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Evaluation of total monomeric anthocyanin, total phenolic content and individual anthocyanins of foam-mat freeze-dried and spray-dried blueberry powder

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Abstract

Anthocyanins in blueberries have generated considerable interest in the scientific community owing to their pleiotropic health benefits against cardiovascular diseases, diabetes and cancer. However, anthocyanins are labile in nature due to their sensitivity to temperature, light, pH, and oxidation and final concentrations in blueberry products is influenced by the processing technique employed. This study evaluated the effect of foam-mat freeze-drying (FMFD) and spray-drying (SD) using a feed rate of 180 and 360 mL/h on the total monomeric anthocyanin (TMA), total phenolic content and contents of individual anthocyanins (cyanidin-3-glucoside (Cyn3Gl), delphinidin-3-glucoside (Del3Gl) and malvidin-3-glucoside (Mal3Gl) in blueberry powders. The protective effect of mixtures of encapsulating agents namely: maltodextrin (MD), trehalose, whey protein isolate (WPI), bovine serum albumin (BSA) and β-lactoglobulin on anthocyanins and phenolic content was also investigated. Anthocyanin content was measured using the pH differential and HPLC methods, while total phenolic content was evaluated using the Folin's assay. The outcome of the study revealed that concentrations of TMA and individual anthocyanins were higher with FMFD (7.11-8.09 mg/g: TMA) compared to SD samples (4.34 -5.69mg/g: TMA). Furthermore, Del3Gl, Cyn3Gl and Mal3Gl retentions were greater, in the order: FMFD > SD 180 = SD 360. Retention of total phenolic ranged from 68-76% and 48-72% in FMFD and SD samples respectively and the choice of the matrix MD/WPI as encapsulating agent was the most effective in protecting blueberry anthocyanins. FMFD is therefore recommended for developing blueberry powders with good retention of anthocyanins.

Keywords: blueberry, phenolic content, anthocyanin, foam-mat freeze-drying, spray-drying

Introduction

Anthocyanins (ACNs) are well-known bioactive compounds found in plants. They are also recognised as natural food colourants. ACN colour varies due to environmental effects such as pH: they are generally red at pH values below 4, colourless at pH 4 to 4.5 and blue at pH 7 and above [1,2]. They usually occur as the glycosides and acyl glycosides of the corresponding anthocyanidins [3]. Wu et al.,[4] recently described 6 of 17 anthocyanidins as particularly prevalent in nature: cyanidin, delphinidin, petunidin, peonidin, pelargonidin and malvidin. The functionality of ACNs had received much attention from researchers due to their possible effects as antioxidant, anticarcinogenic and anti-inflammatory agents [4,5]. Blueberries are classified under the genus Vaccinium and contain considerable amounts of ACNs.[6] The ACN content of blueberries typically ranges from 140 to 820 mg/100 g (of fresh weight), although values well outside this range have also been reported [3].

ACNs in blueberry are labile in nature due to their sensitivity to heating, light, pH, enzymes, metal ions, self-association and oxidation [7]. To improve the retention of ACNs in processed blueberry products a variety of preservation methods have been employed. Among dehydration techniques, spray-drying is the most economical method of drying [8]. In spray-drying natural colourants, etc., are trapped in the coating material added to the solution being sprayed, typically polysaccharides and/or protein. However spray-drying requires high temperatures in operation and allows the feed solution come in contact with hot air for several seconds leading to losses in nutritional quality [9]. Several parameters such as inlet and outlet temperature, feed flow rate, air flow rate, atomizer speed, type of carries or wall materials and concentration of wall materials all influence the physicochemical properties of the final end product and these factors need to be optimized to obtain a proper spray-dried powder [10,11]. Up to now, freeze-drying appears to be best method of dehydration. The benefits of freeze-drying include

reduction of mass and volume, longer shelf life, ease of use of the dry powders as well as retention of colour, shape (of whole berries), aroma, nutrient and phytochemical content [12]. However, the drawbacks of freeze-drying are that it is a much slower process than spray-drying and consumes more energy and thereby increases cost [13]. To overcome these demerits, a novel freeze-drying process known as a foam-mat freeze-drying, which involves a foaming process before freeze-drying, is currently being used [14,15]. This drying process can produce fruit powders that retain all the phytochemicals, sensory attributes and physical properties of the fresh fruit juice [14]. The foaming method also reduces the freeze-drying time and gives greater retention of nutrients [16,17]. A number of encapsulating agents have been employed for the foaming process and several spray-drying experiments have attempted to optimize the type and concentration of carrier agents such as maltodextrin, arabic gum, xanthan gum and whey protein isolate (WPI) [7,10,18–20].

To the best of our knowledge, there is paucity of data on the use foam-mat-freeze-drying and spray-drying for processing of blueberry powder. Furthermore, there are no known studies comparing the impact of these drying techniques (foam-mat-freeze and spray-drying) on bioactive compounds. This is pertinent as the information generated from this kind of research will guide food processors in adopting the best practices to retain bioactive compounds during blueberry processing. In the light of the above, the objective of this study was to determine the changes in total monomeric anthocyanin (TMA), the total phenolic content (TPC), and individual anthocyanin of blueberry powder as affected by foam-mat freeze-drying and spray-drying.

