Scientific paper

Conventional and Ultrasound-Assisted Extraction of Anthocyanins from Blackberry and Sweet Cherry Cultivars

Simona Oancea,[1,*] Cristian Grosu,[2] Otto Ketney[1] and Mihaela Stoia[3]

[1] *Lucian Blaga University of Sibiu, Department of Agricultural Sciences and Food Engineering, 7–9 Ion Ratiu Street, 550012 Sibiu, Romania*

[2] *Lucian Blaga University of Sibiu, Department of Environmental Sciences, 5–7, Ion Ratiu Street, 550012 Sibiu, Romania*

[3] *Lucian Blaga University of Sibiu,"Victor Papilian" Faculty of Medicine, 2A Lucian Blaga Street, 550169 Sibiu, Romania*

** Corresponding author: E-mail: simona.oancea@ulbsibiu.ro, Tel. +40269211338, Fax +40269212558*

Received: 18-02-2013

Abstract

Blackberry and sweet cherry are important plant foods rich in anthocyanins well-known for their pharmacological and antioxidant effects.

The aim of the present paper was to comparatively investigate conventional and ultrasound-assisted extraction procedures in order to isolate an enriched crude anthocyanin extract from blackberry (Thornfree cultivar) and sweet cherry (Black Gold cultivar). Hydroethanolic solution and acidified ethanol were used to conventionally extract anthocyanins by a discontinuous process at 4 °C for 2/ 24 hours. Added hydrochloric acid in ethanol of different concentrations proved to be more efficient in both type of samples. In the ultrasound-assisted extraction, the highest recovered anthocyanin content in blackberry (107.81 mg 100 g^{-1} FM) was obtained with a 10/1 solvent/solid ratio (v/w) at 30 °C for 5 minutes, while a 15/1 solvent/solid ratio (v/w) at 30 °C for 20 minutes lead to an increased antioxidant capacity as determined by ferric reducing antioxidant power in the extract using 0.1% HCl in 80% ethanol. The optimum conditions obtained for ultrasound-assisted extraction from sweet cherry in 0.1% HCl in 60% ethanol at 30 °C include a 15/1 solid/solvent ratio (w/v) and 5 minutes for the maximum yield (36.05 mg 100 $^{-1}$ FM). The final crude anthocyanin extracts may find useful application as dietary supplements, or may be further purified for application as food ingredients.

Keywords: Anthocyanins, blackberry, sweet cherry, ultrasound-assisted extraction, pH differential, FRAP

1. Introduction

Plants have been for long considered an important source of biologically active compounds called »phytochemicals« which provide health-promoting and disease-preventing benefits. Phytochemicals represent a large class of compounds with high structural variability, such as phenolics, carotenoids, alkaloids, vitamins, nitrogen and organosulfur compounds.

Plants from *Rosaceae* family are economically important crops producing fruits known for their high content of bioactive compounds showing a variety of pharmacological effects, which are supported by epidemiological studies.[1–2] In this family, blackberry – the fruit of *Rubus fruticosus* L., and sweet cherry – the fruit of *Prunus avium* L., are plant foods rich in anthocyanins, consumed either as such or in processed foods.

Anthocyanins are water-soluble plant pigments, abundantly consumed by humans, which belong to the class of flavonoids and which display a wide range of beneficial properties based on their free-radical scavenging and antioxidant capacities.[3]

The analysis of anthocyanins in fruits and vegetables represents an important task for estimation of the dietary intake of these biomolecules in particular populations. The analytical strategy initiates with their isolation from plant cells. The isolation procedure which is closely related to the need of obtaining the highest amounts

and of preserving their bioactivity, requires several steps: sample size reduction, appropriate extraction, physicochemical characterization and *in vitro* studies of specific biological activities. The selection of appropriate techniques for each step is essential for the establishment of the structure-activity relationship (SAR) and for the optimization of the composition of the mixed extracts to be used in pharmaceutical, food or cosmetic industry.

