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Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium corymbosum* in the perspective of food applications

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Abstract

Bioflavonoids, in particular anthocyanins have made the topic of many scientific research, mainly for two reasons: their beneficial effects on human health and applications as potential sources of natural food dyes. Natural extracts of these pigments may also find useful application for textiles dying, as an eco-friendly alternative to synthetic dyes.
The aim of the present paper was to apply conventional extraction procedures in order to isolate an enriched crude extract of pigments from highbush blueberries (*Vaccinium corymbosum* L.). Water, hydroalcoholic solution and acidified alcoholic solvent were tested to extract anthocyanins by a discontinuous process. Also, temperatures of 4 ^{o}C, 30 ^{o}C and 50 ^{o}C at 2 hours time of extraction, and storage at –18 ^{o}C were tested. Concentration of monomeric anthocyanins was performed by the spectrophotometric pH differential method.
Added hydrochloric acid in ethanol system was found more efficient than acetic acid regarding the extraction yield. The highest recovered anthocyanin content was obtained with 50 % ethanol (148.51 mg 100 g^{-1} FW), while the lowest one was obtained with water (3.24 mg 100 g^{-1} FW). The applied elevated temperatures showed better extraction yield as diffusion rate and solubility of analytes in solvents are greater. Storage of fresh materials at freezing temperature showed an increase of the initial content of anthocyanins. Through the assessment of the influence of solvents and extraction temperatures, our results showed that anthocyanin extraction from *Vaccinium corymbosum* should be conducted under the following conditions: temperature of 50 °C, extraction time 2 hours, with solvent containing 50 % ethanol, and protection against light.

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1. Introduction

Plants have been for long considered an important source of bioactive compounds useful for traditional and/or alternative medicine as they provide health-promoting and disease-preventing benefits. The term "phytochemicals" refers to a large class of compounds with wide structural variability, such as phenolics, carotenoids, alkaloids, vitamins, nitrogen and organosulfur compounds. Studies of phenolics, in particular anthocyanins have made the topic of many scientific researches, mainly for two reasons: (1) beneficial effects on human health based on their antioxidant properties, and (2) applications as natural alternatives for food ingredients [1].

Anthocyanins are water-soluble vacuolar plant pigments responsible for the bright colors red, purple or blue of flowers, skin, seeds, fruits and leaves. Structurally, these molecules are glycosides of salts of phenyl-2-benzopyrilium, composed of the aglycon called anthocyanidine (for structure see Fig. 1) and carbohydrate residues. They are commonly found in fruits and vegetables in different concentration and composition in relation to both genetic and environmental factors.

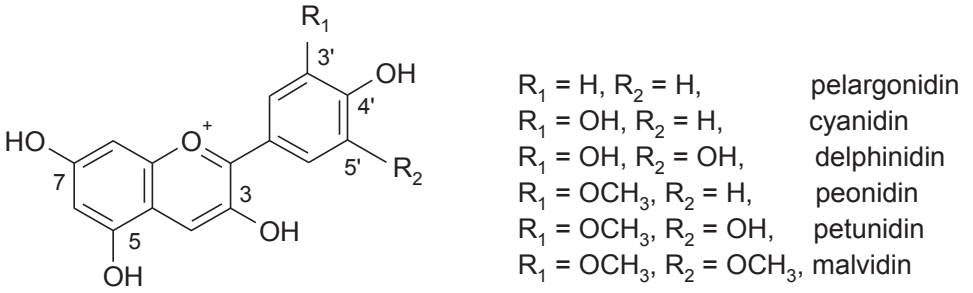

Fig. 1. General chemical structure of common anthocyanidines (flavylium cation).

Isolation of anthocyanins from plant cells becomes an important task closely related to the need of preservation of their bioactivity. Isolation strategy requires several steps: sample size reduction, appropriate extraction, physical-chemical characterization and *in vitro* studies of specific biological activity. Selection of appropriate techniques for each step is essential for the establishment of the structure-activity relationship (SAR) and optimization of composition of mixed extracts to be used in pharmaceutical, food or cosmetic industry.

