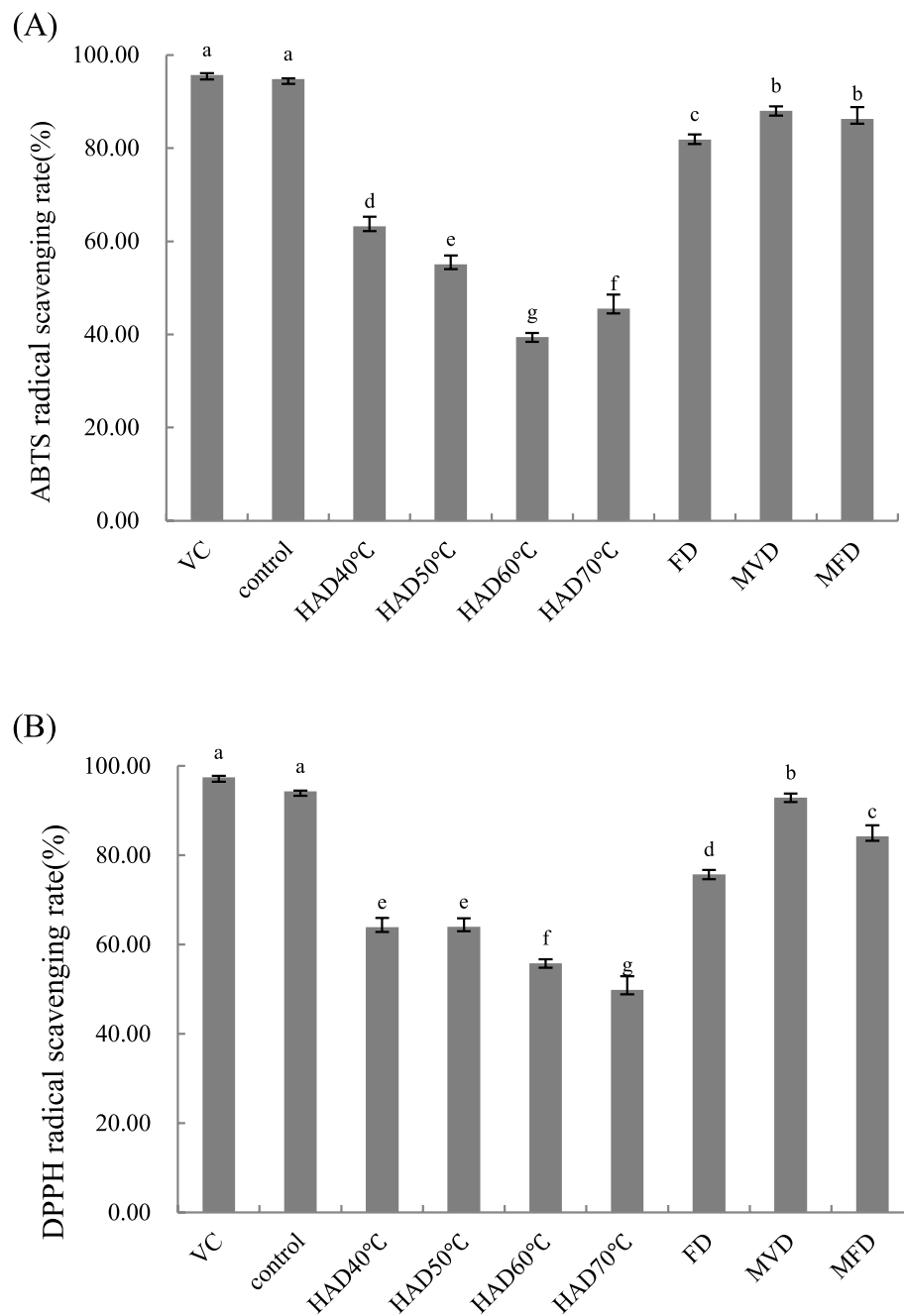


Fig. 2 Effects of different drying methods on the ABTS⁺ (A) and DPPH (B) scavenging ability of blueberry pomace. VC: vitamin C, HAD: hot air drying (40, 50, 60, and 70 °C), FD: freeze drying, MVD: microwave vacuum drying, and MFD: microwave freeze vacuum drying

free radicals up to 95.01%, while HAD-70 °C had the least of only 53%. The results were in consistent with previous reports (Sun et al. 2012).