Materials and Methods

Chemical and solvents

Gallic acid, sodium carbonate and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (USA). Commercial standards of delphinidin-3-glucoside, cyanidin-3-

glucoside and malvidin-3-glucoside were purchased from Extrasynthese (Genay Cedex, France). Acetonitrile and methanol (HPLC grade) were purchased from VWR Int. Chemical (France). Polyphenolic standards were dissolved in methanol (Sigma-Aldrich, UK). All other solutions were prepared using ultra-pure water from a Milli-Q Plus system (Millipore Corporate, MA, USA).

Blueberry juice and drying additives

Concentrated organic blueberry juice was purchased from a local supermarket in Leeds, U.K. The blueberry juice was labelled as not containing added water, sugar or additives or preservatives. The juice was stored at 4 ^{0}C after opening. Maltodextrin (MD) (Sigma-Aldrich, USA) 16.5-19.5 dextrose equivalent (DE) (PubChem CID: 107526), trehalose, whey protein isolate (WPI) (Fonterra, NZ), bovine serum albumin (BSA) and β-lactoglobulin (Sigma Aldrich, USA) were employed as foam stabilizing and foaming agents.

Foam-mat freeze-drying (FMFD) and spray-drying (SD) of blueberry juice

Foam-mat freeze-drying and spray-drying (SD) were conducted according to the method of Darniadi et al.,[21] The total solids fraction for all prepared foams for freeze-drying was fixed at 50 g/kg, while spray-drying was at 100 g/kg. Matrices of MD + WPI were prepared with ratios of MD/WPI = 0.4, 1.0, 1.6, 2.3 and 3.2. Foamed blueberry juice was prepared by whipping blueberry juice + matrices (weight ratio of juice to matrices = 95:5) using a Kenwood KM 330 series mixer (Kenwood, UK) in an 8 L stainless beaker, at maximum speed for 5 min and ambient temperature. 85 g of the foam produced were spread on to a round Teflon-coated pan (diameter = 180 mm, height = 30 mm) for each formulation. The foams were blast frozen using a Valera BF051ET blast freezer (Valera, Italy) at –30 ^{0}C for 6 h and freeze-dried using an Alpha 1-4 LD Plus freeze dryer (Christ Martin, Germany) at –55 ^{0}C and a pressure 0.04 mbar,

for 24 h. The dried layer obtained was then ground for 1 min using a Kenwood CH 180A mini chopper food processor (Kenwood, UK).

Spray-drying was carried out at the feed rate 180 (SD 180) and 360 (SD 360) mL/h in a Buchi B-290 mini spray dryer (Buchi Laborthecnik AG, Switzerland). The drying conditions were kept constant for each run with an inlet temperature of 150 ^{0}C, outlet air temperature of 101 ^{0}C, aspirator rate 100 % (35 $m^3 h^{-1}$), air pressure 0.41 bar and nozzle tip diameter 1.5 mm. After each foam-mat freeze-drying and spray-drying run, the blueberry powders were stored in the dark in pre-weighed airtight containers in a refrigerator at 5 ^{0}C, for further analysis. Foam-mat freeze-drying of blueberry juice was also run with the addition of trehalose + bovine serum albumin and trehalose + β-lactoglobulin using ratio of sugar/protein = 2.8. Freeze-drying conditions were similar to the foam-mat freeze-drying using MD/WPI as stated above.

Extraction of anthocyanins

Extraction of anthocyanins was performed both for concentrated blueberry juice and blueberry powders reconstituted in water. For blueberry juice, 1 mL of blueberry juice was mixed with pure water to give 10 mL in a 15 mL Falcon tube. For blueberry powders, 0.5 g of blueberry powder was dissolved with pure water to give 50 mL at ambient temperature, using a magnetic stirrer for 3 min, and transferred to a 50 mL Falcon tube. Both Falcon tubes, 15 mL and 50 mL, were then centrifuged (at 3000 G for 10 min), filtered through a Whatman no.1 filter paper and the filtrate used for the analysis. The extraction was repeated in duplicate.

pH differential method

Blueberry juice, reconstituted FMFD and SD powders were examined for total monomeric anthocyanin based on a pH differential method [22,23]. Each test portion was diluted with pH 1.0 and pH 4.5 buffers and the absorbance measured at both 520 nm and 700 nm using a 6715 UV /VIS spectrophotometer (Jenway, UK). A 10 mm path length glass cuvette was used and the

diluted test portions were read versus a blank cell filled with distilled water. Anthocyanin pigment concentration was then calculated and expressed as cyanidin-3-glucoside equivalents, as follows:

$$\mathbf{TMA\ (mg/L)} = \frac{(\mathbf{A \times MW \times DF \times 1000})}{\boldsymbol{\varepsilon} \times \mathbf{l}} \qquad \text{Eq. 1}$$

where A = (absorbance at 520 nm – absorbance at 700nm) at pH 1.0 – (absorbance at 520 nm – absorbance at 700nm) at pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (Cyn3Gl); DF = dilution factor; l = path length in cm; Ɛ = 26 900 molar extinction coefficient, in L/mol x cm, for Cyn3Gl; and 1000 = factor for conversion from g to mg.