Several conventional and modern (non-conventional) extraction procedures were described for anthocyanins, finally leading to either an enriched crude pigment extract obtained by solid-liquid partition process, or to a further purified extract. Crude extracts of anthocyanins are used for quantitative analysis by UV-Vis spectroscopy.[4] The extraction techniques may be improved by optimization of various parameters (solvent type, solvent concentration, solvent/solid ratio, temperature, time) in order to obtain high extraction yields.

The conventional extraction of anthocyanins is carried out commonly in acetone or acidified methanolic solutions in order to obtain the red stable flavylium cation,[4–5] but acid and/or methanol evaporation may cause partial hydrolysis of acylated anthocyanins.[6]

Ultrasound-assisted extraction (UAE) – a non-conventional technique, became a very active research topic in particular in food technology.[7] It has been applied in Romania for the extraction of different phytochemicals at industrial scale,[8] being considered a promising tool for an efficient extractive method. Optimization of several ultrasound-assisted extraction conditions by using the response surface methodology was applied to increase the efficiency of phenolics extraction.[9] It was shown that ultrasounds act more efficiently during extraction by an improved mechanical effect and by producing acoustic cavitations in the solvent.[10–11] Other modern extraction techniques, such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SPE) were also applied for anthocyanins but with modest success,[12–14] as anthocyanins are heat-sensitive compounds, and SFE techniques are particular suited for non-polar solvents.

The aim of the present paper was to investigate the optimum conditions for recovering high total anthocyanins content from the selected blackberry and sweet cherry cultivars grown in Romania. Anthocyanins were extracted from the selected fruits under conventional conditions in several solvent systems and under various UAE conditions (extraction time, solvent/solid ratio). In addition, the total antioxidant capacity measured by the ferric reducing antioxidant power (FRAP) assay was evaluated in blackberry anthocyanin extracts prepared under different UAE conditions. The obtained crude anthocyanin extracts may find further application in food, pharmaceutical or cosmetic products, based on the sinergistic effects exerted by phytochemicals.

2. Experimental

2. 1. Plant Samples

Fresh fruits of blackberry Thornfree cultivar (*Rubus fruticosus* L.) and sweet cherry Black Gold cultivar (*Prunus avium* L.) were collected in 2011 from Dragomiresti/Romania cultivated field and Ciresoaia/Romania orchard, respectively. Seeds were removed from sweet cherries. The fresh samples were kept at – 18 °C until analyzed. Reducing sample size of plant material by grinding was performed before extraction.

2. 2. Determination of Moisture, Refractive Index, Refractometric Dry Matter and pH

The moisture content of fruit samples was determined at 105 °C using the ML-50 moisture analyzer (A&D Company Ltd., Japan). The refractive index and the total soluble solids (TSS) of the fruit juices, obtained by manually pressing, were determined by refractometry using an Abbe refractometer (Krüss AR2008, Germany) at a standardized temperature (21 °C). Values are expressed as refractometric TSS (°Brix). The pH of the crude extracts was determined using the S220SevenCompact pH/ionmeter (Mettler Toledo, USA).

2. 3. Anthocyanins Extraction

2. 3. 1. Conventional extraction

The crushed fruits of *Rubus fruticosus* L. and *Prunus avium* L. obtained by using a mortar and pestle were mixed with seven different extraction solvents:

0.1% HCl in 60% ethanol (v/v)
0.1% HCl in 80% ethanol (v/v)
60% ethanol
80% ethanol

The extraction was facilitated by occasional shaking for 2 hours and 24 hours, respectively, at 4 °C. The obtained extracts were filtered and centrifuged at 8000 rpm, at 4 °C for 10 minutes. The refrigerated centrifuge (Nűve NF 800R, Turkey) was used.

2. 3. 2. Ultrasound-Assisted Extraction (UAE)

The ultrasound-assisted extraction (UAE) was carried out in an ultrasonic device (Elmasonic S60H, Germany) with an ultrasonic power effective of 150 W and an ultrasonic frequency of 37 kHz, equipped with a digital timer and a temperature controller.

