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Comparison of methods of extraction of phenolic compounds from American cranberry (*Vaccinium macrocarpon* L.) press residues

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Abstract. American cranberries (*Vaccinium macrocarpon* L.) contain significant quantities of various phenolic compounds. Most of these compounds are recovered when berry juice is produced. However, a considerable part of polyphenols remain in berry press residues and are discarded as food industry waste. The aim of the study was to compare the methods of extraction of polyphenols (ultrasound, microwave-assisted, Soxhlet) from press residues of American cranberry. The impact of main extraction parameters (e.g., extraction time, solid/solvent ratio, solvent type) on the yield of extracted polyphenols. Ultrasound-assisted extraction showed the highest potential from all studied methods, given its fast, convenient use and low cost. Aqueous ethanol and methanol in the presence of acid (anthocyanin extractions should be assisted with trifluoroacetic acid, polyphenol extractions – with HCl) were assessed as the best solvents for extraction. The obtained extracts were characterised using the Folin-Ciocaulteu method for determination of total phenolics and the pH-differential method for determination of total anthocyanins, and UPLC–PDA was used to determine the content of individual anthocyanins. Cyanidin-3-*O*-arabinoside, peonidin-3-*O*-galactoside, peonidin-3-*O*-glucoside and peonidin-3-*O*-arabinoside were identified as the main anthocyanins in cranberry press residue extracts.

Key words: phenolic compounds, antioxidant activity, flavonoids, anthocyanins, *Vaccinium macrocarpon*, press residues

INTRODUCTION

Fruits of American cranberry (*Vaccinium macrocarpon*) are a rich source of phenolic compounds, including flavonoids, phenolic acids and other biologically active substances (Vvedenskaya et al., 2004; White et al., 2010; Kylli, 2011a). The main flavonoids found in berries are anthocyanins, proanthocyanidins, flavonols and catechins (Ancilotti et al., 2016). Flavonoids are responsible for the red colour of fruits and are the most abundant phenolic compounds in various berries. The basic flavonoid structure encompasses the flavan nucleus, containing 15 carbon atoms arranged in three rings. Phenolic acids present in berries are hydroxylated derivatives of benzoic acid and cinnamic acid. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen and hydroxyl radical quenchers. Consumption of natural antioxidants and, inter alia, phenolic compounds is associated with a protective effect against many diseases, such as cardiovascular diseases, obesity, urinary tract diseases, cancer and other

degenerative disorders (Nile and Park, 2014). American cranberry, used fresh or in the form of preserves, juices, wines, liquors or extracts, is among the most consumed berries in the countries of Northern Europe, USA, Canada and Russia (Roopchand et al., 2013).

A widely used approach for the processing of American cranberry is the production of juice, resulting in food industry waste – berry press residues (pomace), containing berry skin and seeds. Due to its acidity and low protein content, it has limited use and usually is discarded (White et al., 2010). From the perspective of valorisation of food industry wastes, berry press residues are a promising source of natural antioxidants – phenolic compounds. Extraction of phenolic compounds from food by-products has been reported for apple pomace (Pingret et al., 2012), black chokeberry wastes (D'Alessandro et al., 2014), chicory grounds (Pradal et al., 2016) and bilberry press residues (Aaby et al., 2013; Kerbstadt et al., 2015). The composition of phenolic compounds in plant material depends on plant species and their distribution in different tissues. Large amounts of phenolic compounds are bound in berry seeds and skin, which makes the release of these compounds difficult. Therefore, an extraction method specifically for berry press residues should be developed. The extraction conditions provided for one plant cannot be directly used for the extraction of phenolics from another plant due to the specific localisation of phenolics in various species. To develop methods for industrial application, the optimisation of extraction conditions is needed.

The aim of the study was (1) to select the best method for the extraction of polyphenols – specifically, anthocyanin – from berry press residues of American cranberry, (2) to elucidate the impact of the main extraction parameters (extraction time, solid/solvent ratio, solvent type and others) on the yield of extracted polyphenols and (3) to identify the anthocyanin composition of American cranberry.

MATERIALS AND METHODS

Berry samples and their processing

Berries of American cranberry (*Vaccinium macrocarpon* L.) were harvested in autumn (September 2016). Cranberries were hand-picked at a commercial farm (Z/S 'Strēlnieki') located on the outskirts of Jūrmala City (Latvia). All berries were frozen at -20 °C to improve the release of juice. Berries were then gently thawed at 5 °C. Once thawed, they were put into a domestic hydraulic 6 L juice extractor (manufactured by Biowin®, Poland) and drained of all juice. At this step, berry press residues (seeds, skins) containing residual moisture (10%) were produced. Berry press cake was frozen once again at -20 °C to prepare it for lyophilisation. Frozen berries were freeze-dried for 3 days in a Labconco® FreeZone benchtop freeze dryer at -45 °C. Finally, dried berries were homogenised to a fine powder using an IKA® M20 analytical mill.