HPLC-PAD (HPLC coupled with photodiode array) for TMA and individual ACNs

HPLC analysis was conducted according to the methods described by Ifie et al., [24]. HPLC identification and quantification of phenolic compounds in blueberry juice and reconstituted powders was carried out using a UFLCXR system (Shimadzu). It consists of a binary pump, a photodiode array with multiple wavelengths (SPD-20A), a Solvent Delivery Module (LC-20AD) coupled with an online unit degasser (DGU-20A3/A5) and a thermostat auto sampler/injector unit (SIL-20A). The photodiode array detector was set to measure at a wavelength of 520 nm.

Identification of anthocyanins in blueberry juice and reconstituted powders was made based on comparison with an external standard of phenolic compounds run under similar conditions regarding the retention time, UV-visible spectrum and spiking of the sample with the corresponding standard phenolic compound. A standard curve was developed using cyanidin-3-glucoside (Cyn3Gl) to express total monomeric anthocyanin (TMA) as mg/g Cyn3Gl solids, while quantification of individual anthocyanins was done by external standards: cyanidin-3-glucoside (Cyn3Gl), delphinidin-3-glucoside (Del3Gl) and malvidin-3-glucoside (Mal3Gl).

Analysis of total phenolic content (TPC)

Analysis of TPC was performed for both the original blueberry juice and reconstituted blueberry powders. The total phenolic content was determined using Folin-Ciocalteau's method [24]. The assay contained 1 mL of concentrated blueberry juice or reconstituted blueberry powder diluted with 80% methanol solution (1:10), 5 mL of diluted Folin-Ciocalteau's phenol reagent (1:10), and 4 mL of 75 g/L sodium carbonate solution. The mixture was then kept in a water bath at 25 ^{0}C, and the absorbance reading measured at 765 nm with a spectrophotometer after 2 h. The estimation of phenolic content was performed using Gallic acid as standard.

Determination of TMA, TPC and individual anthocyanins retention

TPC and ACN retention after drying was calculated according to Fang and Bhandari [11] using the following formula (expressed as dry matter):

$$\textbf{TPC retention}\ (\%) = \mathbf{100\ x}\frac{\textbf{TPC in blueberry powder}}{\textbf{TPC in blueberry juice}} \qquad \text{Eq. 2}$$

$$\boldsymbol{TMA\ retention}\ (\%) = \mathbf{100\ x}\frac{\textbf{TMA in blueberry powder}}{\textbf{TMA in blueberry juice}} \qquad \text{Eq. 3}$$

$$\boldsymbol{Individual\ ACNs\ retention}\ (\%) = \mathbf{100\ x}\frac{\textbf{Del3Gl,Cyn3Gl,Mal3Gl in blueberry powder}}{\textbf{Del3Gl,Cyn3Gl,Mal3Gl in blueberry juice}} \qquad \text{Eq.4}$$

Statistical Analysis

The processing treatments were duplicated, and the means of the results are reported. Two-way ANOVA was performed to establish the presence or absence of significant differences between means. Multiple comparisons were performed using the Tukey's Honest Significant Difference (HSD) test and significance level was set at 0.05. All statistical analysis was carried out using Minitab 17.0.

Results and Discussion

Total monomeric anthocyanin (TMA) measured by pH differential and HPLC methods and TMA retention

Table 1 shows TMA of blueberry juice and foam-mat freeze- and spray-dried blueberry powders measured by the pH differential and HPLC methods. Drying methods and carrier agents significantly ($p < 0.001$) influenced the TMA of blueberry powders. Using the pH differential method, TMA of blueberry juice was 8.92 ± 0.03 mg/g Cyn3Gl blueberry solids, while TMA of FMFD, SD 180, and SD 360 powders ranged from 7.11-8.09, 4.34-5.38, and 5-5.69 mg/g Cyn3Gl blueberry solids, respectively. FMFD powders produced with different MD/WPI ratio showed significant ($p < 0.05$) differences in the TMA compared to those from SD. Regarding FMFD powders, the lowest TMA content was produced with MD/WPI ratio 0.4, while the MD/WPI ratio 3.2 gave the highest TMA. Increasing the MD/WPI ratio resulted in higher monomeric anthocyanin contents of FMFD powders. SD 180 had the lowest TMA (e.g. 4.34 mg/g Cyn3Gl blueberry solids).

The blueberry juice had TMA of 10.03 ± 0.05 mg/g Cyn3Gl solids, while the TMA of FMFD, SD 180, and SD 360 powders ranged between 7.39-8.91, 4.88-7.50, and 5.51-6.12 mg/g Cyn3Gl solids, respectively. The TMA of FMFD powders showed higher levels ($p < 0.05$) than those powders produced via spray-drying, except the sample of SD 180 with MD/WPI 3.2. The TMA of FMFD powders obtained in this study is lower than that of blueberry juice powder (22.69 mg/g Cyn3Gl blueberry solids), and blueberry extract (60.72 mg/g Cyn3Gl blueberry solids) reported elsewhere via freeze-drying [25]. The lower TMA values in SD powders here were in part due to the initially low value of anthocyanins in the original blueberry juice. Furthermore, the inlet air temperature (150 ^{0}C) was higher than in some of these other studies, which is expected to cause greater degradation of anthocyanins in the end product.