The accurately weighed ground samples of frozen blackberry and sweet cherries were mixed with an appropriate amount of extraction solvent which proved to be efficient in the previously performed conventional extraction assay. The vials containing the samples in the selected solvent were immersed into water in the ultrasonic device, and irradiated for the predetermined extraction time

and at various solvent/solid ratios, at 30 °C. The temperature was controlled and maintained at 30 °C by periodical ice addition in the water bath. After the ultrasonic extraction, all samples were filtered first through multiple layers of gauze sheets, and then on Whatman's filter paper #1.

2. 4. Total Anthocyanins Assay

The content of total anthocyanins in the obtained crude extracts was determined spectrophotometrically by the pH differential method.[4] Measurements were made in duplicate. The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena, Germany) was used. The content of anthocyanins was expressed as cyanidin-3-O-glucoside (Cyn-3-O-G) according to its molar extinction.

2. 5. Ferric Reducing Antioxidant Capacity (FRAP Assay)

The total antioxidant capacity of blackberry crude extracts obtained through UAE was determined by the ferric reducing ability assay described by Benzie and Strain.[15] The absorbance of the mixture of anthocyanin extracts and FRAP reagent was measured at 593 nm after 5 minutes. The results were expressed as mg ascorbic acid g^{-1} DM.

2. 6. Statistical Analysis

Data processing consisted in mathematical and statistical methods performed by "Statistica" software, following hypothesis testing and correlation between variables by calculating the Pearson correlation coefficient r ($r = \pm 1$ means perfect correlation), at a significance level of risk $\alpha \leq 5\%$ and probability $P \geq 95\%$.

3. Results and Discussion

3. 1. Conventional Extraction

It is known that extractive technology highly influences the quality of a final herbal product to be used either as supplement or food ingredient.

Anthocyanins occur naturally as glycosides, so that polar solvents are essential for the achievement of a good extraction yield. Methanol has been the most frequently used solvent for the anthocyanin extraction. Because of its toxic effects and in view of the final potential uses of the obtained crude extracts, we have substituted methanol with the environmentally friendly ethanol in all extraction runs. In order to evaluate the solvent concentration influence on the content of total anthocyanins in hereby selected blackberry *Rubus fruticosus* L. Thornfree cultivar and sweet cherry *Prunus avium* L. Black Gold cultivar, different solvent systems based on acidified and non-acidified hydroethanolic solution were investigated for the conventional extraction: *(1)* 0.1% HCl in 60% ethanol (v/v); *(2)* 0.1% HCl in 80% ethanol (v/v); *(3)* 60% ethanol (v/v); *(4)* 80% ethanol (v/v). The extraction was conducted at 4 °C to minimize the anthocyanins degradation. The concentration of anthocyanins was determined by spectrophotometric pH differential method.

Several physicochemical characteristics of the obtained anthocyanin extracts and/or juices are presented in Table 1. The value of total soluble solids represents a quality control parameter useful in processing fruits and represents a measure of the sugar content (indicator of fruit maturity and ripeness). The different phytochemical compositions, in particular organic acids in the studied fruits influence the pH of the final extracts. High content of total phenolics was found for blackberry Thornfree cultivar (257.18 mg GAE $100g^{-1}$ FM) compared to sweet cherry Black Gold cultivar (184.85 mg GAE $100g^{-1}$ FM).

As presented in Figure 1, the results showed that adding hydrochloric acid in ethanol proved more efficient than hydroethanolic solution regarding the extraction yield of anthocyanins from blackberry samples. The highest recovered total anthocyanins content was obtained with 0.1% HCl in 80% ethanol (198.25 mg $100g^{-1}$ FM). The lowest recovered total anthocyanins content was obtained with 80% ethanol (141.27 mg $100g^{-1}$ FM).

Figure 2 presents the total anthocyanins content in sweet cherry samples according to different solvent systems used for their conventional extraction. As noted, 0.1%

Table 1: Physicochemical attributes of the studied blackberry and sweet cherry samples/extracts.

