ORIGINAL PAPER

Microwave-Assisted Extraction of Anthocyanins from Black Currant Marc

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Abstract This paper reports on the process optimization study of anthocyanin extraction from black currant marc by microwave-assisted extraction (MAE) using acidic solvents. Maximum yields of anthocyanins were achieved at pH2 with an extraction time of 10 min with a microwave power of 700 W. The anthocyanin yields in MAE were compared with those obtained by conventional solvent extraction (CE) using citric or hydrochloric acids, and citric acid and water with a sulfur concentration of 50 ppm. A significant reduction of extraction time was achieved using MAE; maximum anthocyanin yield was obtained after 10 min using MAE, while using CE only after 300 min. Furthermore, the amount of solvent used halved; the mass ratio of marc to solvent was 1:40 in CE and 1:20 in MAE.

Additionally, the final anthocyanin concentration in the solvent phase of MAE increased by 20 % compared to the conventional extraction with hydrochloric acid at pH2 and temperature of 80 °C. The results suggest that microwave-assisted extraction is a more efficient technique for the extraction of valuable compounds from black currant residue.

Keywords Black currant marc · Anthocyanin · Extraction · Microwave-assisted extraction · Anthocyanin composition

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Introduction

Berries are rich in health-promoting compounds such as vitamins, anthocyanins, minerals, and amino acids, which are also sensitive to heat and processing. To provide consumers with the maximum benefit of berries in the product, minimal processing technologies are sought. The juices of wild and cultivated berries are rich in these compounds and they can be extracted from the plant tissues into the berry juices during the pressing of the berries. However, this extraction is never complete and, therefore, valuable compounds remain in the pomace (Zheng and Shetty 1998). This by-product of the berry-pressing process, also called marc or press cake, is rich in health-promoting compounds. For example, the black currant marc is abundant in anthocyanins (Kapasakalidis et al. 2006), which are free radical scavengers and potential agents against oxidative stress. Anthocyanins are also known to work in the prevention of cardiovascular illnesses, diabetes, and cancer, and have impressive antibacterial, antiviral and antiallergenic effects (Stintzing and Carle 2004).

Kapasakalidis et al. (2006) reported that the black currant pomace and the black currant press residue can be a potential source for polyphenol extraction. The phenol level in

press residue extract (35–37 mg g^{-1}) was eight to nine times higher than in the pomace extract (3–7 mg g^{-1}) and the main anthocyanins, i.e., delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside constituted the 90 % of the total phenolic content.

Anthocyanins are usually deposited in plant cell vacuoles and in the cell walls, and they are released from the tissue during the commercial juice pressing. However, considerable amounts of phenols (including anthocyanins) are associated with or bound to cell walls and remain in the berry residue (Bengoechea et al. 1997). It was reported that the pretreatment of black currant residue with cellulase enzyme increased the efficiency of phenol extraction (Kapasakalidis et al. 2009). These natural pigments are present in water-soluble form, which allows for their utilization in aqueous food systems (Longo and Vasapollo 2006).

Presently, the common utilization of marc is alcoholic fermentation, fodder production, or composting. During these processes, the bioactive components, antioxidants, and vitamins virtually vanish. The extraction of valuable components can be beneficial from both economic and environmental viewpoints. The berry extracts can also be concentrated by membrane-based technologies (Hodúr et al. 2009; Patil et al. 2009); processes operate at low temperatures, therefore, the heat and processing sensitive anthocyanins are better conserved.

Conventional solvent extraction is the most commonly used extraction method for recovering anthocyanins from plants, but this process is time demanding and requires large volumes of solvents. Furthermore, in the case of black currant press residue, Lapornik et al. (2005) found a considerable loss in total phenol content and in total anthocyanin content after 12 h water extraction. In their work, ethanol extract of black currant marc had higher polyphenol and anthocyanin content than the water extract; however, no significant difference in antioxidant activity was observed. The application of acidic solvent enhances flavonoid yield with the degradation of cell wall polysaccharides but the stability of anthocyanin depends on pH, being the least stable at pHs 3.8 and 5.2, as reported by Nielsen et al. (2003). In strong acidic extractants, the anthocyanins are more degradable due to breaking of the glycoside bonds (Giusti and Wrolstad 2003). As a case in point, Gizir et al. (2008) found that black carrot extracts from pressurized acidified water process with sulfuric and citric acids contained anthocyanin in similar concentration, but the mineral acid accelerated browning, confirming anthocyanin degradation reactions. Cacacae and Mazza (2002) reported that sulfurized water extraction of anthocyanins from black currant showed significantly higher yield compared to water extraction, but the increment was higher at low (300 ppm) SO$_2$ concentration than at high (1,100 ppm) concentration. They also observed that increasing the solvent temperature