Research studies have shown that anthocyanins are unstable, being easily oxidized under various conditions, such as pH, temperature, enzymes, UV radiation, sulfur dioxide, ascorbic acid and chelating metal ions, resulting in color change and degradation [2].

Conventional and modern (non-traditional) 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 [3]. Because of the presence of some contaminants in the crude extract, which may affect stability or may interfere with the qualitative analysis of these pigments, solid-phase purification is

recommended [4]. Extraction techniques may be improved by optimization of various parameters to obtain high extraction yields.

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

Modern extraction techniques, such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SPE) were also applied for anthocyanins [7-9], but with modest success, as anthocyanins are heat-sensitive, and SFE techniques are particular suited for non-polar solvents.

Vaccinium berries are well-known as good sources of bioactives of pharmaceutical interest. Blueberries, in particular northern and southern highbush cultivars, have become an important and major global crop. Regarding blueberry production, Romania ranks among the top ten producers in the world, according to 1970-2008 USDA statistical data (http://faostat.fao.org/). In Romania, harvest of wild blueberries has also increased.

The aim of the present paper was to investigate the anthocyanin content from highbush blueberries (*Vaccinium corymbosum* L.) under various extraction conditions, in order to study the influence of different non-toxic solvent systems and temperatures on the efficiency of anthocyanin extraction through conventional procedures.

2. Materials and methods

2.1. Plant samples

Fresh samples of blueberry (*Vaccinium corymbosum* L.) were purchased from the national market. Fresh samples were kept at 4 ^{o}C until analyzed. Reducing sample size of plant material by grounding was performed before extraction.

2.2. Determination of moisture, refractive index and refractometric dry matter

Moisture content of fruit samples was determined at 105 ^{o}C using the A&D ML-50 moisture analyzer. Refractive index and total soluble solids (TSS) of the blueberry juice obtained by manually pressing, was determined by refractometry using an Abbe refractometer (Kruss AR2008) at a standardized temperature (21 ^{o}C). Values are expressed as refractometric TSS (^{o}Brix).

2.3. Anthocyanins extraction

Grounded samples of *Vaccinium corymbosum* L. were homogenized using seven different extraction solvents, at 4 ^{o}C:

1. Water
2. Ethanol/acetic acid/water (50/2/48)
3. Ethanol/0.1 N hydrochloric acid (95/15)
4. 80 % ethanol (v/v)
5. 70 % ethanol (v/v)
6. 60 % ethanol (v/v)
7. 50 % ethanol (v/v)

Extraction was facilitated by occasional shaking for 2 hours. The obtained extracts were filtered and centrifuged at 8000 rpm, at 4 ^{o}C for 10 minutes. The NF800R refrigerated centrifuge (HT) was used.

Three other temperatures were investigated for optimization of the extraction, as follow: 4 ^{o}C, 30 ^{o}C, and 50 ^{o}C, respectively.

2.4. Anthocyanins assay

The content of monomeric anthocyanins in extracts was determined spectrophotometrically by the pH differential method and the content of total anthocyanins by single pH method [3]. Measurements were made in duplicate. The T80 UV-Vis spectrophotometer (PG Instruments Ltd) was used. Content of anthocyanins was expressed as cyanidin-3-O-glucoside (Cyn-3-O-G) according to its molar extinction. The degradation index (DI) was calculated as the ratio between total and monomeric anthocyanins.

3. Results and discussions

It is known that the type of extraction highly influence the quality of a final plant product to be used either as herbal supplement or food ingredient.

In order to determine the solvent influence on anthocyanins extracted from blueberry (*Vaccinium corymbosum* L.) samples, different solvent systems based on water, acidified ethanol and hydroethanolic solution were investigated in the present study.

The determined physical chemical characteristics of the obtained anthocyanin extracts and/or juices are as follow: 85.8 moisture (%), 1.3484 refractive index (n) and 10.3 refractometric TSS (^{o}Brix). TSS value represents a quality control parameter useful in processing fruits and represents a measure of the sugar content (indicator of fruit maturity and ripeness).