Sample	Physicochemical characterization				Extraction solvents	pH of extract
	Moisture (%)	Total soluble solids (°Brix)	Refractive index (n)	Total phenolics (mg GAE $100g^{-1}$ FM)		
Blackberry	84.4	10.4	1.3477	257.18	0.1% HCl in 60% EtOH (v/v)	2.17
Thornfree					0.1% HCl in 80% EtOH (v/v)	2.18
cultivar (*Rubus*					60% EtOH	4.44
fruticosus L.)					80% EtOH	4.94
Sweet cherry	78.9	19.4	1.3621	184.85	0.1% HCl in 60% EtOH (v/v)	1.72
Black Gold					0.1% HCl in 80% EtOH (v/v)	1.71
cultivar					60% EtOH	4.74
(*Prunus avium* L.)					80% EtOH	5.14

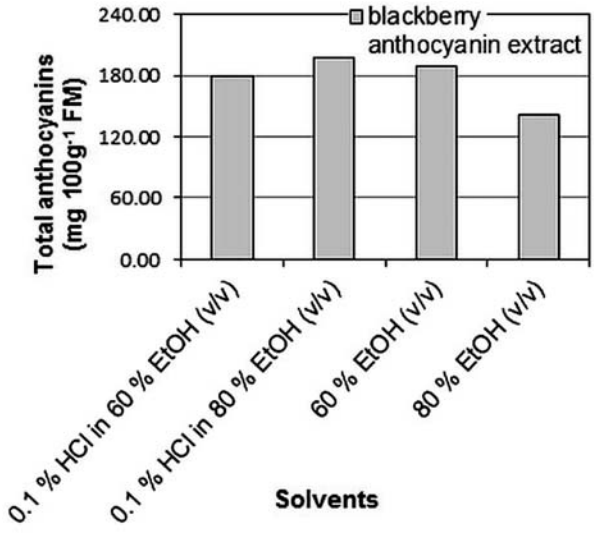

Figure 1: Total anthocyanins content in blackberry Thornfree cultivar (*Rubus fruticosus* L.) fresh samples according to different extraction solvent systems, at 4 °C, 2 hours

HCl in 60% ethanol proved to be the most efficient solvent system for high anthocyanins recovery (44.19 mg 100 g^{-1} FM). The lowest recovered total anthocyanins content was obtained with 60% ethanol (28.22 mg 100 g^{-1} FM).

Acids are essential to stabilize anthocyanins in the form of the flavylium cation, but excess may lead to the partial hydrolysis of the glycosidic bonds or cause

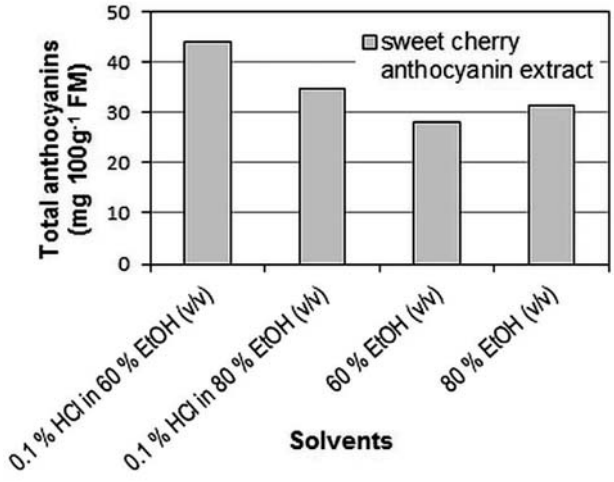

Figure 2: Total anthocyanins content in sweet cherry Black Gold cultivar (*Prunus avium* L.) fresh samples according to different extraction solvent systems, at 4 °C, 2 hours

breaking linkages with metals or co-pigments. The formation of furfural and hydroxymethylfurfural generated by acid hydrolysis of the sugar residues, which is accelerated by heat, has been shown to favor the pigment decay.

3. 2. Ultrasound-Assisted Extraction (UAE)

In this investigation, the extraction solvent, which determined the highest total anthocyanins content, was selected in accordance with previous experiments of conventional extraction, as follow: 0.1% HCl in EtOH 80% in case of blackberry Thornfree cultivar, and 0.1% HCl in EtOH 60% for sweet cherry Black Gold cultivar. Other studies regarding the optimization of anthocyanins extraction showed that despite elevated extraction temperatures improve the process efficiency, at a critical temperature of 35 °C anthocyanin degradation initiates.[16] Considering this and for a better anthocyanin extraction yield, the UAE investigation was performed at 30 °C. As solvent volumes highly influence the content of bioactives in extracts, the most suitable solvent/solid ratio was evaluated. Extraction time affects extraction yield as well, so that we investigated three time point between 5 and 20 minutes. The experimental design for UAE of anthocyanins from blackberry and sweet cherry is shown in Table 2.