Lee et al., [26] reported that the amount of TMA of five berries determined by pH differential and HPLC methods had a high correlation ($r = 0.98$, $p < 0.001$). The TMAs of blueberry from their study were 10.45 and 8.59 mg/g Cyn3Gl dry weight via the pH differential and HPLC methods, respectively. The TMA of blueberry by the pH method were significantly greater than that by HPLC and it was attributed to the variety of anthocyanins present in blueberry. On the contrary, Lee et al., [27] reported lower values of TMA of berry juices using the pH method compared to HPLC method. This results coincides with our study where TMA measured by HPLC showed slightly higher values than pH differential method.

TMA retention values are also presented in Table 1. The TMA retentions measured by HPLC showed no difference compared to those measured by the pH differential method. It is observed that the FMFD samples had better TMA retention (pH method 80-90%, HPLC method 70-80%) compared to SD samples (pH method 49-63%, HPLC method 48-76%). Turan et al., [25] also reported that freeze-drying resulted in up to 90% TMA retention in blueberry powder, while spray-drying gave lower retentions, e.g., 73%.

Total phenolic content (TPC) and TPC retention

The total phenolic content of blueberry juice and reconstituted blueberry powders is also presented in Table 1. Results showed that together both drying methods and carrier agents did not significantly ($p > 0.05$) influence the TPC of the powders obtained. However, ANOVA indicated that the total phenolic content significantly ($p < 0.05$) varied with drying methods.

The average TPC of FMFD powders was 31.3 mg/g GAE solids, which was statistically ($p < 0.05$) higher than SD 180 samples (25.4 mg/g GAE solids) but not significantly different with SD 360 samples (26.7 mg/g GAE solids) (Data not shown). MD/WPI ratios did not appear to be a major factor for TPC values in FMFD process. However, increasing the MD/WPI ratio resulted in increasing TPC levels with SD 180 and SD 360. Maltodextrin is responsible for

forming a dry crust around the droplets and the phenolic compounds might be protected from heat exposure by this dry crust during spray-drying [20]. Overall, it is seen that SD processing resulted in considerably lower TPC compared to the FMFD method.

As observed in Table 1, the TPC retention was 68-76%, 48-69%, and 54-72% for FMFD, SD 180, and SD 360, respectively. From this table, it is also observed that increasing the MD/WPI ratio improved the TPC retention. There was no significant ($p > 0.05$) effect of drying methods and carrier agents on the TPC retention. However, drying methods significantly ($p < 0.05$) influenced the TPC in blueberry powders, where average FMFD powders gave the highest TPC retention (73%). SD 180 and SD 360 gave low TPC retention, i.e., 60 and 62%, respectively (Data not shown). Franceschinis et al., [28] reported comparison of freeze- and spray-drying using maltodextrin as a carrier agent in producing blueberry powders. According to the authors, total phenolic content retention of these two drying methods was 73 and 68%, for freeze-drying and spray-drying, respectively. Our results are in agreement with this study.

Individual anthocyanins

Anthocyanins extracted from blueberry juice and reconstituted powders were analysed by HPLC-PAD. In this study, three external standards were utilised: delphinidin-3-glucoside (Del3Gl), cyanidin-3-glucoside (Cyn3Gl) and malvidin-3-glucoside (Mal3Gl). The retention times of these Del3Gl, Cyn3Gl and Mal3Gl standards were 15.2, 17.5 and 21.5 min, respectively (Figure 1A) with peak heights 12.3, 15.2 and 12.2 $mAUx10^{-4}$, respectively. More than 10 peaks were visible between retention times 13.5 to 34.0 min in blueberry juice (Figure 1B), and compounds with retention times (15.2, 17.5 and 21.5 min) with corresponding peak heights of 3.7, 3.5 and 2.5 $mAUx10^{-4}$, respectively, were identified as Del3Gl, Cyn3Gl and Mal3Gl respectively based on similar retention time with standard compounds, spiking of samples and previous descriptions of the main anthocyanins in blueberry juice [26,29].

The concentration of individual anthocyanins from blueberry juice and reconstituted powders are presented in Table 2. Del3Gl of the blueberry juice sample (1.38 mg/g blueberry solids) was higher than that measured in all reconstituted powder samples. FMFD samples had 1.09-1.17 mg/g solids, while 0.62-0.77 mg/g solids was observed with SD samples. All Del3Gl concentrations measured from FMFD samples were significantly ($p < 0.001$) higher than SD powders, most probably due to the higher temperatures of spray-drying.