SEM analysis of blueberry pomace

The quality of blueberry pomace was closely related to its microstructure which was altered during drying.

Blueberry pomace particles were blocky with a relatively tight, surface texture and small tissue voids following HAD (Fig. 3A-D). The surface showed a certain degree of folds and grooves whereas the internal microstructure was more loose and more porous due to increased temperatures. In contrast, the microscopic particles of blueberry pomace following FD was smaller and relatively

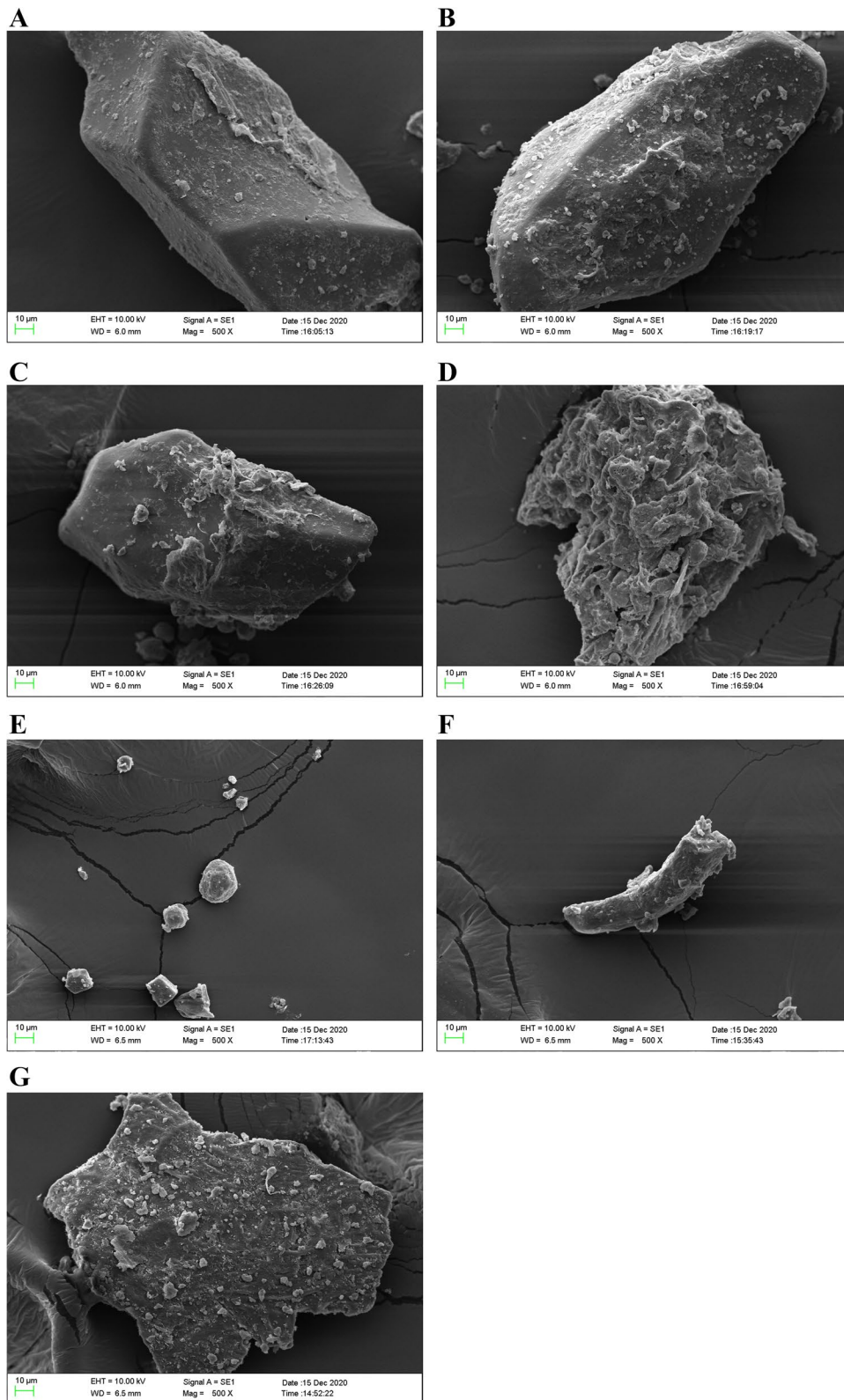


Fig. 3 SEM micrographs of blueberry pomace dehydrated using different drying methods and observed at 5000× magnification. **A** HAD-40 °C, **B** HAD-50 °C, **C** HAD-60 °C, **D** HAD-70 °C, **E** freeze drying (FD), **F** microwave vacuum drying (MVD), and **G** microwave freeze vacuum drying (MFD)

