SHORT COMUNICATION

NATURAL ANTIOXIDANTS IN BLACK CHOKEBERRY MARC EXTRACTS DEPENDING ON THE EXTRACTION METHOD

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Abstract

The aim of this study was to find out the optimal conditions for extraction of natural antioxidants of grinded black chokeberry marc. The marc was extracted using microwave and ultrasound-assisted extraction methods in water/ethanol solutions with different degrees of concentration, using different particle sizes of the marc and different extraction times. Total content of polyphenols and anthocyanins was determined by spectrophotometry. The highest amount of total polyphenols was found in the extracts obtained using the ultrasound method, a 40% ethanol/water solution and a 1 mm diameter particle size extracted for 30 minutes. The microwave method presented the highest amount of anthocyanins in black chokeberry marc extract, using a 70% ethanol/water solution, a 2 mm diameter particles and 20 minute extraction time. It is necessary to find the most effective extraction method in order to develop a production technology for black chokeberry marc extracts rich in natural antioxidants.

Keywords: black chokeberry, natural antioxidants, polyphenols, anthocyanins.

Introduction

Black chokeberry (*Aronia melanocarpa*, Elliott**)** is a natural, rich source of phenolic antioxidants, such as anthocyanins, quercetin derivatives, and, in smaller amounts, vitamin C. Many reports have suggested its anti-proliferative effects against cancer cells, as well as antimutagenic, hepatoprotective, cardioprotective, and antidiabetic activities. Moreover, a series of papers reported the antioxidant properties of black chokeberry extracts or their phenolic constituents, using various, well established in vitro and in vivo models for direct antioxidant capacity, as well as their protective effects against oxidative stress. Recently, the neuroprotective effects of cyanidin-3-O-glycosides, commonly present in black chokeberry, have been tested in mice. (Gironés-Vilaplana, 2012)

The solid waste streams generated during fruit juice production can be alternative and attractive sourcesof valuable bioactive compounds due to their low cost and biorenewable nature. The utilization of wastes represents a sustainable approach to the integral benefit of raw materials minimizing the environmental problems caused by their disposal.

Berries contain high levels of phytochemicals with phenolic structure (phenolic acids, flavonoids, hydrolysable and condensed tannins) that can act as antioxidants and have health-promoting activities. The solid pressing wastes originated during separation of peels, seeds and pulp from the fruit juice are an abundant source of flavonoids, colour pigments and pectins. Berries are rich sources of anthocyanins, which impact the dark red or blue colour to the fruit, and are strong antioxidants (Laroze at al., 2010).

The phenolic content and composition greatly differs with the type of berry, and the extraction yield is greatly affected by the solvent. The recovery of compounds from the solid residue after berry processing into juice has been investigated using both conventional and alternative technologies, such as ultrasound or microwave assisted alcoholic extraction.

It is widely known that the efficiency of solid / liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time. This means that each plant matrix / extraction solvent pair behaves in a unique way, so it should be studied as such. On the other hand, both the particle size of the plant matrix and the temperature on the extraction process are easily manipulated physical conditions. In general, a smaller size and a higher temperature facilitate mass transfer, but quantification of such heuristic rules for each plant source is required before optimisation efforts can be rationally developed (Giao et al., 2009).

The present study was aimed at evaluating the effect of extraction method and particle size on the extraction and recovery of antioxidant compounds remaining in the pressing pomace of black chokeberry.

Materials and Methods

Fruit pomace of black chokeberry, grown in Latvia, was dehydrated for three days at +50 °C and milled. The ground material was passed through a sieve with different meshes. The fractions were separated and packed in polyethylene bags and kept in dark place at room temperature before use. Solid-solvent ratio 1 : 5. All the samples were assayed in triplicate.

The pressing pomaces from berries were ground in a coffee grinder, and four different particle sizes (0.5, 1, 2 and 3 mm) were selected to evaluate the effect on the yield extraction. Different ethanol solutions were used for the pomace extraction using microwave (70% and 40%) and ultrasound-assisted (50% and 40%) extraction methods. Extraction lasted for 30 minutes at 30 °C with ultrasound bath Elmasonic S30H, 50/60 Hz and 1–20 minutes for microwave oven at 90 W (for longer exposure time samples were already boiling).