Cyn3Gl of the blueberry juice was 1.33 mg/g blueberry solids. FMFD samples had 1.32-1.37 mg/g solids, while 0.79-0.98 mg/g solids was observed with SD samples. The blueberry juice and FMFD samples had higher Cyn3Gl concentration than the SD samples. SD 180 caused a 35% reduction of Cyn3Gl except for the MD/WPI 3.2 samples, and SD 360 gave a slightly lower reduction (< 32%). Mal3Gl concentration in the blueberry juice was measured as 1 mg/g blueberry solids. FMFD samples had Mal3GL concentration of 0.80-0.88 mg/g solids, while 0.50-0.58 mg/g solids was observed with SD samples. FMFD samples exhibited significantly ($p < 0.001$) higher Mal3Gl concentrations compared to SD samples.

Cyn3Gl was found to be at a higher level, followed by Del3Gl and Mal3Gl in FMFD reconstituted powders, which is in line with the findings of Trost et al., [30]. However, Lee et al., [26] found that levels of Del3Gl, Cyn3Gl, and Mal3Gl from conventional freeze-dried blueberry powder were 1.43, 0.27 and 2.0 mg/g solids respectively.

In the case of individual anthocyanin retention, FMFD samples had higher Del3Gl retention (79-85%) compared to SD samples (45-55%). Cyn3Gl retention of FMFD was the highest (>95%), while Cyn3Gl of SD samples was 58-72%. Mal3Gl retention was calculated as 80-88% in FMFD samples but only 50-58% in SD samples.

Properties of FMFD powders produced with trehalose and pure proteins

Foam-mat freeze-drying was also conducted with alternative matrices of trehalose + bovine serum albumin/BSA (T3A1) and trehalose + pure β-lactoglobulin (T3B1) at the ratio of sugar: protein = 2.8. Trehalose was chosen because of its supposed special properties in acting as a cryoprotective agent [31] and pure β-lactoglobulin because it is the main surface active ingredient of WPI [32]. BSA has been used in other model studies of foaming [33] and although these agents are more expensive than maltodextrin or WPI, it was of interest to see if the more pure ingredients conferred any particular advantages. The FMFD powder produced with MD/WPI (M3W1) was used for comparison.

Figure 2-A shows total phenolic content (TPC) and total monomeric anthocyanins (TMA) of foam-mat freeze-dried made with three different carrier agents. It was observed that M3W1 was superior ($p < 0.05$) in TPC and TMA content compared to both samples made with pure proteins. The M3W1 had 31.5 mg/g GAE solids and 8.5 mg/g Cyn3Gl solids for the TPC and TMA, respectively. The TPC of T3BL1 and T3A1 was calculated as 17.7 ± 0.08 and 14.5 ± 0.3 mg/g GAE solids, respectively. In the case of TMA content, T3BL1 and T3A1 had 5.9 ± 0.33 and 5.1 ± 0.09 mg/g Cyn3Gl solids, respectively. This occurence could be attributed to the maltodextrin used in M3W1 sample, which was better at preserving phenolic compounds and anthocyanins compared to trehalose-treated samples [28,34]. This provoked higher retention of TPC and TMA in M3W1 powder recorded as 73 and 95% respectively compared with T3BL1 and T3A1 samples which showed lower retention of TPC and TMA, i.e. < 60% (Figure 2-B). Franceschinis et al., [28] reported 73 and 75% of TPC and TMA retention respectively of freeze-dried blackberry powder made with maltodextrin. However, the M3W1 sample in our study showed lower TPC retention when compared to the freeze-dried blueberry powder applying the same wall material (TPC retention: 95%) reported elsewhere [25]. This may be related to the blueberry cultivar that was used to produce the juice.

Individual anthocyanins of foam-mat freeze-dried produced with T3BL1, T3A1, and M3W1 are presented in Figure 3A. Del3Gl, Cyn3Gl and Mal3Gl concentration were found to be highest in M3W1. The concentration of Del3Gl, Cyn3Gl, and Mal3Gl were recorded as 1.17., 1.38, and 0.85 mg/g solids, respectively. Retention of individual anthocyanins is shown in Figure 3B. M3W1 prevented the degradation of anthocyanins best in the foam-mat freeze-dried samples. Del3Gl retention by M3W1 was calculated as 85%, while 46 and 48% was recorded for T3BL1 and T3A1 powders respectively. In the case of Cyn3Gl retention, M3W1 recovered >95%, whereas T3BL1 and T3A1 recovered 64 and 69%, respectively. Again, M3W1 gave a high retention of Mal3Gl, i.e. 85%. Conversely, both powder samples made with pure protein recovered 52-53%.

Conclusion

Blueberry powders were successfully prepared with MD (DE 16.5-19.5) and WPI using foam-mat freeze-drying (FMFD) and spray-drying (SD). Concentrations of total monomeric anthocyanin (TMA) and individual anthocyanins were higher with FMFD compared to SD samples. The choice of matrix MD/WPI as encapsulating agent was the most effective in protecting blueberry anthocyanins. However, it is recommended that the efficiency of MD/WPI as FMFD additive needs studied further. Overall, FMFD is recommended for developing blueberry powders with good retention of anthocyanins.