from 20 to 60 °C did not result in significant increment of anthocyanin yield, but the time demand of extraction decreased since the diffusion coefficient and the solubility of the components were higher. An increase in solvent ratio from 20 to 60 mL g^{-1} increased the total phenol yield and the anthocyanin yield due to the higher concentration gradient.

Recently, microwave-assisted extraction (MAE) has become one of the most popular extraction processes for organic pollutants, pesticides, phenols, polymers, pharmaceuticals, and natural products. It is a promising alternative to conventional extraction, due to the shortened process time and lower solvent demand (Eskilsson and Bjorklund 2000). In this type of extraction, microwave energy is used to heat solvents in contact with samples, to accelerate the extraction of valuable and soluble compounds from the sample into the extractant (Morales-Muñoz et al. 2006). Several authors reported on the advantages of microwave-assisted extraction compared to conventional extraction, such as reduced process time, lower solvent and energy demand, and higher yield (Hemwimon et al. 2007; Proestos and Komaitis 2008; Chen et al. 2008). As water molecules have a polar character, the applied microwave radiation can be absorbed efficiently. Therefore, water is an appropriate and environmentally friendly solvent in MAE processes. In some cases, the solubility of organic compounds is not as high as that of conventionally available organic solvents, but the interaction of water molecules with microwaves is higher, due to its high dielectric constant and effective loss factor (Jones et al. 2000).

During microwave irradiation, the cells are thermally stressed. As a result, the temperature and pressure in the cell increase to levels that result in the rupture of cell walls. Liu et al. (2005) reported that, due to the stronger destructive effect of microwave radiation on the structure of orange skin organization, the swelling effect eventually forces the cell to split.

Because of the large energy demand of microwave generation and the heat sensitivity of bioactive components, the process needs to be optimized. The most frequently used process parameters for modeling and optimization are the temperature, the extraction time and the solid/solvent ratio (Pensado et al. 2000; Pizarro et al. 2006; Khajeh 2009).

Therefore, the aim of this study was to screen the effects of the applied microwave power, the extraction time, the pH of the solution and the marc to solvent ratio on the anthocyanin yield, and to optimize the extraction process using response surface methodology.

Materials and Methods

Raw Materials

Black currants (*Ribes nigrum* L.) were cultivated and processed into juice in Tolcsva (Hungary) by Fitomark 94 Ltd.

The berries were treated with pectinase enzyme preparation to increase the yield of juice during the squeezing process. After squeezing, the marc was stored deep frozen (at −22 °C) in polyethylene until the extraction process. The average total solid content of the marc (on a wet basis) was 23.5 w/w%.

Extraction Units and Procedures

Armfield pilot plant solvent extractor (Hampshire, UK) was used for the conventional extraction (CE). The extraction was carried out at a constant temperature of 80 °C. The solvents in use were aqueous HCl at pH2, and citric acid solutions at pH2, and a solution containing 50 ppm SO_2 and 1 % citric acid. 100 g of black currant press residue was used as an experimental batch, and the marc to solvent ratio was 1:40, the flow rate of solvent circulation was adjusted to 0.33 L/min.

The microwave-assisted extraction test was carried out using a single-mode cavity resonator, at a magnetron frequency of 2.45 GHz. The power output of magnetron can be adjusted between 100 and 700 W, with varying of anode voltage. In all experiments, a constant 28 g of marc was used. Response surface methodology was used to determine the optimum condition for extraction to achieve the highest anthocyanin content of the extracts. During the extraction, the microwave power varied between 140 and 700 W, the pH of the solvent between pHs 2 and 7, the marc to solvent ratio between 1:10 and 1:20 and the extraction time between 10 and 30 min. Polytetrafluorethylene vessels were used to minimize the energy loss of microwave irradiation. The vessel was covered to prevent the evaporation during the irradiation without pressure increase.