Total phenolic content of the extracts were measured using Folin-Ciocalteu method as described by Almey et al. (2010). Gallic acid was used as standard. 0.5 mg·mL^{-1} stock standard solution of gallic acid was prepared by dissolving 35 mg of dry gallic acid in 50 mL of 70% ethanol and then diluted to 100 mL with the same solvent. Working standards of between 0.0014 and 0.007 mg mL^{-1} were prepared by diluting the stock solution with distilled water, adding 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) and after 2 minutes adding 2 mL of 7.5% sodium carbonate solution. Samples were prepared likewise from the extract stock solution prepared at concentration of 2–4 mg·mL^{-1} (2 mL of sample solution was taken for 25 mL test tube).

All the samples were incubated at 50 °C in water bath for 15 min, cooled quickly at cold water and filled up with purified water. Then the absorbance was read at 760 nm using Camspec M550 spectrophotometer. The standard calibration curve of gallic acid (0.0014–0.007 mg·mL^{-1}) was plotted.

The pH differential method was conducted for detection of total anthocyanin content as described in detail by Lee et al. (2005) using a spectrophotometer. It is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to colour change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0.

Results and Discussion

Total polyphenols extraction by ultrasound-assisted extraction using different particle sizes lasted for 30 minutes at 30 °C temperature and showed the highest concentration (60±1 GAE g^{-1}) when 40% ethanol solution and 1 mm particle size was used (Figure 1).

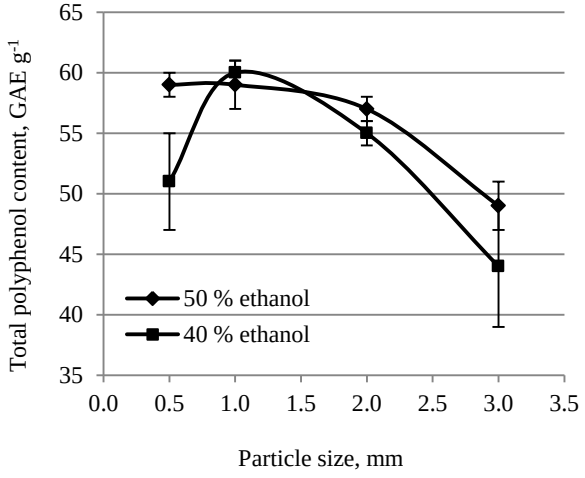

Figure 1. Relevance of particle size and polyphenol content by ultrasound-assisted extraction (30 min)

However almost the same concentration (59±2 GAE g^{-1}) was reached with 50% ethanol solution and 1 mm particle size.

Meanwhile anthocyanins were extracted and the highest amount of total monomeric anthocyanins (4594 ± 412 mg·L^{-1}) was found at 50% ethanol extract when 1 mm particles were used (Figure 2). The extraction solvent concentration was more important in this case comparing to polyphenol extraction. The most effective extraction in both experiments was obtained using 1 mm particle size of black chokeberry marc.

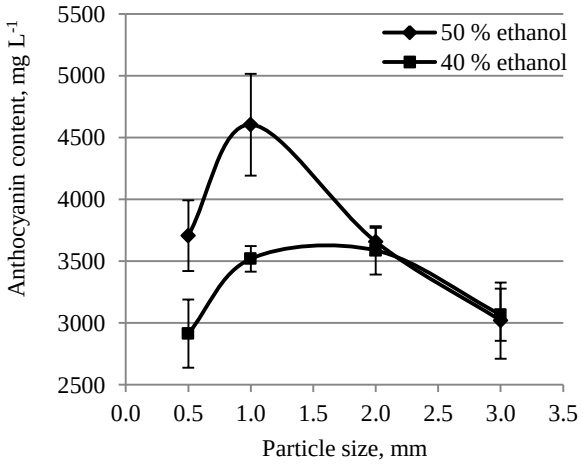

Figure 2. Relevance of particle size and anthocyanin content by ultrasound-assisted extraction (30 min)

The results obtained using microwaves showed at figures 3 and 4. Two particle sizes (2 mm and 3 mm) and two ethanol concentrations (40% and 70%) were investigated.