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Table 1. Effect of drying methods and MD/WPI ratios on total monomeric anthocyanin (TMA), total phenolic content (TPC), TMA retention, and TPC retention by pH and HPLC methods

Sample	MD/WPI	TMA*		TMA retention (%)		TPC**	TPC retention (%)
		pH	HPLC	pH	HPLC		
Blueberry juice	0	8.92 ± 0.03 ^{a}	10.03 ± 0.50 ^{a}	--	--	43.25 ± 0.01	--
FMFD powder	0.4	7.11 ± 0.04 ^{c}	8.91 ± 0.66 ^{b}	80 ^{b}	89 ^{a}	29.43 ± 5.13	68 ± 8
	1.0	7.26 ± 0.13 ^{c}	8.42 ± 0.33 ^{b}	82 ^{b}	84 ^{a}	30.53 ± 4.66	71 ± 8
	1.6	7.28 ± 0.16 ^{c}	7.60 ± 2.18 ^{bc}	82 ^{b}	76 ^{bc}	32.18 ± 4.84	74 ± 8
	2.3	7.85 ± 0.07 ^{b}	7.39 ± 1.38 ^{bcd}	83 ^{b}	74 ^{ab}	32.84 ± 4.21	76 ± 7
	3.2	8.09 ± 0.10 ^{b}	8.45 ± 0.11 ^{b}	90 ^{a}	84 ^{ab}	31.73 ± 6.36	73 ± 10
SD 180 powder	0.4	4.34 ± 0.08 ^{g}	5.35 ± 0.11 ^{de}	49 ^{e}	53 ^{d}	20.84 ± 3.62	48 ± 6
	1.0	4.84 ± 0.21 ^{f}	5.21 ± 0.16 ^{e}	55 ± 2 ^{de}	52 ^{d}	21.86 ± 3.26	51 ± 5
	1.6	5.13 ± 0.21 ^{ef}	4.88 ± 0.19 ^{e}	57 ± 2 ^{cd}	49 ^{d}	25.27 ± 0.39	58 ± 1
	2.3	5.29 ± 0.22 ^{def}	5.37 ± 0.04 ^{de}	59 ± 2 ^{cd}	54 ^{d}	29.10 ± 3.88	67 ± 6
	3.2	5.38 ± 0.19 ^{de}	7.50 ± 0.41 ^{bc}	60 ^{cd}	75 ^{ab}	29.87 ± 6.42	69 ± 11
SD 360 powder	0.4	5.00 ± 0.15 ^{ef}	5.51 ± 0.01 ^{cde}	55 ± 1 ^{de}	55 ^{d}	23.31 ± 3.73	54 ± 6
	1.0	5.27 ± 0.17 ^{def}	5.84 ± 0.04 ^{cde}	60 ^{cd}	58 ^{cd}	24.99 ± 1.88	58 ± 3
	1.6	5.46 ± 0.26 ^{de}	5.78 ± 0.01 ^{cde}	63 ± 2 ^{c}	58 ^{cd}	25.38 ± 2.44	59 ± 4
	2.3	5.65 ± 0.15 ^{d}	5.93 ± 0.02 ^{cde}	61 ± 3 ^{cd}	59 ^{cd}	28.52 ± 2.62	66 ± 5
	3.2	5.69 ± 0.17 ^{d}	6.12 ± 0.06 ^{cde}	63 ± 1 ^{c}	61 ^{cd}	31.14 ± 6.35	72 ± 10

Means ± SD values of n=4 measurements followed by different single letter in a column are significantly different ($p < 0.05$, Tukey HSD test). *Results expressed in mg/g Cyn3Gl equivalent (blueberry solids). ** mg/g GAE blueberry solids