The temperature for the conventional extraction was chosen based on preliminary MAE experiments. In these experiments, the temperature of the extracts was approximately 80 °C at the end of the extraction, measured with an infrared temperature probe from the surface of the solvent.

Analytical Measurements

Monomeric Anthocyanin Pigments

Monomeric anthocyanin pigments (MAP) was determined using a pH difference protocol, as described in Current Protocols in Food Analytical Chemistry, Unit F1.2. This method relies on the structural transformation of the anthocyanin chromosphere as a function of pH, which can be measured by spectroscopy.

Samples from all the 54 experimental runs were collected and the 100 μL extract samples were diluted to 1,000 μL by distilled water. Two dilutions of the samples have been made, one with potassium chloride buffer pH1.0 and the other with sodium acetate buffer pH4.5. The pH of the buffers was adjusted with HCl solution (Spketrum-3D, Hungary). The dilution factor (DF) was determined in accordance with linear range of spectrophotometers (absorbance range of 0.2–1.0). Diluted samples were thermostated for 30 min to equilibrate at 20 °C, and the absorbance was measured at 520 and 700 nm with an UV/VIS spectrophotometer (WPA Lightwave S2000, UK) in quartz cuvettes against distilled water. The differences of absorbance of the diluted samples were calculated using Eq. 1.

$$A = (A_{1vis-\max} - A_{700})pH_{1.0}$$
$$- (A_{1vis-\max} - A_{700})pH_{4.5} \tag{1}$$

The concentration of MAP were calculated from absorbance difference using Eq. 2 and expressed in cyanidin-3-glucoside equivalents.

$$MAP(mg/L) = \frac{A \times MW \times DF \times 1,000}{\varepsilon \times 1} \tag{2}$$

where MW is the molecular weight (MW=449.2), DF is the dilution factor, ε is the molar absorptivity (ε=26 900).

HPLC Analysis of the Anthocyanin Extracts

A random selection of different extracts from the conventional extraction and microwave assisted extraction, as well as the extracts obtained under optimum extraction conditions in MAE were further analyzed by high-performance liquid chromatography (HPLC) to determine the composition and profile of anthocyanins. For this aim, a mixture of 5 mL of extracted sample and 5 mL 2 % acetic acid were vortexed for 15 min, followed by homogenization in an ultrasonic bath. The samples were prefiltered by 0.45 μm polyamide filter (Macherey-Nagel) to a vial, prior to injection into the HPLC equipment. The HPLC method used by Goiffon et al. (1999) was followed with modification to separate and quantitatively determine anthocyanins on Sphinx RP (Macherey-Nagel, Germany) 100 C18 3 μm 150×4.6 column, using gradient elution of (a) 10 % formic acid in water and (b) 10:45:45 formic acid–water–acetonitrile as the mobile phase (flow rate, 0.7 mL/min). The elution started with 100 % (a), changed to 100 % (b) in 25 min and turned to 100 % (a) in 5 min. For analytical analysis of anthocyanins, a Waters (Alliance) HPLC system was used, which consist of a 2695 Separation Module and a 2996 photodiode-array detector. The operation and data processing was performed by Waters Empower software. For quantification, the HPLC chromatogram was displayed and integrated at 525 nm. Diluted solutions of cyanidin-3-rutinoside (from Sarsynthese Marignac, France) were used as external standard to quantify the peaks as well as in the calibration and recovery test. The total anthocyanin content was measured by spectrophotometric method.

The anthocyanin yield for both the HPLC and pH differential methods was expressed as milligrams per gram wet weight marc, and was calculated using the following formula:

$$c(mg/g) = \frac{c_{anthocyanin}(mg/L) \times V_{solvent}(L)}{m_{marc}(g)} \qquad (3.)$$

Experimental Design and Optimization

Response surface methodology was used to determine the optimum conditions of the anthocyanin extraction from black currant marc. The effects of the following factors were investigated: microwave power (X_1), the marc to solvent ratio (S/L, X_2), the pH of the solvent (pH, X_3), and the extraction time (t, X_4). As a response function, the monomeric anthocyanin pigments content of the extracts was measured. The monomeric anthocyanin pigments yield

was calculated and expressed in milligrams per gram as cyanidin-3-glucoside equivalents. The data were analyzed using MODDE 8.0 experimental design software (Umetrics, Sweden).