The results indicate that using 0.1% HCl in 80% ethanol, at a 10/1 solvent/solid ratio (v/w) at 30 °C for 5 minutes was adequate for UAE anthocyanin extraction in blackberry, while a 15/1 solvent/solid ratio (v/w) at 30 °C for 20 minutes lead to an increased antioxidant capacity as measured by ferric reducing antioxidant power (FRAP) assay. However, short extraction time favours high anthocyanins recovery but longer UAE extraction time and greater solvent/solid ratio lead to the increase of total antioxidant capacity given probably by the presence of other bioactives extracted under the process conditions.

As some reported results showed that the antioxidant activity of different cultivars of sweet cherry is not related only with total phenolics or anthocyanins,[17] in this case we have limited the optimization of UAE only to the evaluation of total anthocyanins.

The results have shown that using 0.1% HCl in 60% ethanol at a 15/1 solvent/solid ratio (v/w) at 30 °C for 5 minutes was adequate for UAE anthocyanin extraction in sweet cherry. As experimented previously with conventional extraction of anthocyanins from sweet cherry, a rapid UAE extraction at 30 °C is also recommended, as longer extraction time determined a highly decrease in total anthocyanins probably due to their decomposition. The final E9 UAE experimental run showed that at 20/1 solvent/solid ratio (v/w) for 20 minutes, 53.8% of total anthocyanins was recovered.

The results of statistical analysis performed by comparison between groups of experiments highlight the optimum values for total anthocyanins and FRAP antioxidant capacity at a solvent/solid ratio (v/w) of 15/1, but in different relationship with extraction time (Table 3). While the total anthocyanins content varies inversely with the UAE extraction time in both fruit samples ($r = –0.81$ for blackberry, and $r = –0.90$ for sweet cherry, respectively), the relationship between FRAP antioxidant capacity and extraction time is a direct proportional one ($r = 0.98$).

Table 2: Experimental data (extraction time, solvent/solid ratio) and the observed response value (total anthocyanins,antioxidant capacity) through the ultrasound-assisted extraction of anthocyanins from blackberry and sweet cherry.

			Blackberry Thornfree cultivar		Sweet cherry Black Gold cultivar
Experiment no.	**Extraction time (min)**	**Solvent/solid ratio (v/w)**	**Total anthocyanins mg (100 g^{-1} FM)**	**FRAP antioxidant capacity (mg ascorbic acid g^{-1} DM)**	**Total anthocyanins (mg 100 g^{-1} FM)**
E1	5	10	107.81	22.80	31.16
E2	5	15	105.23	22.88	36.05
E3	5	20	91.67	22.27	28.24
E4	10	10	87.00	20.11	22.08
E5	10	15	101.93	23.14	24.21
E6	10	20	85.09	23.19	25.99
E7	20	10	88.23	21.25	19.98
E8	20	15	101.54	24.45	19.50
E9	20	20	92.75	24.02	19.40

The values of FRAP antioxidant capacity of blackberry showed the highest homogeneity (0.75% variation), while the values of total anthocyanins content in sweet cherry showed the lowest one (32% variation).

The analyzed parameters have different trends in relationship to the experimental design conditions (extraction time and solvent/solid ratio), as follow: FRAP antioxidant activity and total anthocyanins extracted from blackberry depend on the solvent/solid ratio (optimum values at 15/1), while total anthocyanins extracted from sweet cherry depend on the time extraction (optimum values at 5 minutes) at a significance level of $\alpha \leq 5\%$ and $P \geq 95\%$ (Figures 3 and 4).