Table 2 Effect of drying methods and MD/WPI ratios on individual anthocyanins

Sample	MD/WPI	Del3Gl*	Del3Gl retention (%)	Cyn3Gl*	Cyn3Gl retention (%)	Mal3Gl*	Mal3Gl retention (%)
Blueberry juice	0	1.38 ± 0.06 ^{a}	--	1.33 ± 0.07 ^{a}	--	1 ± 0.08 ^{a}	--
FMFD powder	0.4	1.19 ± 0.11 ^{b}	85 ± 7 ^{a}	1.39 ± 0.08 ^{a}	103 ± 6 ^{a}	0.90 ± 0.08 ^{ab}	88 ± 7 ^{a}
	1.0	1.11 + 0.05 ^{b}	82 ± 1 ^{a}	1.34 ± 0.04 ^{a}	103 ± 2 ^{a}	0.84± 0.04 ^{b}	86 ± 2 ^{a}
	1.6	1.13 + 0.08 ^{b}	79 ^{a}	1.39 ± 0.06 ^{a}	103 ± 3 ^{a}	0.90 ± 0.05 ^{ab}	88 ± 3 ^{a}
	2.3	1.09 ± 0.01 ^{b}	79 ± 1 ^{a}	1.32 ± 0.01 ^{a}	100 ^{ab}	0.79 ± 0.03 ^{b}	80 ± 1 ^{a}
	3.2	1.12 ± 0.01 ^{b}	85 ± 3 ^{a}	1.34 ± 0.01 ^{a}	102 ^{a}	0.84 ± 0.02 ^{b}	83 ± 1 ^{a}
SD 180 powder	0.4	0.74 ± 0.01 ^{cd}	53 ^{b}	0.85 ± 0.01 ^{def}	63 ^{cde}	0.57 ± 0.02 ^{c}	58 ± 1 ^{b}
	1.0	0.72 ± 0.04 ^{cd}	53 ± 3 ^{b}	0.82 ± 0.01 ^{ef}	60 ^{de}	0.56 ± 0.02 ^{c}	55 ± 1 ^{b}
	1.6	0.66 ± 0.03 ^{d}	49 ± 1 ^{b}	0.78 ± 0.04 ^{f}	58 ± 2 ^{e}	0.51 ± 0.03 ^{c}	52 ± 3 ^{b}
	2.3	0.76 ± 0.01 ^{c}	55 ^{b}	0.85 ± 0.0 ^{def}	63 ^{cde}	0.57 ± 0.01 ^{c}	57 ^{b}
	3.2	1.12 ± 0.06 ^{b}	79 ± 3 ^{a}	1.18 ± 0.04 ^{b}	87 ± 2 ^{b}	0.79 ± 0.06 ^{b}	76 ± 4 ^{c}
SD 360 powder	0.4	0.61 ± 0.01 ^{cd}	45 ^{b}	0.85 ± 0.01 ^{def}	63 ^{cde}	0.49 ± 0.01 ^{c}	50 ± 1 ^{b}
	1.0	0.67 ± 0.01 ^{cd}	49 ^{b}	0.92 ± 0.01 ^{cde}	68 ± 1 ^{cde}	0.52 ± 0.0 ^{c}	52 ^{b}
	1.6	0.66 ± 0.0 ^{cd}	48 ^{b}	0.91 ± 0.01 ^{cde}	67 ^{cde}	0.51 ± 0.0 ^{c}	51 ^{b}
	2.3	0.69 ± 0.01 ^{cd}	50 ^{b}	0.93 ± 0.0 ^{cd}	69 ^{cd}	0.54 ± 0.01 ^{c}	54 ^{b}
	3.2	0.72 ± 0.04 ^{cd}	52 ± 2 ^{b}	0.97 ± 0.01 ^{c}	72 ^{c}	0.56 ± 0.02 ^{c}	57 ^{b}

Means values ± SD of n=4 *measurements followed by different single letter in a column are significantly different ($p < 0.05$, Tukey's HSD test).* *mg/g blueberry solids

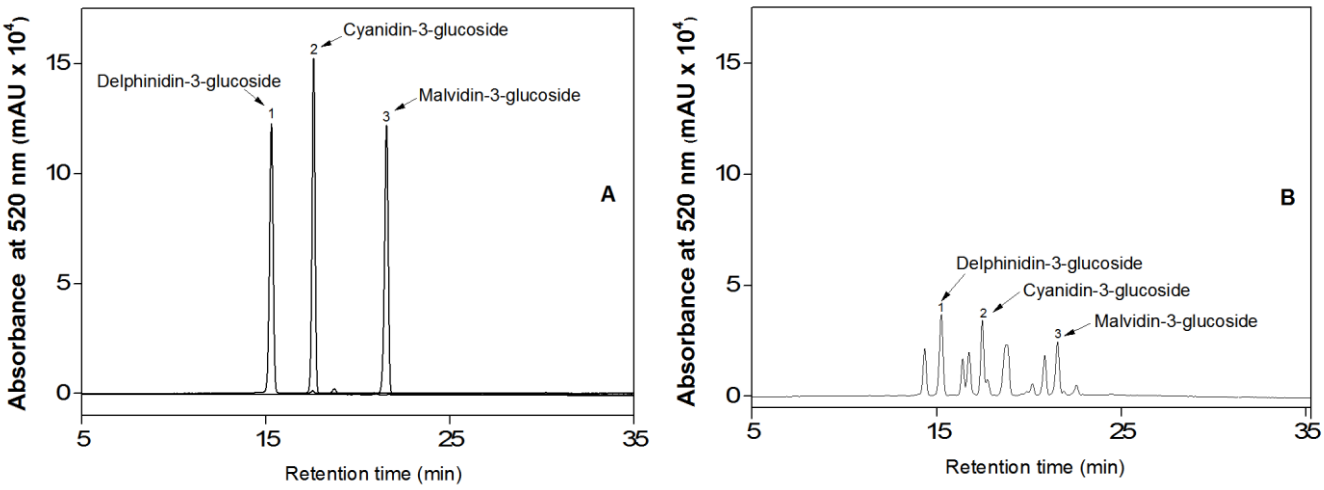


Figure 1. HPLC chromatogram of anthocyanin profiles from anthocyanins standards (A) and blueberry juice (B)