The complete design consisted of 29 runs (Table 1) including five replicates in the center points (exp. no 25–29) to evaluate the model validity. The experiments were carried out in a randomized order to avoid the unexplained variability in the response due to systematic error. The design factors and the obtained response values are illustrated in Table 1.

A central composite face centered design was applied in this study, and a polynomial equation was fitted by multiple linear regression since this method is suitable when there is only one response at a time.

The multiple linear regression model is described as:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon \qquad (4)$$

Table 1 Statistical design of experiments for the extraction of anthocyanins from black currant marc

Experiment number	Factors MWP (W)	S/L	pH	t (min)	Response MAP (mg/g)
N1	140	0.05	2	10	13.1±0.6
N2	700	0.05	2	10	24.6±0.8
N3	140	0.1	2	10	12.9±0.5
N4	700	0.1	2	10	21.9±0.4
N5	140	0.05	7	10	9.3±0.2
N6	700	0.05	7	10	21.0±0.2
N7	140	0.1	7	10	9.7±0.6
N8	700	0.1	7	10	20.1±0.4
N9	140	0.05	2	30	18.6±0.5
N10	700	0.05	2	30	24.0±0.1
N11	140	0.1	2	30	18.8±0.2
N12	700	0.1	2	30	22.0±0.3
N13	140	0.05	7	30	22.4±0.4
N14	700	0.05	7	30	21.6±0.4
N15	140	0.1	7	30	20.4±0.9
N16	700	0.1	7	30	20.1±0.1
N17	140	0.075	4.5	20	16.5±0.2
N18	700	0.075	4.5	20	20.4±0.4
N19	420	0.05	4.5	20	19.1±0.1
N20	420	0.1	4.5	20	16.9±0.2
N21	420	0.075	2	20	19.4±0.4
N22	420	0.075	7	20	17.3±0.0
N23	420	0.075	4.5	10	17.1±0.1
N24	420	0.075	4.5	30	20.5±0.1
N25	420	0.075	4.5	20	18.3±0.1
N26	420	0.075	4.5	20	18.5±0.1
N27	420	0.075	4.5	20	18.6±0.1
N28	420	0.075	4.5	20	20.6±0.1
N29	420	0.075	4.5	20	19.4±0.1

Table 2 Analysis of variance for the quadratic model in the extraction of anthocyanins from black currant marc

Source of variation	DF[a]	SS[b]	MS[c]	F value	p value	SD[d]
Total corrected	28	376.28	13.43			3.67
Regression	6	350.17	58.36	49.18	0.000[e]	7.64
Residual	22	26.11	1.19			1.09
Lack of fit	18	22.51	1.25	1.39	0.409[e]	1.12
Pure error	4	3.58	0.89			0.95

[a] Degrees of freedom

[b] Sum of squares

[c] Mean square

[d] Standard deviation

[e] 5 % significance level

where Y is the dependent variable, β_i, $j=0,1,\ldots,k$, are called the regression coefficients of the model, and x_i $i=1,2,\ldots k$ are the independent variables in the model. The optimization procedure was performed after the refinement of model with the aim of defining the processing conditions for the maximum recovery of anthocyanins from black currant berry marc.

Validation of the Model

The optimum conditions for the microwave-assisted extraction of the anthocyanins from black currant marc were determined using the optimizer function. Further experiments were carried out in duplicates at the optimum conditions and analyzed by HPLC to determine the composition of the extract.

Results and Discussion

Model Fitting and Refinement

The experimental data were fitted to a polynomial equation and the model was refined by the removal of nonsignificant terms. After refitting the model, the regression coefficients

were obtained and tested for accuracy by the analysis of variance (Table 2). The coefficients of linear terms, i.e., the microwave power, the marc to solvent ratio, the pH of the solvent and the extraction time have significant effect on the amount of anthocyanins in the extracts. The interaction terms of microwave power with extraction time ($X_1 \times X_4$) and the pH of the solvent with extraction time ($X_3 \times X_4$) have also a significant contribution to the maximum recovery of anthocyanins from black currant marc by MAE ó. The importance of process variables is presented in Fig. 1. The order of the variables was as follows: microwave power (X_1) >microwave power with extraction time interaction term ($X_1 \times X_4$)>extraction time (X_4)>the pH and extraction time interaction effect ($X_3 \times X_4$)>pH of the solution (X_3)>marc to solvent ratio linear term. Similarly, Sun et al. (2007) observed that the microwave power and the extraction time were the most important variables in the recovery of anthocyanins from red raspberries, and the effect of solvent to solid ratio was less significant during the MAE process.