Chemicals and reference substances

Ethanol, acetone (Enola*)*, methanol and acetonitrile (ChemPur) used for extractions were of analytical grade. Demineralised water was obtained from a Milli Q system (Millipore*)*. Trifluoroacetic acid (99.5%), formic acid (99.9%), acetic acid (99.9%), gallic acid (97%) and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich. Sodium carbonate, potassium chloride, sodium acetate, trichloroacetic acid (all of ≥99.5% purity) as well as hydrochloric acid (37%), and sulphuric acid (98%) were purchased from Enola. Methanol (99.9% HPLC grade, Sigma-Aldrich) used for UHPLC

and reference standards of cyanidin-3-*O*-galactoside (≥97%), cyanidin-3-*O*-glucoside (≥96%), cyanidin-3-*O*-arabinoside (≥95%) and peonidin-3-*O*-glucoside (≥95%) were purchased from Extrasynthese (France). Peonidin-3-*O*-galactoside (≥97%) and peonidin-3-*O*-arabinoside (≥97%) were obtained from Polyphenols Laboratories AS (Norway).

Extraction methods

Four extraction methods were tested for an impact on the anthocyanin/polyphenol yields. Microwave and ultrasound-assisted extractions were performed using 0.50 g of lyophilised, homogenised cranberry powder, and 1.0 g of berry powder was used for Soxhlet extraction. Microwave, ultrasound and maceration-assisted extractions were done using 50 mL of solvent, while Soxhlet extraction, due to the larger extraction volume needed, was done using 100 mL of solvent. After the extraction procedure, extracts were filtered through cellulose filter paper with a pore size of 20 μm to remove berry particles and insoluble matter and stored in dark at 4 °C.

Soxhlet extraction

Soxhlet extraction was performed using 100 mL of 96% ethanol and 0.5% trifluoroacetic acid (TFA) (v/v). Berry powder (1.0 g) was weighed into a cellulose thimble and set for the extraction at 80 °C for 12 hours with a condenser. 25 cycles were completed during the extraction period. A five-sample block heater was used for simultaneous extractions in a *Behr ET2* Soxhlet extraction unit (Labor-Technik).

Microwave-assisted extraction

Microwave-assisted extraction was done using a Milestone Ethos One microwave extraction unit. Samples were weighed (0.5 g) straight into the microwave extraction capsules, and 50 mL of appropriate solvent was added. The applied extraction programme consisted of 10-minute heat-up time at 600 W to reach 80 °C, in which, the sample solution was held for 20 minutes.

Ultrasound-assisted extraction

Extraction from cranberry press residues was optimised using the ultrasound-assisted extraction method. A berry sample (0.5 g) was weighed, and 50 mL of solvent was added. 100 W ultrasound was used for the optimisation experiments, and, to compare the impact of the capacity of ultrasound on the efficiency of extraction, an experiment with ultrasound of a higher capacity (360 W) was done (Cole-Parmer*)*. The temperature of ultrasound bath was monitored and not allowed to exceed 30 °C, at which point the water was replaced (every 20 minutes). After the treatment with ultrasound, the samples were left shaking for 24h in the dark. For comparison, to show the efficiency of ultrasound treatment, an experiment was performed where a sample was left shaking for 24h without ultrasound treatment.

Chemical analysis

Determination of dry residue

To determine dry residue of each extract, a set of glass vials were dried at 80 °C overnight. The vials were cooled down in a desiccator for at least 3 hours prior to use. Each vial was then marked and weighed on analytical scales. 1 mL of each extract was measured with a volumetric pipette and transferred into a dry vial. The vials were dried at 40 °C overnight to evaporate the solvents and moisture. The vials with dry residue

were put into a desiccator and cooled down for at least 3 hours. After that, the vials were weighed. The difference in weight of the vial before and after the addition of extract was expressed as a dry residue of the extract of 100 g^{-1} berry powder.

Determination of total carbohydrates

Total carbohydrates were determined by the phenol-sulphuric acid method using glucose as a standard (Dubois et al., 1956). 1 mL of the berry extract or 1 mL of an appropriate dilution of the berry extract was mixed with 1 mL of 5% (w/v) phenol solution. 5 mL of sulphuric acid was added to the mixture and carefully mixed by shaking. The samples were incubated for 40 minutes at room temperature and measured at 488 nm. A calibration curve was prepared in the same manner using glucose standard solutions (0–200 mg L^{-1}). All measurements were made using a *Shimadzu UV-1800 UV-VIS* spectrophotometer against a reagent blank, where the sample volume was substituted with de-mineralised water.