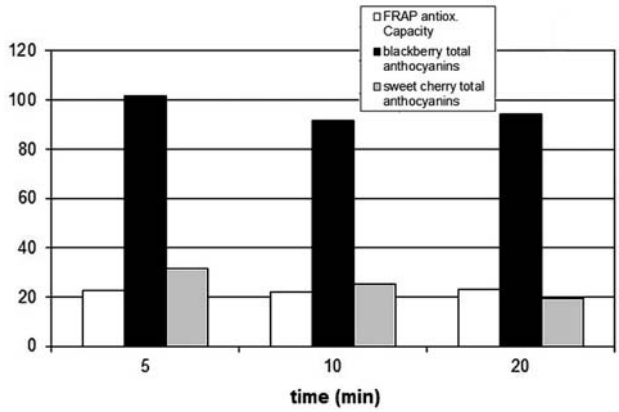

Figure 3: Mean values trend of total anthocyanins content and FRAP antioxidant capacity related to extraction time

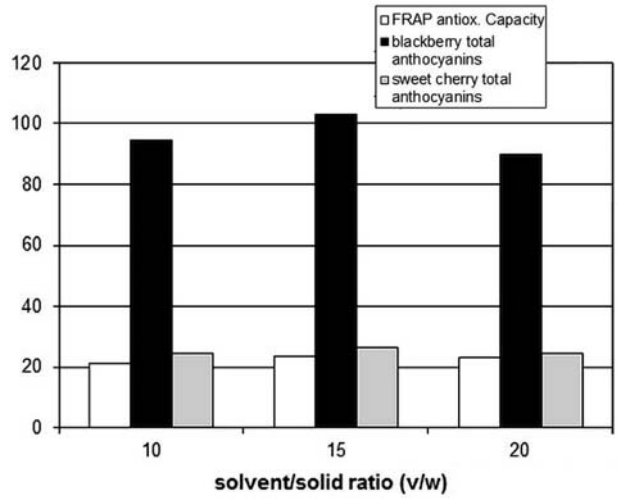

Figure 4: Mean values trend of total anthocyanins content and FRAP antioxidant capacity related to solvent/solid ratio (v/w)

3. 3. Comparison of UAE to Conventional Extraction

Comparison of UAE with conventional extraction of anthocyanins from blackberry and sweet cher-

Table 3: Mean values of total anthocyanins content and antioxidant capacity of blackberry and sweet cherry at 15/1 solvent/solid ratio (v/w) in relationship with the extraction time (r).

Variables	**Mean value**	**Standard deviation**	**Coefficient of variation (%)**	**Correlation between variable and extraction time (r)**
Blackberry total anthocyanins (mg 100 g^{-1} FM)	102.90	2.00	1.97	– 0.81
Sweet cherry total anthocyanins (mg 100 g^{-1} FM)	26.59	8.50	32.00	– 0.90
Blackberry FRAP antioxidant capacity (mg ascorbic acid g^{-1} DM)	23.49	0.18	0.75	0.98

ry was done on similar treated samples – the frozen ones.

As transfer of the quality-relevant constituents from the plant material to the crude extract may be improved by increasing the time of conventional extraction, we have investigated the extraction of anthocyanins from frozen samples of blackberry and sweet cherry, using the appropriate solvent and solvent/solid ratio, for 2 and 24 hours, respectively. The results are shown in Figures 6 and 7. Longer time of conventional extraction lead to a better recovery of total anthocyanins in blackberry Thornfree cultivar (*Rubus fruticosus* L.), but not in case of sweet cherry Black Gold cultivar (*Prunus avium* L.), as shown in Table 4. This is possibly due to the abundance of native enzymes in sweet cherries, particularly polyphenoloxidase (PPO) which has been shown to be stable also at freezing temperatures causing anthocyanins destruction in frozen samples.[18] The browning process is accelerated by organic acids found in these fruits which are substrates for PPO. However, we observed that anthocyanins from sweet cherry are less stable than those isolated from blackberry, and rapid handling is required for achieving a good extraction.