As anthocyanin composition of the obtained crude extract may vary according to the type, concentration and elution strength of the solvent, the following seven different solvent systems were used in our investigation: (1) water; (2) ethanol/acetic acid/water (50/2/48); (3) ethanol/0.1 N hydrochloric acid (95/15); (4) 80 % ethanol (v/v); (5) 70 % ethanol (v/v); (6) 60 % ethanol (v/v), and (7) 50 % ethanol (v/v). Extraction was conducted at 4 ^{o}C. Concentration of anthocyanins was performed by the spectrophotometric pH differential method.

As presented in Fig. 2, added hydrochloric acid in ethanol system was more efficient than acetic acid regarding the extraction yield. Extraction with ethanol:water (50:50, v/v) appeared more efficient to the selected samples of *Vaccinium* than those with acidified alcoholic solvent (ethanol:acetic acid:water 25:1:24, and ethanol:0.1 N HCl 85:15). The anthocyanin level in 50% ethanol was found 148.51 mg 100 g^{-1} FW. The lowest recovered anthocyanin content was obtained with water (3.24 mg 100 g^{-1} FW).

Anthocyanins occur naturally as glycosides, so polar solvents are essential for good extraction. Methanol has been the most used reported solvent for anthocyanin extraction. Because of the toxic effects and in view with the final potential uses of the crude extracts, we have substituted methanol with ethanol in all extraction runs. Acids are essential to stabilize anthocyanins in the form of flavylium cation, but excess may lead to partial hydrolysis of glycosidic bond or breaking linkages with metals or co-pigments. The formation of furfural and hydroxymethylfurfural by acid hydrolysis of sugar residues, which is accelerated by heat, has been shown to favor pigment decay. In our investigation, good recovery of anthocyanins from blueberry samples were obtained with 50 % ethanol, a safe solvent system which also minimize the pigment decomposition favoring the extraction of anthocyanins in their native form.

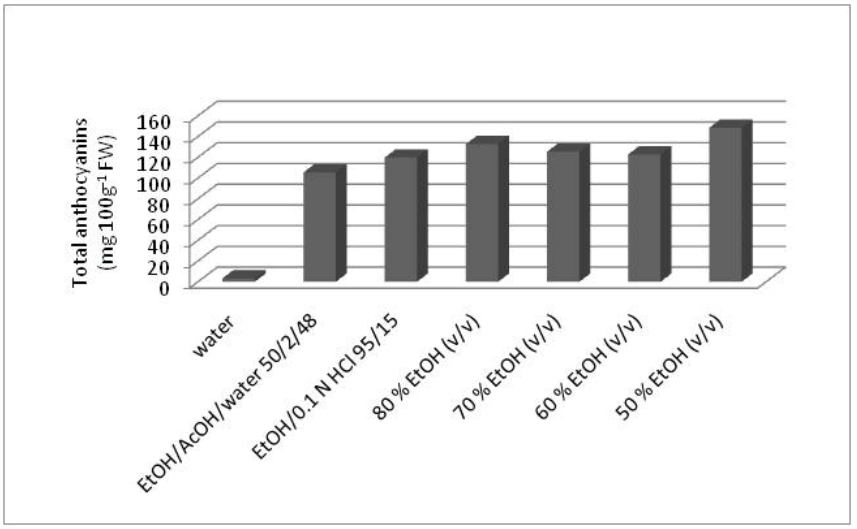

Fig. 2. Anthocyanin content in blueberry (Vaccinium corymbosum L.) samples according to different extraction solvent systems, at 4 °C, 2 hours.

As transfer of quality-relevant constituents from plant material to the crude extract may be improved by increasing temperature, we have investigated the extraction of anthocyanins from samples kept at -18 ^{o}C for three weeks, in 50 % ethanol (v/v) under three different temperatures: 4 ^{o}C, 30 ^{o}C, and 50 ^{o}C, respectively. As expected (see Fig. 3), elevated temperatures (50 ^{o}C) showed better extraction yield, because diffusion rate and solubility of analytes in solvents are greater [7]. At this extraction temperature, anthocyanins with higher degradation index DI (1.16) were obtained compared to low extraction temperatures (1.13). However, temperatures higher than 70 °C have been shown to rapidly decompose anthocyanins [10]. Brown products may be formed, in particular in the presence of oxygen. Mechanism of thermal degradation of monolgycosides at low pH is presented in Fig. 4.