Determination of total phenolic compounds (TPC)

Total polyphenols were quantified using the Folin-Ciocalteu colorimetric method (Folin and Ciocalteu, 1927; Siriwoharn et al., 2004). A standard curve was prepared (R^2 = 0.999) using gallic acid in the range of 0–0.350 g mL^{-1}. Standards were prepared by solution in water and measured at 765 nm after a 20–30-minute incubation period at room temperature in the dark. Appropriate sample dilutions were prepared in the same manner as the standards and measured against a de-mineralised water blank. All measurements were made using a *Shimadzu UV-1800 UV-VIS* spectrophotometer. Total polyphenols in g of gallic acid equivalents (GAE) 100 g^{-1} berry powder (BP) were calculated using the following equation (Eq. 1),

$$TPC\ g\ GAE\ 100\ g^{-1}\ BP = \frac{C.\ of gallic\ acid\ (g\ mL^{-1}) \times Volume\ of\ extract\ (mL)}{Weighed\ amound\ of\ the\ sample\ (g)} \times 100 \quad (1)$$

where: *C*. of gallic acid (g mL^{-1}) is calculated using a calibration curve regression equation.

Determination of total anthocyanins

The spectrophotometric pH-differential method (Lee et al., 2005) was used to determine the total amount of anthocyanins in the prepared extracts. In this method, the ability of anthocyanin molecules to change colour at different pH levels is used. Two buffer solutions with different pH were prepared: 0.025 M potassium chloride solution at pH 1.0 and 0.4 M sodium acetate solution at pH 4.5; pH was adjusted with concentrated hydrochloric acid (HCl). Two dilutions of the same sample were prepared using the buffers described above, so that the Abs at 520 nm falls within the linear range of the spectrophotometer (0.1–1.4 AU) and does not exceed the 1:5 sample/buffer ratio. The diluted samples were left in the dark for 20–30 minutes and measured within 20–40 minutes. The absorbance of each dilution was measured at 520 nm and 700 nm against a de-mineralised water blank using *Shimadzu UV-1800 UV-VIS* spectrophotometer. The total anthocyanin content was calculated using following equation (Eq. 2),

$$Anthocyanin\ content\ (cyanidin-3-glucoside\ eq.,\ g\ L^{-1} = \frac{A \times MW \times DF}{\varepsilon \times l} \quad (2)$$

where: A = ($A_{520nm} - A_{700nm}$) pH 1.0 – ($A_{520nm} - A_{700nm}$) pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor; l = cuvette path length in cm (1cm); ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1} for cyd-3-glu.

The extraction yield in g of anthocyanin 100 g^{-1} berry powder (BP) was calculated using following equation (Eq. 3),

$$g\ anthocyanin\ 100\ g^{-1}BP = \frac{Anthocyanin\ (g\ L^{-1}) \times Volume\ of\ extract(L)}{weighed\ amount\ of\ the\ sample\ (g)} \times 100 \quad (3)$$

Ultra-high performance liquid chromatography analysis

Ultra-performance liquid chromatography (UPLC) identification and quantification analyses of anthocyanins were carried out using a Waters ACQUITY UPLC system equipped with a Quaternary Solvent Manager (QSM), a Sample Manager – Flow-through Needle (cooled to 4 °C) (SM–FTN), a column heater (CH–A) and a photodiode array (PDA) λ detector. PDA data were collected using a Waters Empower data systems software.

The analyses were carried out at 35 °C using a C18 column (Acquity UPLC BEH C18 2.1×50 mm i.d., 1.7 μm) with a column pre-filter (frit and nut 0.2 μm and 2.1 mm). The mobile phase consisted of aqueous 5.0% formic acid (A) and methanol/1.0% formic acid in water (70:30 v/v) (B). The flow rate was 0.250 mL min^{-1}, and the gradient elution was from 80% to 75% of solvent A in 15 minutes, from 75% to 60% in 7 minutes and from 60% to 0% in 18 minutes, followed by 10 min of stabilisation at 80%. The total sample run time was 40 minutes. The injection volume for samples was 2.0 μL. Identity assignment was carried out considering the retention times and by PDA analysis. Anthocyanins were quantified using external calibration curves prepared form anthocyanin standard mixture (3–100 mg L^{-1}).

Statistics and data analysis

All measurements were made in triplicate and expressed as a mean. Measurement standard deviations were calculated for each result. Standard curves were prepared in MS Excel software in the linear range of measurements, with the correlation coefficient (R^2) of at least 0.999. Statistical (significance at α = 0.05) tests (*Student's t-test, ANOVA, Tukey's HSD*) and calculations were performed in JMP® (SAS) software for statistics.