uniform likely due to the low temperature employed compared to other drying methods (Fig. 3E). The blueberry pomace particles appeared flaky following MFD (Fig. 3G). Use of MVD resulted in sample particles that were drier on the surface creating an internal water diffusion rate that was lower than the surface water evaporation rate thereby hindering the internal water diffusion and making the particles more dense in texture (Fig. 3F).

Analysis of anthocyanin components using HPLC and HPLC-ESI-MS

Figure 4 shows that the blueberry pomace contained twelve anthocyanin monomers before drying comprising two main and ten minor peaks with retention times ranging from 20.067 to 31.006 min. Compound 10 with a retention time of 28.74 min was the predominant one (peak 10) followed by compounds with retention times of 25.097 min and 31.006 min (peaks 6 and 13), respectively. Our results are consistent with the reports of Chai et al. (2021) and Hutabarat et al. (2019).

The molecular ion peaks and major ion fragments of blueberry anthocyanins are shown in Table 2. From Table 2, it could be seen that the molecular ion at peak 10 of the major component ($t_R=28.74$ min) has m/z 493.1 and fragment ions appeared at m/z 331, which can be inferred that it is a malvidin derivative. The loss of neutral fragment 162 was consistent with the molecular weight of hexose. According to literature reports (Bueno et al. 2012), the sugar in malvidin galactoside in blueberries is mainly bonded at the C-3 position, and that the glycoside formed by connecting glucose at the C-5 position is very unlikely. Accordingly, it was speculated that peak 10 was malvidin-3-*O*-galactoside.

The molecular ion of peak 3 ($t_R=23.394$ min) has m/z 449.2, and the fragment ion has m/z 287, which is likely formed through loss of a neutral fragment with mass number 162 at m/z 449. However, 132 is the resultant mass number from a molecule of water lost when a five-carbon sugar (150) and an anthocyanin are converted into glycosides. Similar to the anthocyanin in peak 1, the glycoside-forming position is at C3 when there is only one sugar, so it can be inferred that peak 3 is cyanidin-3-*O*-galactoside. The molecular ion and fragment ions of these anthocyanins were compared with those of the anthocyanin standards and literature data. Peaks 10, 3 and 13 were identified as malvidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside and malvidin-3-*O*-galactoside, respectively. By analogy, peaks 1–2, 4–9, and 11–12 were identified in sequence as shown in Table 2.

From Table 2, the three anthocyanins with relatively high content in fresh samples of blueberry pomace were malvidin-3-*O*-galactoside (901.31 $\mu\text{g/g}$) ($t_R=28.740$ min), malvidin-3-*O*-glucoside (515.23 $\mu\text{g/g}$) ($t_R=31.006$ min), and

petunidin-3-*O*-galactoside (269.23 $\mu\text{g/g}$) ($t_R=25.097$ min), respectively. The composition and content of monomeric anthocyanins in blueberry pomace were altered significantly ($P<0.05$) following drying. The levels of 7 anthocyanins decreased with an increase in temperature of HAD. Malvidin-3-*O*-galactoside and malvidin-3-*O*-glucoside were significantly decreased with the increase of drying temperature from 40 to 70 °C. At 70 °C, the levels of two anthocyanins were only 3.92% and 20% while petunidin-3-*O*-galactoside could no longer be detected and seven others were completely degraded. In contrast, FD, MVD, and MFD resulted in significantly higher anthocyanin levels than HAD ($P<0.05$).

The results indicated that drying temperatures severely affected the stability of anthocyanins in blueberry pomace. According to literature (Orsat et al. 2014), anthocyanins are easily degraded during heat treatment at high temperatures. Moreover the high-temperature drying process leads to a decrease in anthocyanin levels in blueberries due to thermal degradation in which high temperature promote the hydrolysis of C3 glycosides in anthocyanins, and /or that the anthocyanin glycosides undergo a hydration reaction to form the pseudoalkaloid form of anthocyanins and then isomerized to form a chalcone and its isomer α -diketone (Sun et al. 2012).