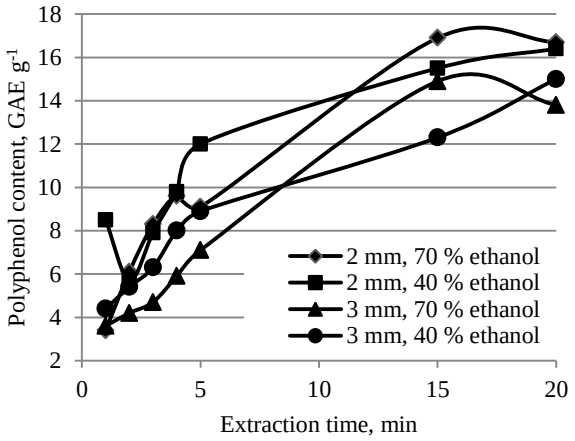

Figure 3. Polyphenol content depending on the extraction time by microwave-assisted extraction

The polyphenol concentration increases by increasing of extraction time, however after 15 minutes it decreases in 70% ethanol extracts. The highest level of polyphenols (16.9±1.2 mg L^{-1}) was achieved with 2 mm particles in 70% ethanol extraction after 15 min. The anthocyanin concentration increases in the same way – by increasing extraction time. The highest total anthocyanin concentration (6517±182 mg·L^{-1}) was obtained with 2 mm particles in 70% ethanol extract

after 20 min. Longer extraction times would not be accurate due to the rise of temperature and solvent evaporation.

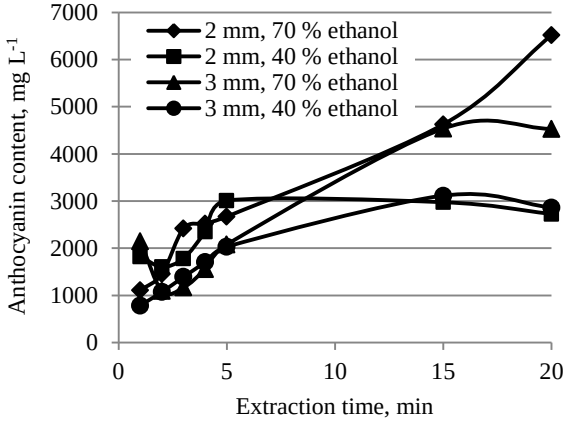

Figure 4. Anthocyanin content depending on the extraction time by microwave-assisted extraction

Microwave technology was more effective for 2 mm than 3 mm particles and 70% ethanol was more effective than 40% ethanol solution for both – polyphenols and anthocyanins

Some authors (Landbo, Mayer, 2001, Wang et al., 2011) investigated extraction efficiency using different solvents, temperature conditions, solvent-solid ratios and particle size and concluded that higher yields in extraction resulted from a decrease in particle size. Other researcher groups working with plant material found out that the most effective extraction is achieved when 0.2 mm (Giao et al., 2009; Goula 2013) and <0.5 mm (Fonseca et al., 2006) particle sizes were used. However smaller particles than 0.5 mm were not investigated in this research due to the available meshes, 1 mm particles were more effective than 0.5 mm. Researcher group investigating black chokeberry found >2 mm particles to be the most effective for ultrasound extraction of polyphenols (d'Alessandro et al, 2012), but it was reached in water extraction at 60 °C temperature and solid-solvent ratio 1:20. Hence each plant material and extraction solvent pair behaves in different way as mentioned above and effect of particle size, time, temperature, solid-solvent ratio, solvent composition and an impact of extraction method need to be evaluated separately. These results are significant for new product developing from black chokeberry juice pressing residues – rich in natural antioxidants.

Conclusions

Polyphenols and anthocyanins were successfully extracted from black chokeberrymarc of different particle size, with different extraction methods, different solvent concentrations and different extraction time. The highest polyphenol yield was obtained from the marc having 1 mm particle size in 40% ethanol extract using ultrasound for 30 minutes. The highest anthocyanin yield was obtained from the marc having 2 mm particle size in 70% ethanol by microwave extraction for 20 minutes. Both extraction methods may be combined to find the most effective extraction method for both – polyphenols and anthocyanins.

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