RESULTS AND DISCUSSION

Despite polyphenolic and anthocyanin extractions from different berries being intensively studied and performed, there is still substantial inconsistency in the way how it is done. Considering the differences in chemical and physical properties of different polyphenols, these polar molecules are usually extracted with methanol (Lätti et al., 2008; Corrales et al., 2010; Sójka et al., 2013; Wiczkowski et al., 2013), ethanol (Chen et al., 2007; d'Alessandro et al., 2012; Ćujić et al., 2016), acetone (Vatai et al., 2008; Kylli et al., 2010; Kylli et al., 2011b; Šliumpaitė et al., 2013; Chen et al., 2016), acetonitrile (Lätti et al., 2008; Li et al., 2011) or water (Kim et al., 2009; Denev et al., 2010; d'Alessandro et al., 2012). Despite methanol and acetone being the most effective extraction solvents, their use is limited in food industry due to toxicity. Ethanol, in turn,

is a solvent more suitable for food industry. To assist the extraction of anthocyanins, various acids are used, i.e. trifluoroacetic acid (Li et al., 2011; Wiczkowski et al., 2013), HCl (Burdulis et al., 2007; Chen et al., 2007), formic acid (Lätti et al., 2008; Sójka et al., 2013), citric acid (Denev et al., 2010) and acetic acid (Chen et al., 2016). The addition of acids in anthocyanin extraction stabilises these molecules in the flavylium cation form, which produces red colour at low pH. The choice of acid can influence the stability of anthocyanins. For example, hydrochloric acid (HCl) can catalyse hydrolysis of acetylated anthocyanins. Therefore, organic acids are preferred for this type of extraction (Denev et al., 2010).

Selection of solvent for polyphenol extraction

Various extraction conditions suggested in other studies were considered in order to compare the different solvent systems used for the extraction of phenolic compounds. The suggested solvent mixtures were tested on the same type of sample, i.e. press residues of American cranberry, to find the best solvent for the specific type of sample used in this study. Ultrasound-assisted extraction for 40 minutes was used for each sample. The content of dry residue, which indicates the overall efficiency of extraction, showed variations among the different solvents used. For example, water and 1% HCl extraction shows significantly lower extraction yields ($\alpha = 0.05$) in all the measured parameters, except total carbohydrates (14.82 g 100 g^{-1} berries). Thus, considering the application of the extract, a solvent system where water is used should be avoided, as the low levels of phenolics (0.89 g 100 g^{-1} berries) and anthocyanins (0.098 g 100 g^{-1} berries) and high levels of total carbohydrates might not be applicable for further analytical study of extract composition. As the extracts obtained in this study were analytically characterised using liquid chromatography, the amount of total carbohydrates (7.82–19.52 g 100 g^{-1} berries) does not interfere with the methods used to characterise them. However, if extracts are intended to be used for production purposes, the high amounts of sugars might be an inconvenience, as sugars make the final product a thick, viscous mass, which could be hard to handle and process (Table 1).

Table 1. Comparison of different solvent mixtures used for the extraction of phenolic compounds/anthocyanins. Uncertainty represents standard deviation. All solvents were used as v $v\%^{-1}$. Asterisk (*) represents a significant difference in the results ($p \leq 0.05$, Student's t-test)

Solvent	Dry residue, g 100 g^{-1} berry powder	Total carbohydrates, g 100 g^{-1} berry powder	Anthocyanins, g 100 g^{-1} berry powder	Total polyphenols, g 100 g^{-1} berry powder
Acetonitrile 49.5%, TFA 0.5%, water 50%	37.24 ± 1.53	7.82 ± 0.27*	0.228 ± 0.006	3.84 ± 0.12*
Acetone 50%	34.29 ± 1.41	12.17 ± 0.43	0.151 ± 0.004	2.70 ± 0.08
Acetone 75%	36.01 ± 1.48	18.52 ± 0.65*	0.156 ± 0.004	2.69 ± 0.08
Methanol 60%, acetone 30%, water 10%	37.94 ± 1.56	16.86 ± 0.59	0.184 ± 0.005	2.34 ± 0.07
Methanol, HCl 1%	48.38 ± 1.98*	17.93 ± 0.63	0.451 ± 0.011*	4.80 ± 0.14*
Water, HCl 1%	16.91 ± 0.69*	14.82 ± 0.52	0.098 ± 0.002*	0.89 ± 0.03*
Ethanol 70%, HCl 1%	39.62 ± 1.59	16.85 ± 0.51	0.204 ± 0.005	3.43 ± 0.09

In our experiments, the highest extraction yields (48.38 g 100 g^{-1} berries) were obtained by the use of methanol and 1% HCl (v/v). This extraction also gave the highest amount of total anthocyanins (0.451 g 100 g^{-1} berries) and polyphenols (4.8 g 100 g^{-1} berries). However, the stability of anthocyanin molecules must be considered when using this system. The easy use and low costs of ethanol and the high phenolic yield obtained from the use of this solvent (3.43 g 100 g^{-1} berries) support the selection of lower alcohols (ethanol, methanol) for further optimisation of extraction (Table 1). Increasing varieties of products containing berry press residues are available for consumers. In this situation, there is a need for efficient extraction strategies to control the quality of such products.