As Table 2 shows, the model is significant at a confidence level of 95 %, and no lack of fit was observed (F value of 1.4136, p value of 0.403), i.e., the model error is smaller than the pure error. The model showed excellent prediction ability for the extraction of the anthocyanin compounds with an R^2 value of 0.930, R^2 adj. value of 0.911,

Fig. 1 Effects of process variables in microwave-assisted extraction of anthocyanins from black currant marc. *MWP* microwave power (W), *MWP*t* microwave power and extraction time interaction term, *t* extraction time, *pH*t* pH of the solvent and extraction time interaction term, *pH* pH of the solvent, *S/L* marc to solvent mass ratio
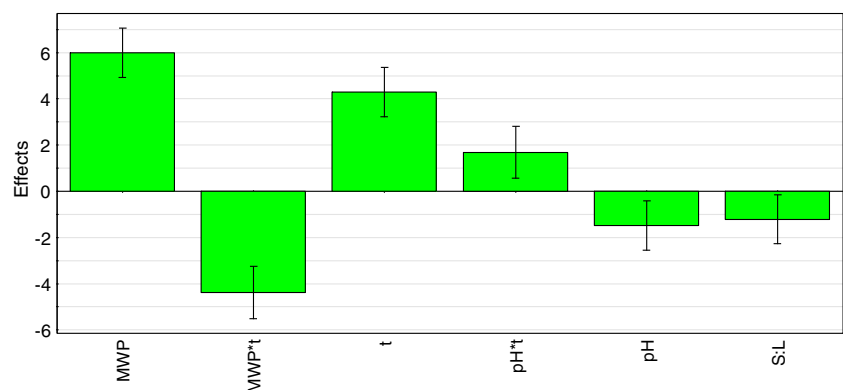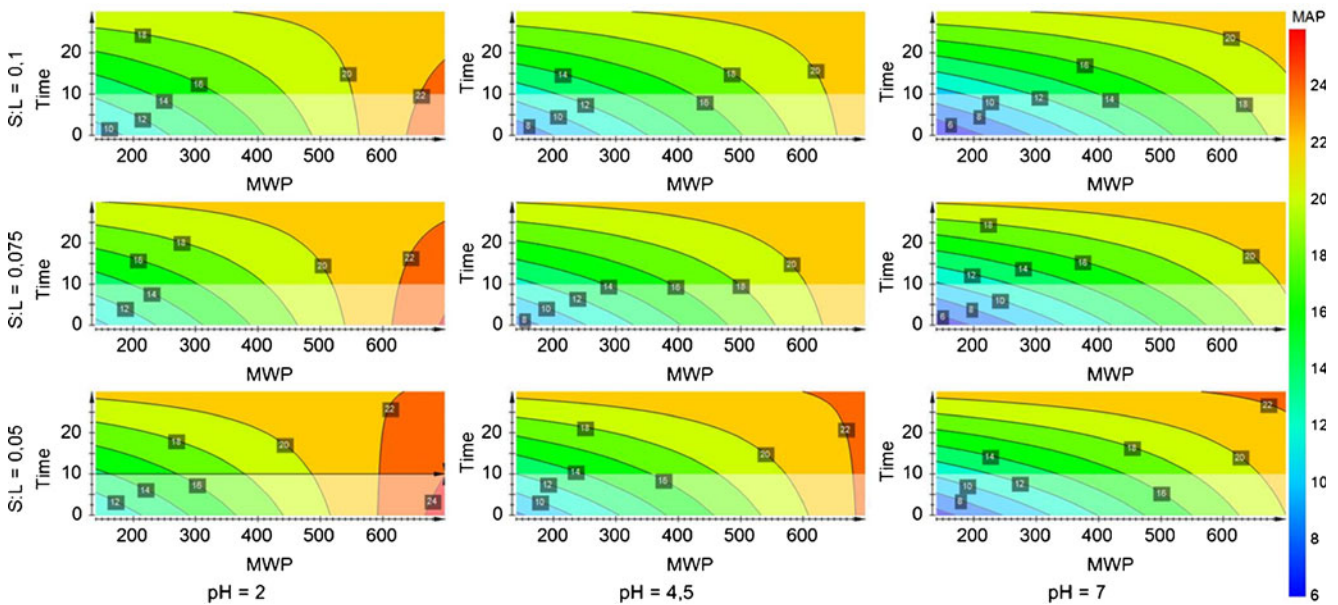
Fig. 2 Response surface plots of anthocyanin yield by microwave-assisted extraction from black currant marc at different pH. *MWP* microwave power (W), *Time* extraction time in minutes, *pH* pH of the solvent, *S/L* marc to solvent mass ratio