Unlike HAD, more anthocyanins were retained in the samples dehydrated using FD, MVD, and MFD. Three anthocyanins were completely degraded during FD, whereas MVD and MFD retained anthocyanin species. Similar findings on decreased anthocyanin levels were reported recently (Polat et al. 2021) with black carrots dehydrated using FD. Concerning the three major anthocyanins of blueberry pomace, MVD resulted in the highest retention of malvidin-3-*O*-glucoside at 75.94%, while FD and MFD retained 58.86% and 72.4%. The content of malvidin-3-*O*-galactoside (630 $\mu\text{g/g}$) in the residue following MVD and MFD was not significantly different ($p>0.05$). Interestingly, three new anthocyanin compounds were found in blueberry pomace following dehydration using FD, MVD, and MFD. It is possible that the hydroxyl groups on anthocyanin molecules and sugar residues were further combined with some fatty acids and aromatic acids to form acylated anthocyanins (Zhang et al. 2012). Anthocyanins are the main antioxidants in blueberries. Therefore, pomace samples with high anthocyanin content also had high antioxidant activity (Fig. 2). However, further studies are needed regarding which anthocyanins play a major role. Sun et al. (2012) studied the chemical properties and biological activities of different blueberry cultivars, where delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside and delphinidin-3-*O*-arabinoside were relatively more effective in scavenging