It is worthy to mention that the total anthocyanins content under UAE at an ultrasonic power effective of 150 W was found comparable to that obtained through conventional extraction, especially for blackberry, but with significant reduction in extraction times. As conventional extraction procedures of anthocyanins require longer extraction time (2–24 hours) which may lead to their decomposition in particular when elevated temperatures are employed, the hereby applied UAE technique proved to be a useful rapid tool for extraction of the greatest amounts of these biomolecules in shorter time (extraction at 30 °C for 5 minutes).

Our results will complete the present knowledge regarding the status of UAE of blackberry anthocyanins, as other reported studies focused on experiments with higher temperature (58–70 °C) and extraction time (30–40 minutes) at higher ultrasonic power (300–500 W),[19–20] while microwave-assisted extraction (MAE) was most frequently applied for sweet cherry anthocyanins as non-conventional methods compared to UAE method.[21] Some recent reported studies indicate that microwave-assisted extraction of anthocyanins investigated in *Schisandra chinensis* fruit has a greater color degradation effect on these molecules compared to the ultrasound-assisted extraction.[22] A threshold extraction time of 60 minutes at 25 °C and a ultrasonic power of 250 W for UAE and a threshold extraction time of 5 minutes for MAE is reported in this study. However, higher extraction temperature and longer extraction time might lead to increased extracted impurities and increased energy costs.

4. Conclusions

The effects of solvent type, solvent/solid ratio and time on extraction yield were comparatively studied through conventional and ultrasound-assisted extraction procedures at laboratory-scale in order to isolate an enriched crude anthocyanin extract from blackberry (Thornfree cultivar) and sweet cherry (Black Gold cultivar). The results indicate that added hydrochloric acid in ethanol of different concentration lead to highest amounts of anthocyanins in both type of samples. In the ultrasound-assisted extraction experiments, the highest recovered anthocyanins content in blackberry was obtained with a 10/1 solvent/solid ratio (v/w) at 30 °C for 5 minutes, while a 15/1 solvent/solid ratio (v/w) at 30 °C for 20 minutes lead to an increased antioxidant capacity as determined by ferric reducing antioxidant power. The optimum ultrasound-assisted extraction conditions to obtain a maximum anthocyanins extraction yield from sweet cherry were 15/1 solid/solvent ratio (w/v) and 5 minutes at 30 °C.

The ultrasound-assisted extraction technology may be of practical interest since it can be used as a rapid method that precedes quantitative evaluation of anthocyanins from different varieties of crops or wild flora, or from by-products of food industry. Enhancement in the design of efficient ultrasonic devices will become of great future interest, which may lead to the development of a standard technique.

The final hydroalcoholic crude extract of anthocyanins from selected fruits may find useful application as dietary supplement, or may be further purified for application as food ingredient.

5. Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0474.

Table 4: Total anthocyanins content in blackberry and sweet cherry according to different times of conventional extraction, at 4 °C.

Experiment no.	Extraction time (hours)	Solvent/solid ratio (v/w)	Blackberry Total anthocyanins (mg $100g^{-1}$ FM)	Sweet cherry Total anthocyanins (mg $100g^{-1}$ FM)
E10	2	10	111.62	53.43
E11	24	15	122.87	44.06