Comparison of polyphenol extraction methods

The most often used polyphenol extraction methods are ultrasound-assisted extraction (Chen et al., 2007; Lätti et al., 2008; Ghafoor et al., 2009; Ćujić et al., 2016) and extraction where the sample is shaken in the extraction solvent for extended periods of time (Pinelo et al., 2005; Makris et al., 2008; Yi et al., 2009; Denev et al., 2010).

In addition to the two most often used methods, microwave-assisted extraction and Soxhlet extraction were also tested, as these methods prove to be reliable for routine use and produce repeatable results. All extractions were done with 96% ethanol and 0.5% TFA, v/v. Soxhlet and microwave extractions gave significantly lower overall yields than ultrasound and shaking-assisted extractions (23.88 g and 21.01 g 100 g^{-1} berries, respectively). In addition, the latter two methods also gave lower levels of extracted anthocyanins (0.054 and 0.065 g 100 g^{-1} berries) and polyphenols (1.21 g and 1.09 g 100 g^{-1} berries) (Table 2). Soxhlet extraction is primarily performed with a solvent (ethanol) rather than acid, as trifluoroacetic acid forms an azeotropic mixture with water in ethanol, increasing the boiling point of the mixture and not allowing the acid to come in contact with the sample. Ultrasound-assisted extractions at different capacities (100 W or 360 W) showed similar extraction yields (34.05–34.53 g 100 g^{-1} berries), total carbohydrates (11.46–12.15 g 100 g^{-1} berries), anthocyanins (0.136–0.147 g 100 g^{-1} berries) and polyphenols (1.59–1.68 g 100 g^{-1} berries) (Table 2).

Table 2. Comparison of different extraction methods. All extractions were done with 96% ethanol and 0.5% TFA, v/v. Uncertainty represents standard deviation. Asterisk (*) represents significant difference in the results ($p \leq 0.05$, *Student's t-test*).

Method	Dry residue, g 100 g^{-1} berry powder	Total carbohydrates g 100 g^{-1} berry powder	Anthocyanins, g 100 g^{-1} berry powder	Total polyphenols, g 100 g^{-1} berry powder
Microwave	21.01 ± 0.86*	8.80 ± 0.36*	0.054 ± 0.001*	1.09 ± 0.04*
Soxhlet	23.88 ± 1.80*	8.33 ± 0.34*	0.065 ± 0.002*	1.21 ± 0.05
100W ultrasound	34.05 ± 1.40	11.46 ± 0.47	0.135 ± 0.003	1.59 ± 0.07
360W ultrasound	34.53 ± 1.42	12.15 ± 0.50	0.147 ± 0.004	1.68 ± 0.07
24h shaking	33.01 ± 1.35	11.78 ± 0.48	0.098 ± 0.002	1.12 ± 0.06*

When the sample is treated with ultrasound, the cell wall matrix is disrupted, which ensures the release of various compounds, including polyphenols, into the surrounding medium, which is very important in this case, as the type of the sample (berry skins,

seeds) has thick cell walls (Ćujić et al., 2016). The results obtained from ultrasound-assisted extractions showed that this method has the greatest potential from all of the methods used. Given its fast, convenient use and low cost, it is the method of choice for phenolic extractions from berry press residues.

Selection of acid for polyphenol extractions

The extraction of polyphenolic substances is influenced not only by the choice of solvent (Table 1) but also the choice of acid used to assist the extraction. To identify the most optimal acid for the extraction of anthocyanin and phenolics, a series of ultrasound-assisted extractions were performed, where the solvent (96% ethanol) was mixed with various acids (at 1%, v/v) used for polyphenol extractions and routine work in a laboratory (Chen et al., 2007; Denev et al., 2010; Li et al., 2011; Sójka et al., 2013; Chen et al., 2016).

Extractions with no added acid, formic acid, acetic acid and sulphuric acid gave similar results for anthocyanins (0.122–0.145 g 100 g^{-1} berries) and polyphenols (1.1–1.4 g 100 g^{-1} berries) (Fig. 1).