and a goodness of fit Q^2 value of 0.853. The reproducibility was 0.9337. Therefore, based on these results, the model is adequate to determine the optimum process parameters.

The polynomial equation obtained based on Eq. 4 was:

$$Y = 18.714 + 2.99778X_1 - 0.608X_2 - 0.73916X_3$$
$$+ 2.15806X_4 - 2.195X_1X_4 + 0.8331X_3X_4 \tag{5}$$

Optimization of Anthocyanin Extraction and Model Verification

Based on the constructed mathematical model, response surfaces and contour plots were generated to evaluate the effect of processing factors on the anthocyanin content of the extracts. The plots were generated by keeping the acidity of the solvent at pH2 and the marc to solvent ratio at 0.05. The results are illustrated in Figs. 2 and 3. The low marc to solvent value (0.05) represents the higher amount of solvent per berry marc, i.e., the addition of 560 mL of solvent liquid to 28 g of berry marc. The higher solvent ratio increases the driving force during the mass transfer (Pinelo et al. 2005) and thus reduces the extraction time. The chemical forms and, thereby, the colors of anthocyanins depend on the pH of the solution (Castañeda-Ovando et al. 2009). In unnatural forms, after extraction or isolation, the anthocyanins are unstable. The stability of anthocyanins significantly depends on pH, oxygen concentration, temperature, light, the type of solvents, and the presence of other compounds (Rubinskiene et al. 2005). At low pH, good stability of anthocyanins is observed. Cabrita et al. (2000) reported that, in very acidic

aqueous solutions, the anthocyanin 3-glucosides occurred in the most reddish color typical for their flavylium form, and showed stability over 70 % after 60 days storage at 10 °C.

In this study, the response contour and the response surface plot indicated that the anthocyanin yield increased with extraction time and applied microwave power. When higher microwave power was applied, shorter extraction time was suggested to reach the maximum anthocyanin yield. This is in accordance with the model, which showed that the microwave power and the extraction time have a
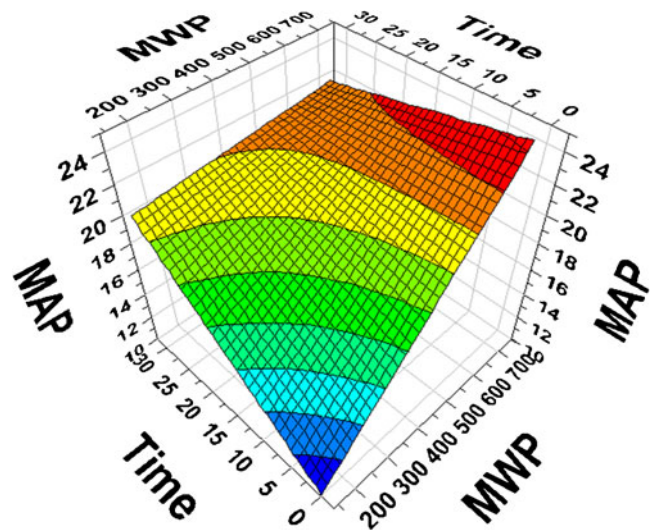