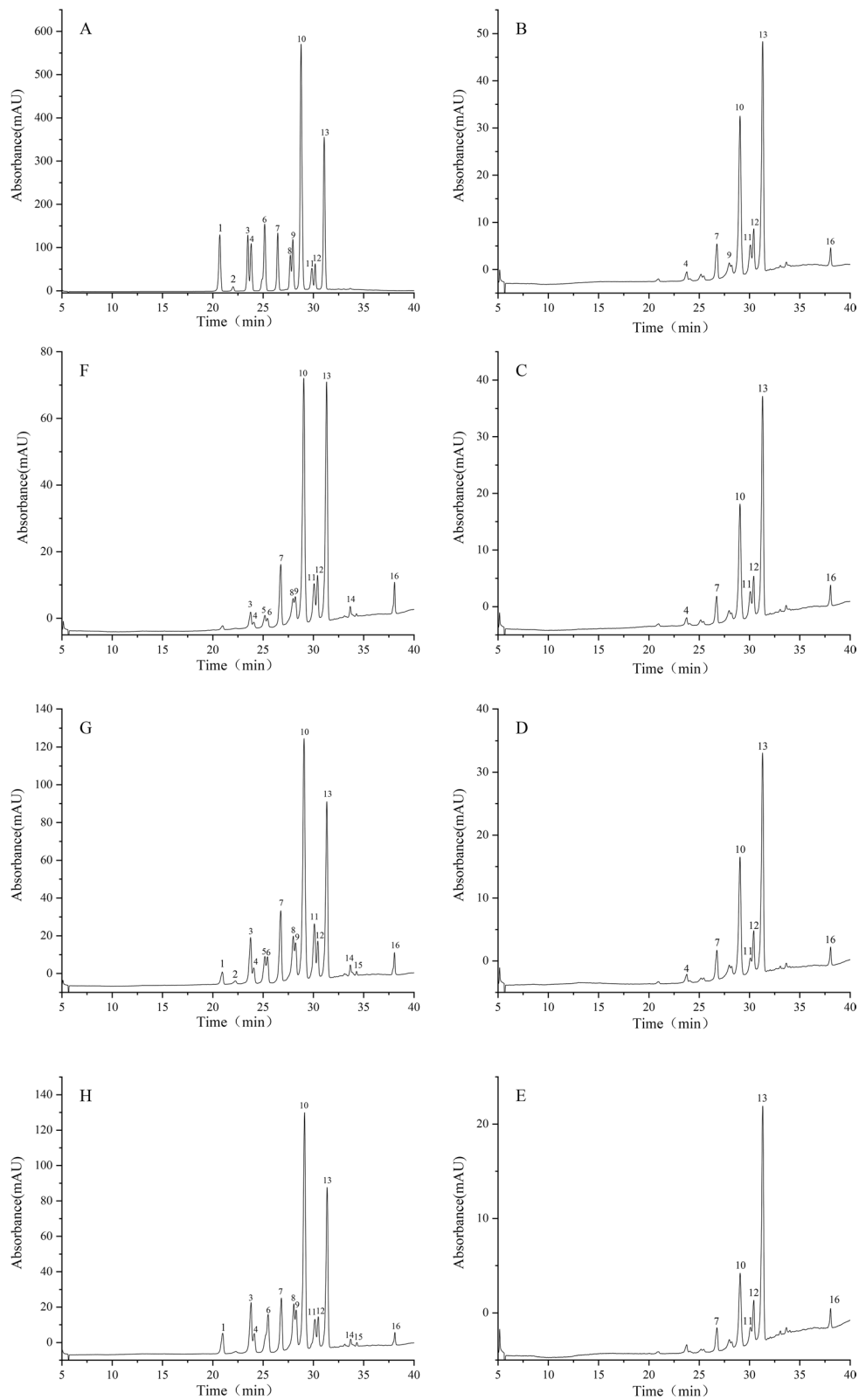


Fig. 4 HPLC chromatograms of blueberry pomace dehydrated using different drying methods. **A** Control, **B** HAD-40 °C, **C** HAD-50 °C, **D** HAD-60 °C, **E** HAD-70 °C, **F** freeze drying (FD), **G** microwave vacuum drying (MVD), and **H** microwave freeze vacuum drying (MFD). The peak number in this figure matches what is displayed in Table 2

Table 2 Individual anthocyanin profile and content of blueberry pomace of different drying methods^{A,B,C}

HPLC peak	Retention time(min)	[M+H(m/z)]	[MS/MS(m/z)]	[Δa.m.u.(m/z)]	Anthocyanin compound	Anthocyanin content (μg/g DW)							
						Control	HAD (40 °C)	HAD (50 °C)	HAD (60 °C)	HAD (70 °C)	FD	MVD	MFD
1	20.905	465.1	303	162	Delphinidin-3-O-galactoside	212.46 ± 3.93 ^b	ND	ND	ND	ND	ND	28.89 ± 0.40 ^c	52.85 ± 1.10 ^b
2	22.254	465.1	303	162	Delphinidin-3-O-glucoside	16.48 ± 1.06 ^a	ND	ND	ND	ND	ND	6.88 ± 0.31 ^b	ND
3	23.761	449.2	287	162	Cyanidin-3-O-galactoside	175.11 ± 1.11 ^a	ND	ND	ND	ND	19.43 ± 0.19 ^d	111.35 ± 0.55 ^c	128.80 ± 0.13 ^b
4	24.073	435.1	303	132	Delphinidin-3-O-arabinoside	155.43 ± 487 ^a	3.91 ± 0.06 ^e	2.00 ± 0.06 ^f	2.01 ± 0.06 ^f	ND	4.32 ± 0.01 ^d	29.68 ± 0.07 ^c	41.48 ± 0.05 ^b
5	25.174	449.2	287	162	Cyanidin-3-O-glucoside	ND	ND	ND	ND	ND	14.40 ± 0.11 ^b	62.86 ± 0.06 ^a	ND
6	25.437	479.1	317	162	Petunidin-3-O-galactoside	269.23 ± 7.12 ^a	ND	ND	ND	ND	7.94 ± 0.35 ^d	51.99 ± 0.83 ^c	127.18 ± 1.00 ^b
7	26.752	479.1	317	162	Petunidin-3-O-glucoside	178.29 ± 152 ^a	28.77 ± 0.36 ^e	17.72 ± 0.32 ^f	17.35 ± 0.02 ^g	7.39 ± 0.04 ^h	76.05 ± 0.14 ^d	165.12 ± 0.61 ^b	127.38 ± 0.42 ^c
8	27.996	463.2	301	162	Peonidin-3-O-galactoside	107.31 ± 9.86 ^c	ND	ND	ND	ND	32.70 ± 0.55 ^d	112.85 ± 1.86 ^b	124.48 ± 3.81 ^a
9	28.229	449.2	317	132	Petunidin-3-O-arabinoside	158.22 ± 3.31 ^a	1.69 ± 0.02 ^e	ND	ND	ND	22.24 ± 0.32 ^d	71.71 ± 1.49 ^c	82.26 ± 1.36 ^b
10	29.071	493.1	331	162	Malvidin-3-O-galactoside	901.31 ± 22.27 ^a	158.95 ± 1.49 ^d	96.91 ± 0.87 ^e	86.40 ± 0.91 ^f	35.35 ± 0.05 ^g	343.48 ± 1.47 ^c	630.73 ± 1.11 ^b	629.75 ± 0.28 ^b
11	30.099	463.1	301	162	Peonidin-3-O-glucoside	80.35 ± 2.85 ^b	23.71 ± 0.02 ^e	17.76 ± 0.27 ^f	7.15 ± 0.11 ^g	4.47 ± 0.19 ^h	48.30 ± 0.48 ^d	132.55 ± 0.87 ^a	73.65 ± 0.59 ^c
12	30.442	463.2	331	132	Malvidin-3-O-arabinoside	69.40 ± 4.93 ^a	27.62 ± 0.68 ^e	21.06 ± 0.34 ^f	19.83 ± 0.23 ^g	12.50 ± 0.24 ^h	41.48 ± 0.33 ^d	59.48 ± 0.76 ^b	53.45 ± 0.60 ^c
13	31.334	493.1	331	162	Malvidin-3-O-glucoside	515.23 ± 26.84 ^a	204.07 ± 0.90 ^e	161.59 ± 0.49 ^f	146.26 ± 0.55 ^g	104.35 ± 0.09 ^h	303.26 ± 0.19 ^d	391.31 ± 1.17 ^b	373.14 ± 0.52 ^c
14	33.676				Unknown acetyl-anthocyanin	ND	ND	ND	ND	ND	8.87 ± 0.70 ^c	19.72 ± 0.55 ^a	13.03 ± 0.47 ^b
15	34.289				Unknown acetyl-anthocyanin	ND	ND	ND	ND	ND	ND	2.29 ± 0.07 ^a	2.40 ± 0.30 ^a
16	38.056				Malvidin-3-O-(6'-acetyl)galactoside	8.42 ± 0.79 ^d	7.73 ± 0.75 ^e	5.79 ± 0.69 ^f	3.25 ± 0.55 ^g	25.02 ± 1.64 ^b	33.09 ± 2.17 ^a	18.77 ± 1.67 ^c	