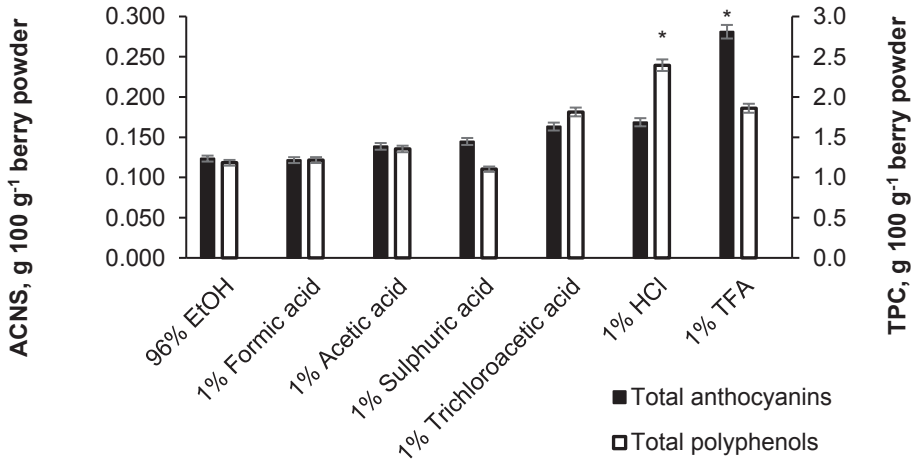

Figure 1. Comparison of total polyphenol (TPC) and total anthocyanin (ACNS) extraction efficiency using various acids at the concentration of 1% with 96% ethanol (v/v). Error bars represent 95% confidence interval. Asterisk (*) represents a significant difference in the results (*ANOVA, Tukey's HSD*).

Formic acid is one of the acids most often used in the extraction of anthocyanins. However, the extraction yields are lower in this solvent/acid system (no significant difference from 96% ethanol and no acid extraction) compared to using other acids. Another acid frequently used in the extraction of anthocyanins is trifluoroacetic acid (TFA), although at lower concentrations (0.1% and 0.5%). When using 1% TFA, the detected anthocyanin yield was approximately two times higher (0.280 g 100 g^{-1} berries) than that of the extractions with carboxylic acids and sulphuric acid. The phenolic compounds extracted with 1% TFA were in similar concentrations to those extracted with trichloroacetic acid (1.9 g and 1.8 g 100 g^{-1} berries, respectively). However, due to the difficult removal of trichloroacetic acid from the extracts, the use of this acid might

be problematic. The highest amount of extracted polyphenols was observed when using HCl (2.4 g 100 g^{-1} berries). At the same time, the amount of extracted anthocyanins (0.169 g 100 g^{-1} berries) was comparable with sulphuric acid and trichloroacetic acid extractions, indicating that HCl assists the extraction of non-anthocyanin phenolics (Fig. 1). Overall, the use of acids to increase the yields of polyphenol extractions is a necessary step. Taking into consideration that the acids used will not interfere with the analytical measurements, anthocyanin extractions should be assisted with trifluoroacetic acid and polyphenol extractions – with HCl.

Ultrasound treatment kinetics

Comparing the prospective extraction methods, it was concluded that ultrasound extractions give the highest yields of phenolics (Table 2) at the two different ultrasound capacities used. To optimise the procedure of ultrasound-assisted extraction, the duration of ultrasound treatment was investigated as one of the main factors influencing the extraction efficiency. A series of 7 experiments were done, taking three different concentrations of ethanol (96%, 40% and 70%) and 5% formic acid (v/v) and performing ultrasound-assisted (100 W) extraction for 3, 5, 8, 10, 15, 25 and 40 minutes. Two parameters – total anthocyanins and total polyphenols – were measured for each time-point. The amount of total anthocyanins was plotted against the ultrasound treatment time, revealing that 40% and 70% ethanol extractions give higher extraction yield than the extractions done with 96% ethanol (Fig. 2).

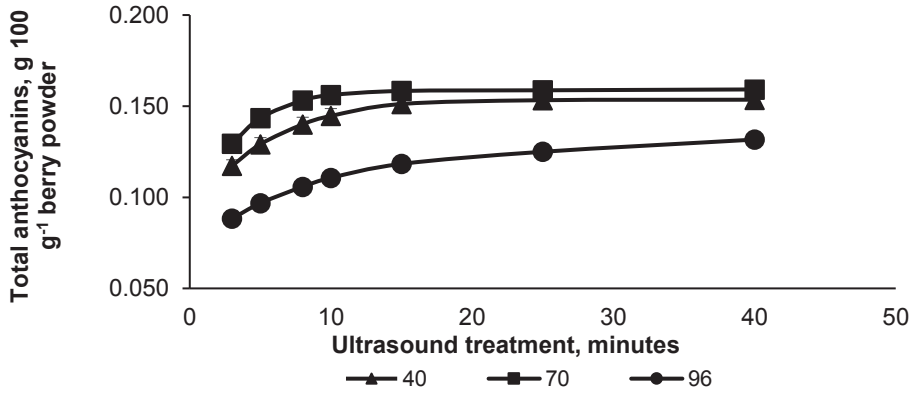

Figure 2. Total extracted anthocyanins from cranberry press residues depending on the duration of ultrasound treatment. Three different ethanol concentrations were used with 5% formic acid (v/v). Error bars represent 95% confidence interval.