Fig. 3 Response surface plot of anthocyanin yield by microwave-assisted extraction from black currant marc. *MWP* microwave power (W), *Time* extraction time in minutes, *MAP* monomeric anthocyanin pigments as milligrams per gram cya-3-glu Eq
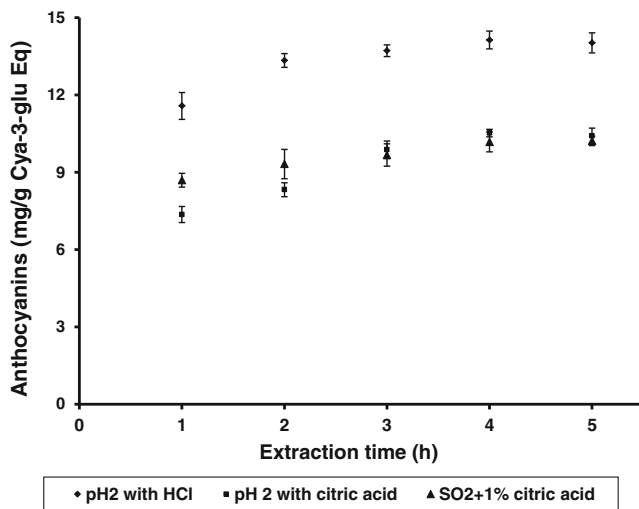
Fig. 4 Monomeric anthocyanin pigments content of the extracts during conventional extraction using different acidic solvents

positive effect, but a negative interaction effect of these factors as described by Eq. 5.

Meanwhile, the optimal operating parameters for extraction were obtained from the response surface analysis by keeping the marc to solvent ratio at a constant 0.05 value and the acidity of the solution at pH2. By these circumstances, the optimum process variables for the maximum recovery of anthocyanins from black currant marc were determined at the microwave power of 700 W and the extraction time of 10 min.

With the application of these parameters, further runs were conducted in duplicates to verify the accuracy of the model. The values obtained for the anthocyanin content were 24.0±0.0 and 25.1±0.1 mg/g, which were close to the value of 23.9 mg/g predicted in the optimization process and, therefore the model was found adequate for the optimization process.

Comparison of Microwave-Assisted Extraction with Conventional Extraction of Anthocyanins

The results showed that MAE was suitable to reduce the extraction time, minimize the volume of solvent used and to increase the final concentration of anthocyanins in the extracts, when compared to conventional solvent extraction process. The most effective solvent in the CE was the aqueous solution of hydrochloric acid at pH2. Changes of the monomeric anthocyanin pigment concentration during the CE process are illustrated in Fig. 4.

In all cases of conventional solvent extraction, the concentration of the monomeric anthocyanin pigments increased during 5 h of extraction until it reached a maximum value, but the extraction rate was higher in the first 3 h. The reason behind this might be the continuous decrease in the driving force, i.e., the concentration difference between the soluble and solid phase. Alternatively, it is also possible that a prolonged exposure time at high temperatures caused the thermal degradation of heat-resistant anthocyanins.

In the experiments when the microwave extraction was carried out using the optimum parameters of 700 W power level and 10 min of extraction time, the recorded temperature was 69.7 °C, measured by an infrared temperature sensor. Accordingly, the yield of anthocyanins obtained by the CE method was about 20 % lower than the yield by MAE under optimized conditions (16.7 and 20.4 mg/g for CE and MAE extracts, respectively). In addition, to reach the anthocyanin concentration of 16.7 mg/g, 300 min process time is needed using CE, while higher anthocyanin concentration was achieved after 10 min irradiation during MAE.

The anthocyanin composition of three samples from CE, and four randomly chosen samples from MAE were

Table 3 Composition of anthocyanin extracts in microwave-assisted and conventional extraction determined by HPLC