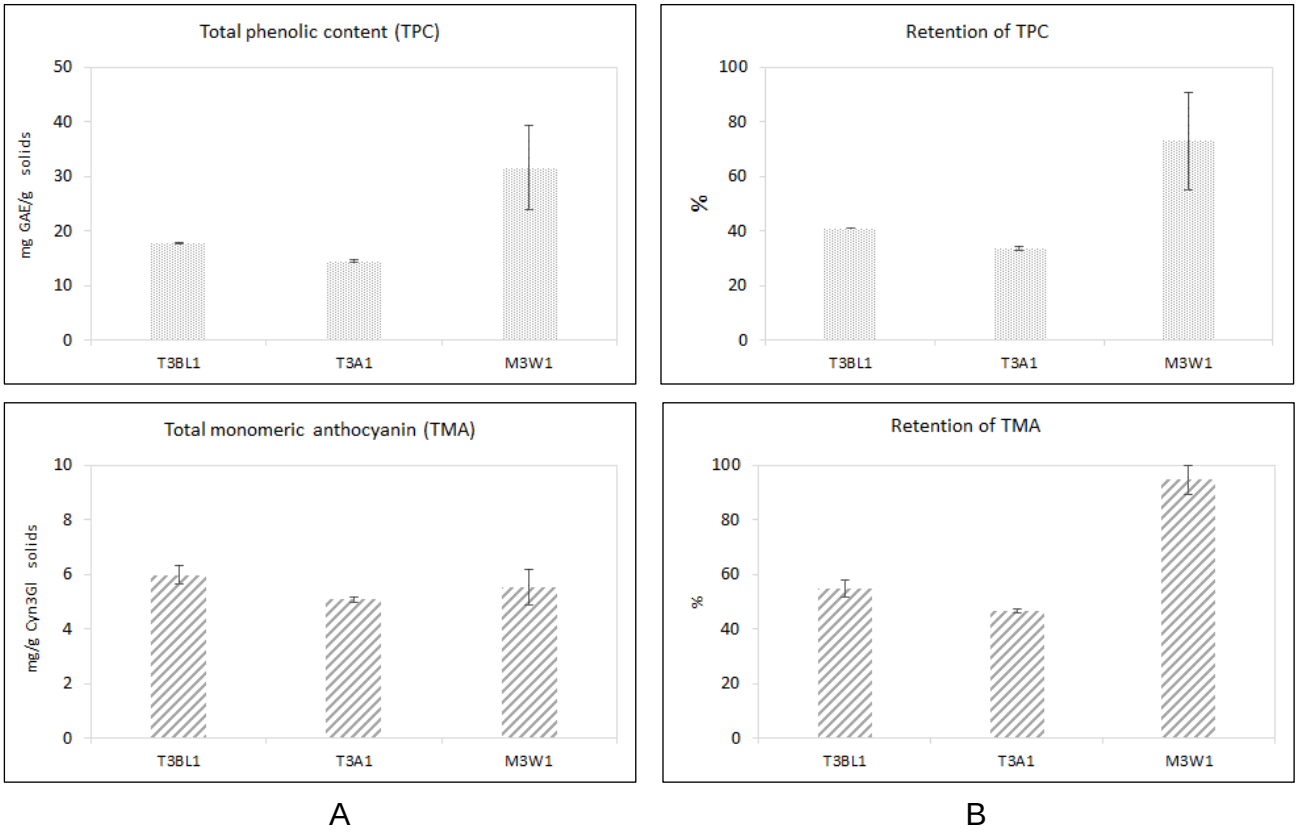
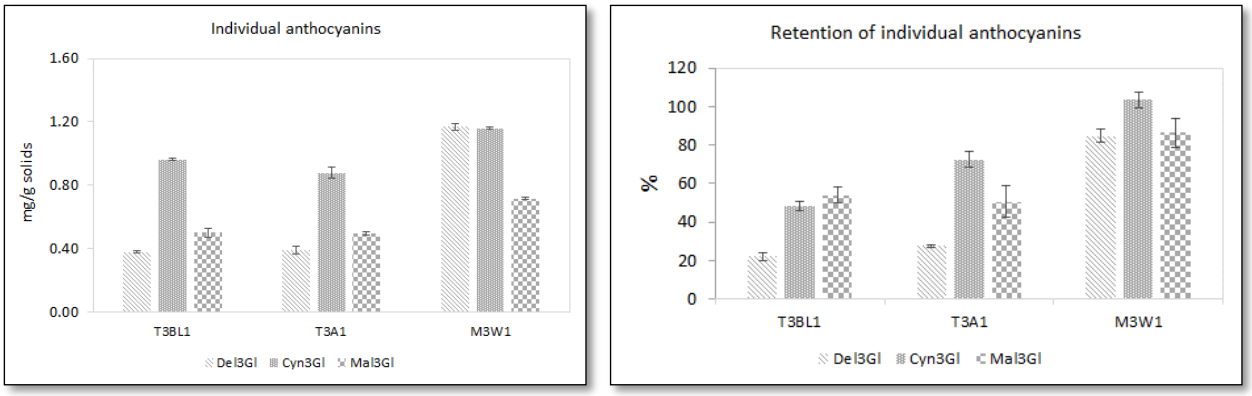


Figure 2 A: Total phenolic content/TPC and total monomeric anthocyanins/TMA of FMFD powders. B: Retention of TPC and TMA of FMFD powders. Results are expressed as means ± range of duplicate determinations.



A B

Figure 3 A: Concentration of Del3Gl, Cyn3Gl, and Mal3Gl of FMFD powders. B: Retention of Del3Gl, Cyn3Gl, and Mal3Gl of FMFD powders. Results are expressed as means ± range of duplicate determinations.