The same pattern was also observed with the total polyphenols (Fig. 3). The amount of total anthocyanins extracted with 96% ethanol was significantly lower than that of the 40% and 70% ethanol extractions. The extraction kinetics revealed that the amount of extracted anthocyanins and polyphenols did not increase after 15 min of ultrasound treatment. In fact, there was no significant difference between the 15-, 25- and 40-minute time points as well as the 40% and 70% ethanol extractions (Figs 2 and 3). These results suggest that, first, the extraction solvent should contain water to increase the efficiency

of extraction of both anthocyanins and polyphenols, and, second, the optimal length of ultrasound treatment is 15–25 minutes.

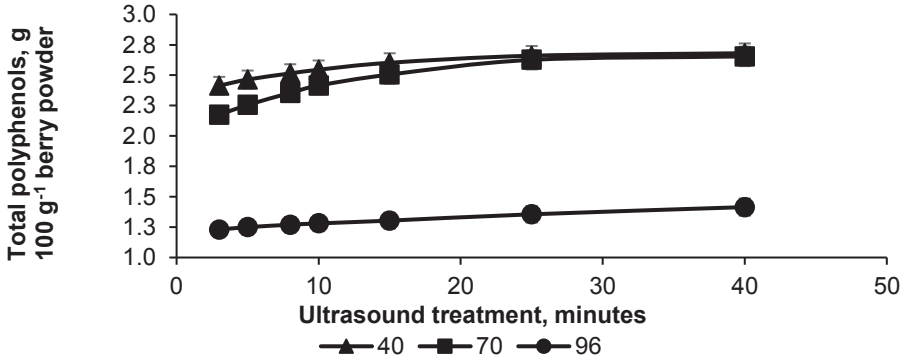

Figure 3. Total extracted polyphenols from cranberry press residues depending on the duration of ultrasound treatment. Three different ethanol concentrations were used with 5% formic acid (v/v). Error bars represent 95% confidence interval.

Optimisation of solid/solvent ratio

Maximisation of the total yield of extractions is an important step in optimisation. Not only it saves time but also resources, as the same result can be achieved by using less solvents and sample material. To investigate the capacity of ethanol and methanol for polyphenol extraction, a set of experiments with different berry press residue/solvent ratios was done. Four different solid/solvent ratios were tested: 1:30, 1:60, 1:90 and 1:120. In the experiments where ethanol was used, no significant difference could be seen between the different solid/solvent ratios. However, in the extractions where methanol was used, the optimal solid/solvent ratio was between 1:90 and 1:120, as these experiments resulted in significantly higher amounts of extracted anthocyanins (0.174 g and 0.169 g 100 g^{-1} berries) and total polyphenols (2.34 g and 2.29 g 100 g^{-1} berries) (Fig. 4).

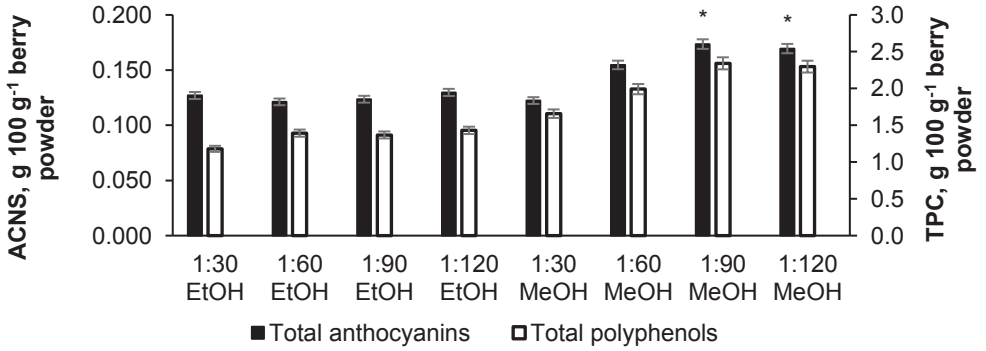

Figure 4. Effect of the solvent/solid ratio on the extraction of total polyphenol (TPC) and total anthocyanin (ACNS) using ethanol (EtOH) and methanol (MeOH) with 5% formic acid (v/v). Error bars represent 95% confidence interval. Asterisk (*) represents a significant difference in the results ($p \leq 0.05$, *Student's t-test*).