Extraction parameters				Anthocyanin compounds										Total
Extraction method	MWP (W)	Time (min)	pH	Delphinidin-3-glucoside		Delphinidin-3-rutinoside		Cyanidin-3-glucoside		Cyanidin-3-rutinoside		Malvidin-3-rutinoside		
				(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)
CE+CA	–	300	2.0	3.2±0.1	25.4	4.7±0.1	37.5	1.2±0.0	9.5	3.3±0.1	26.4	0.2±0.0	1.2	12.5±0.4
CE+HCl	–	300	2.0	4.0±0.2	24.4	6.2±0.2	37.5	1.6±0.1	9.7	4.6±0.1	27.9	0.1±0.0	0.5	16.7±0.5
CE+SO$_2$+CA	–	300	2.0	2.7±0.2	22.1	5.5±1.0	44.6	0.9±0.0	6.9	3.1±0.1	25.1	0.2±0.0	1.3	12.2±1.2
MAE	140	10	4.5	3.8±0.3	27.9	5.5±0.2	39.8	1.0±0.0	7.3	3.3±0.1	23.9	0.1±0.0	1.0	13.7±0.5
MAE	140	10	2.0	2.7±0.1	28.1	3.9±0.1	40.1	0.7±0.0	7.2	2.3±0.1	23.7	0.1±0.0	0.9	9.7±0.3
MAE	140	30	2.0	3.4±0.1	20.2	7.5±0.3	44.3	1.2±0.0	7.3	4.5±0.1	26.9	0.2±0.0	1.4	16.9±0.5
MAE*	700	10	2.0	5.6±0.3	27.5	8.4±0.2	41.2	1.5±0.1	7.1	4.8±0.2	23.3	0.2±0.0	0.9	20.4±0.8

CE conventional extraction, *CA* citric acid, *HCl* hydrochloric acid, *SO$_2$* sulfur dioxide, *MAE* microwave assisted extraction, *MAE** microwave assisted extraction carried out under the optimized process conditions, *MWP* microwave power

determined by HPLC as described before and the abundance of delphinidin-3-glucoside (dp-3-glu), delphinidin-3-rutinoside (dp-3-rut), cyanidin-3-glucoside (cy-3-glu), cyanidin-3-rutinoside (cy-3-rut), and malvidin-3-rutinoside (mal-3-rut) were calculated. The results are summarized in Table 3.

Landbo and Meyer (2004) reported that the four major anthocyanin pigments in black currant juice were dp-3-rut, cy-3-rut, dp-3-glu and cy-3-glu, with a relative distribution of 46–48, 31–32, 15–17, and 5–6 %, respectively, and a similar anthocyanin distribution was also reported by Iversen (1999) in black currant berries and juices.

The results of the analysis showed that the composition of the extracts were similar to that reported in the literature. The most abundant anthocyanin compound was dp-3-rut with a relative distribution 37.5–47.1 % in the different extracts, followed by cy-3-rut (23.7–27.9 %) and dp-3-glu (18.5–28.3 %), while cy-3-glu and mal-3-rut was present in all extracts with much lower concentration, between 5.8 and 9.7 % and 0.5 and 1.4 %, respectively. These results confirmed that the microwave-assisted extraction has only increased the efficiency and the rate of the extraction procedure, but neither the application of microwave, nor the conventional extraction did compromise significantly the composition of the extracts.

Conclusions

Microwave-assisted extraction was found a suitable and intensive method to extract the heat-resistant anthocyanins from black currant marc. Optimum condition for MAE was determined at pH2, extraction time of 10 min, marc to solvent ratio of 1:20 and microwave power of 700 W using aqueous hydrochloric solution as solvent. These process conditions were determined using response surface methodology, to have maximum yields of anthocyanins in the extracts as a response. The results showed that a polynomial equation fitted well to the data and, therefore, the optimum conditions for MAE could be established. Compared to conventional solvent extraction, the anthocyanin yield was increased by 20 % applying optimized MAE process, and the extraction time needed to reach maximum achievable yield was reduced from 300 to 10 min. Taking into consideration the shorter process time, the lower demand for solvent and the higher yield of extracted anthocyanins, the MAE process was proved to be useful in the recovery of heat-resistant bioactive components from food industry by-products. The lower use of solvent also benefits the further processing of the anthocyanin extracts; evaporation, membrane concentration, or drying can be performed much more economically. The results of this study also revealed that the composition of the product processed by microwave was not different than the one obtained from the commercially used solvent extraction process. In addition, once the optimum parameters are established, taking into account the microwave power, the pH and amount of the solvent and the extraction time, the scale-up of microwave-assisted extraction process becomes economically more feasible. Considering the above facts, it is suggested that microwave-assisted extraction can be used for the recovery of valuable compounds from berry juice processing by-products to obtain anthocyanin extracts. Optionally, the quality of the anthocyanin extract could be further improved and the extracts concentrated by membrane technology, to obtain a valuable dietetic product.

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