ND not detected

A: Data are mean ± S.D. Values in the same row with different superscript letters are significantly different ($P < 0.05$) using ANOVA with Duncan's multiple comparison test, $n = 3$

B: Individual anthocyanin content is expressed as micrograms of Cyanidin-3-O-glucoside per gram of sample

C: HAD Hot air drying (40, 50, 60, and 70 °C), FD Freeze drying, MVD Microwave vacuum drying, and MFD Microwave freeze vacuum drying

DPPH free radicals. In addition, the higher antioxidant activity of delphinidin may also be attributed to the substitution of three hydroxyl groups on its B-ring, which is consistent with previously published results on multivariate correlation analysis (Noda et al. 2002). Overall, the loss of anthocyanins in blueberry pomace was minimal following FD, MVD, and MFD with MVD proving to be the best.

Conclusions

In the present study, blueberry pomace powder was obtained using four drying methods. The non-thermal treatment gave higher quality pomace compared to the thermally-treated. FD, MVD, and MFD resulted in higher anthocyanin levels and better antioxidant capacity than HAD. MVD was best at retaining phenolic substances. Considering the lengthy drying times associated with MFD and FD, MVD is a novel, efficient and economical way to dry blueberry pomace. MVD has the advantages of high thermal efficiency of microwave vacuum and high retention of antioxidants while saving costs and time, therefore, providing a theoretical basis for the preparation of dehydrated, high-quality blueberry pomace products during industrial production.

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Authors' contributions

Lixia Zhang: Conceptualization, Methodology, Original draft; Writing-Original draft preparation Supervision. Chenyan Zhang: Data curation, Writing, Software. Wuyang Huang and Zheng Yan: Visualization, Investigation. Zisheng Luo: Software, Validation. Trust Beta and Xueming Xu: Writing- Reviewing and Editing. The author(s) read and approved the final manuscript.

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Availability of data and materials

Data will be made available on request by the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors hereby declare no conflict of interest.

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