Chromatographic identification of anthocyanins in cranberry extracts

To characterise the composition of anthocyanins in the obtained extracts, UPLC-PDA chromatography was used. Considering the potential application and significance of anthocyanin extracts that have influence on human health, the anthocyanins found in cranberry extracts were identified and quantified.

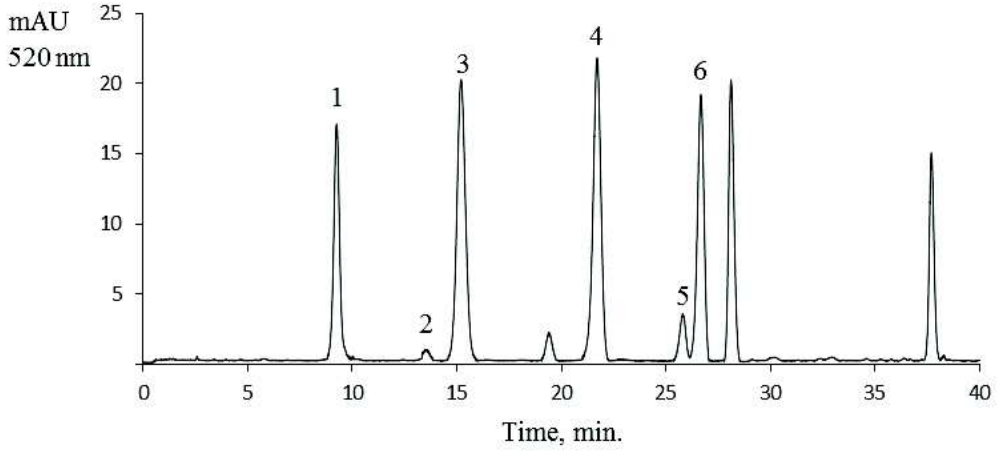

Figure 5. UPLC chromatogram (520 nm) of 96% ethanol, 5% formic acid (v/v) cranberry press residue extract. Peak numbers represent the anthocyanins quantified in Table 3.

Chromatographic analysis revealed the presence of a total of 9 major substances, and 6 of them were identified and quantified by comparison with reference substances (Fig. 5, Table 3). Cyanidin-3-*O*-arabinoside, peonidin-3-*O*-galactoside, peonidin-3-*O*-glucoside and peonidin-3-*O*-arabinoside were found in the highest concentrations. Similar grouping of anthocyanins in cranberries has been found in the previous studies with whole berries (Vvedenskaya et al., 2004), but the total concentrations of anthocyanins obtained in our study were higher than those found by other researchers, thus supporting the applicability of the extraction conditions used in this study.

Table 3. Profiling of anthocyanins in cranberry press residue extract by UPLC/PDA (selective wavelength 520 nm). Retention time (Rt) on PDA chromatogram.

Peak No.	Compound name	Rt, min.	λ_{max}, nm	Amount, mg g^{-1} dry powder)
1	cyanidin-3-*O*-galactoside	9.26	278.15; 512.50	1.73 ± 0.17
2	cyanidin-3-*O*-glucoside	13.61	278.15; 328.11; 516.15	0.06 ± 0.01
3	cyanidin-3-*O*-arabinoside	15.21	278.15; 513.72	3.07 ± 0.31
4	peonidin-3-*O*-galactoside	21.71	278.15; 512.50	3.04 ± 0.21
5	peonidin-3-*O*-glucoside	25.81	278.15; 361.22; 514.93	0.36 ± 0.04
6	peonidin-3-*O*-arabinoside	26.68	278.15; 517.34	2.31 ± 0.23

[a] Data expressed as mean values ± standard deviation (n = 3), mg g^{-1} berry powder.

CONCLUSIONS

The present study determined the most optimal extraction parameters for the extraction of polyphenols and anthocyanins from press residues of American cranberry. Several solvent/acid combinations, solid/solvent ratios and extraction methods were examined.

The performed experiments revealed that the use of ethanol and methanol with 1% trifluoroacetic acid for the extraction of anthocyanins and 1% HCl for the extraction of non-anthocyanin polyphenols are the optimal solvents for extraction. Ultrasound-assisted extraction with the ultrasound treatment for 15–25 minutes gave the highest extraction yields when the solid/liquid ratio was between 1:90 and 1:120. The results obtained in this study provide a reliable and repeatable method for the extraction of polyphenols and anthocyanins from press residues of American cranberries and, possibly, other berry powders.

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