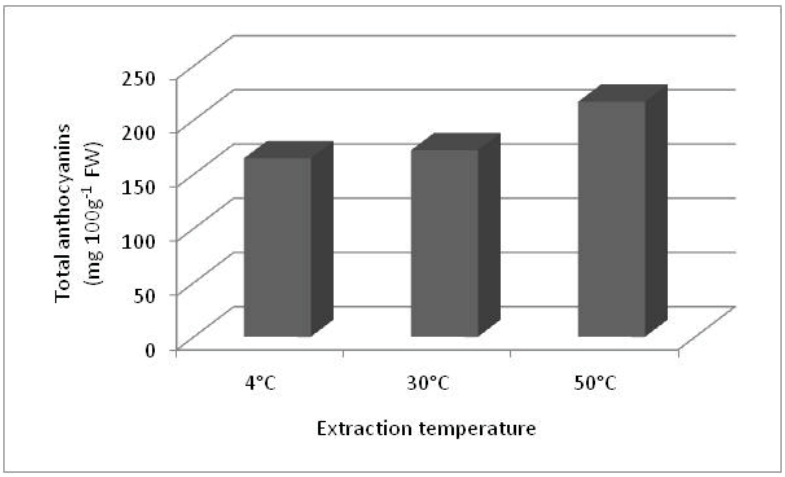

Fig. 3. Anthocyanin content in blueberry (*Vaccinium corymbosum* L.) samples according to different extraction temperatures in 50 % ethanol (v/v), 2 hours.

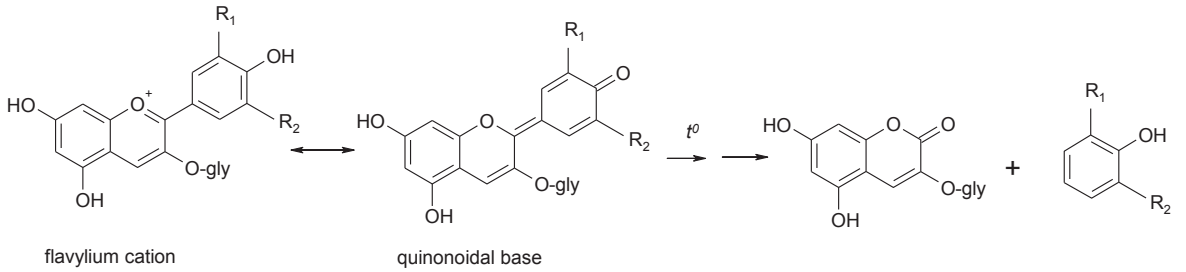

Fig. 4. Thermal degradation of anthocyanin monoglycoside.

Through these experiments we have also investigated the effect of low temperature storage (-18 ^{o}C) on the level of anthocyanins. We have found an increase of 20 % in anthocyanins after 6 months of storage of the original samples at freezing temperatures (see Fig. 5).

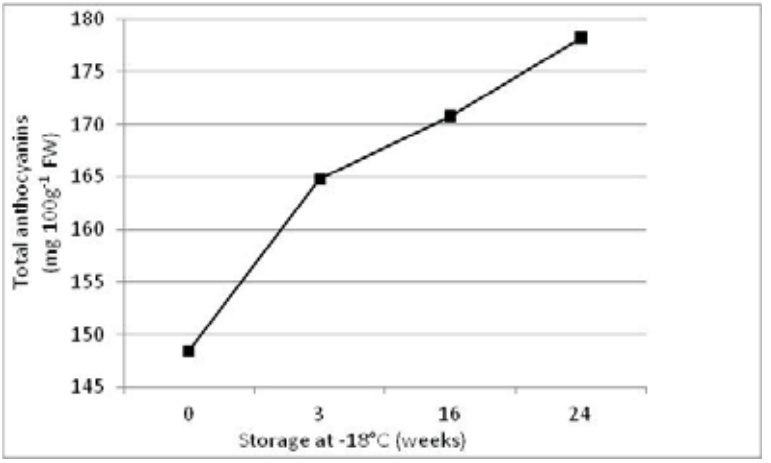

Fig. 5. Anthocyanin content of blueberry (Vaccinium corymbosum L.) samples after freezing storage (1-6 months).