6. References

1. G. Block, B. Patterson, A. Subar, *Nutr. Cancer* **1992**, *18*, 1–29.
2. S. Y. Kang, N. P. Seeram, M. G. Nair, L. D. Bourquim, *Cancer Lett.* **2003**, *194*, 13–19.
3. E. Kowalczyk, P. Krzesinski, M. Kura, B. Szmigiel, J. Blaszczyk, *Pol. J. Pharmacol.* **2003**, *55*, 699–702.
4. M. M. Giusti, R. E. Wrolstad, in: R. E. Wrolstad, S. J. Schwartz (Ed.): Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc. New York, NY, **2001**.
5. C. Garcia Viguera, P. Zafrilla, F. Artes, F. Romero, P. Abellan, F. A. Tomas-Barberan, *J. Sci. Food Agric.* **1998**, *78*, 565–573.
6. E. Revilla, J.-M. Ryan, G. Martin-Ortega, *J. Agric. Food Chem.* **1998**, *46*, 4592–4597.
7. R. K. Bhaskaracharya, S. Kentish, M. Ashokkumar , *Food Engineering Reviews* **2009**, *1*, 31–49.
8. M. Vinatoru , *Ultrason. Sonochem.* **2001**, 8, 303–313.
9. X. Wang, Y. Wu, G. Chen, W. Yue, Q. Liang, Q. Wu, *Ultrason. Sonochem.* **2013**, *20*, 846–854.
10. K. Ghafoor, Y. H. Choi, J. Y. Jeon, I. H. Jo, *J. Agric. Food Chem.* **2009**, *57*, 4988–4994.
11. M. A. Rostagno, M. Palma, C. G. Barroso *J. Chromatogr. A* **2003**, *1012*, 119–128.
12. Z. Y. Ju, L. R. Howard, *J. Agric. Food Chem.* **2003**, *51*, 5207–5213.
13. C. Mantell, E. Martinez de la Ossa, M. Rodriguez, in: M. Cox, M. Hidalgo, M. Valiente (Ed.) Solvent Extraction for the 21st Century, Society of Chemical Industry, London, 1999, pp. 1365–1369.
14. M. Bleve, L. Ciurlia, L. Rescio, *NutraCos* **2005**, *4*, 23–26.
15. F. F. I. Benzie, J. J. Strain, *Anal. Biochem.* **1996**, *239(1)*, 70–76.
16. J. E. Cacace, G. Mazza, *J. Food Eng.* **2003**, *59*, 379–389.
17. V. Usenik, J. Fabči, F. Štampar , *Food Chem.* **2008**, *107*, 185–192.
18. A. Chaovanalikit, R. E. Wrolstad, *J. Food Sci.* **2004**, *69(1)*, 73–83.
19. L. Li, Y. Liu, X. Yue, Q. Peng, D. Duo, *Natural Product Research and Development*, **2011**, *23(5)*, 939–945.
20. X. Huaide, Y. Ninghuan, C. Wei, L. Lifang, *Transactions of the Chinese Society of Agricultural Engineering* **2008**–02.
21. C. G. Grigoras, E. Destandau, S. Zubrzycki, C. Elfakir, *Sep. Purif. Technol.* **2012,** *100(24)*, 51–58.
22. C. Ma, L. Yang, F. Yang, W. Wang, C. Zhao, Y. Zu, *Int. J. Mol. Sci.* **2012**, *13*, 14294–14310.

Povzetek

Robide in česnje so pomemben prehrambeni vir antocianinov, ki so znani po farmakološkem in antioksidativnem delovanju. Namen članka je primerjava postopkov, konvencionalne ekstrakcije in ekstrakcije z ultrazvokon (UAE) za izolacijo surovga ekstrakta robide (sorta Thornfree) in česnje (sorta Black Gold). Raztopini etanola v vodi in etanola z dodatkom kisline sta bili uporabljeni v konvencionalnih šaržnih postopkih pri temperaturi ekstrakcije 4 °C in času ekstrakcije 2/24 ur. Dodatek klorovodikove kisline k etanolu je bil zelo učinkovit pri ekstarkciji obeh vzorcev. Pri ultrazvočni ekstrakciji je bil najvišji dobitek antocianinov pri robidi (107.81 mg 100 g^{-1}) pri razmerju topilo/trdna snov 10/1 (v/w) pri temperaturi 30 °C in času ekstrakcije 5 min, medtem ko je bila pri razmerju topilo/trdna snov 15/1 (v/w) pri 30 °C in 20 minutni ekstrakciji povišana antioksiadtivna kapaciteta ekstrakta (FRAP) pri uporabi 0.1 % HCl v 80 % etanolu. Optimalni procesni parametri za ekstrakcijo česnje z ultrazvokom so 0.1 % HCl v 60 % etanolu pri 30 °C, razmerje trdna snov/topilo 15/1 (w/v) in čas ekstrakcije 5 min. Maksimalni dobitek je bil 36.05 mg 100 g^{-1}. Surov ekstrakt antocianinov se lahko uporablja v industriji hrane kot prehransko dopolnilo ali pa se ga frakcionira in uporabi kot sestavino hrane.