Through the assessment of the influence of solvents and extraction temperatures, our results showed that anthocyanin extraction from *Vaccinium corymbosum* L. should be conducted under the following conditions: temperature of 50 °C at extraction time of 2 hours, using solvent 50 % ethanol/water (v/v), and protection against light. Anthocyanin contents of frozen samples of blueberries were found higher than those of the original fresh samples. Nevertheless, comparison of levels of anthocyanins from different reported data should be conducted with caution as reported values highly depend on the extraction strategies and quantitative methods used. Also, the great variability of anthocyanin content according to genetic and environmental conditions of plants should be considered.

The final hydroalcoholic crude extract (tincture) may find useful application as dietary supplement, or may be further purified for application as food ingredients.

4. Conclusions

In order to obtain high extraction yields of anthocyanins from blueberry, which may influence the quality of the final product, various parameters were optimized (extraction solvent, temperature, time,

storage at freezing temperatures). In our investigation, we substituted the most used potent solvents such as methanol, acetone and diethyl ether, with ethanol, because of their toxicity that may interfere with the final purpose of the obtained crude extract (pharmaceutical and food applications).

The findings of this work showed that increased anthocyanin recovery from highbush blueberries (*Vaccinium corymbosum* L.) may be obtained under the following conditions: discontinuous extraction process at temperature of 50 °C, extraction time 2 hours, with solvent containing 50 % ethanol (v/v), and protection against light. The investigated samples showed an increase of 20 % of the anthocyanin level during long-term storage at -18 °C.

The results may be of interest for food manufacturers, as any processing method may influence the content and composition of these pigments and consequently their health benefits.

Acknowledgements

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References

[1] Delgado-Vargas F, Jiménez AR, Paredes-López O. Natural pigments: carotenoids, anthocyanins, and betalains-characteristics, biosynthesis, processing and stability. *Crit Rev Food Sci Nutr* 2000;**40**:173–289.
[2] Santos-Buelga C, Williamson G. *Methods in polyphenol analysis*. Cambridge: The Royal Society of Chemistry; 2003.
[3] Giusti MM, Wrolstad RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad RE, Schwartz SJ, editors. *Current Protocols in Food Analytical Chemistry*. New York: John Wiley & Sons, p. F1.2.1-F1.2.13.
[4] Jackman RL, Yada RY, Tung MA. A review: Separation and chemical properties of anthocyanins used for their qualitative and quantitative analysis. *J Food Biochem* 1987;**11**:279-308.
[5] Garcia Viguera C, Zafrilla P, Artes F, Romero F, Abellan P, Tomas-Barberan FA. Colour and anthocyanin stability of red raspberry jam. *J Sci Food Agric* 1998;**78**:565-573.
[6] Revilla E, Ryan JM, Martin Ortega G. Comparison of several procedures used for the extraction of anthocyanins from red grapes. *J Agric Food Chem* 1998;**46**:4592-4596.
[7] Ju ZY, Howard LR. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *J Agric Food Chem* 2003;**51**:5207-5213.
[8] Mantell C, Martinez de la Ossa E, Rodriguez M. Supercritical fluid extraction of anthocyanins from grape pomace. In: Cox M, Hidalgo M, Valiente M, editors. *Solvent Extraction for the 21st Century*, Barcelona, Spain, 1999.
[9] Bleve M, Ciurlia L, Rescio L. Supercritical carbon dioxide extracts from red fruits: a “natural” cosmeceutical application. *Nutra Cos* 2005;**4**:23.
[10] Markakis P, Livingston GE, Fellers CR. Quantitative aspects of strawberry-pigment degradation. *Food Research* 1957;**22**: 117-130.