ANNALS OF THE UNIVERSITY OF ORADEA
FASCICLE OF TEXTILES, LEATHERWORK

EXTRACTION OF ANTHOCYANINS FROM ARONIA MELANOCARPA, AS A POTENTIAL NATURAL TEXTILE DYE

PRUNEANU Melinda, BUCIȘCANU Ruxandra, BUCIȘCANU Ingrid

"Gheorghe Asachi" Technical University of Iași, Faculty of Textiles, Leather and Industrial Management, Department of Textile and Leather Chemical Technology, 29 Prof. dr. docent Dimitrie Mangeron Blvd, 700050, Iași, Romania, E-Mail: decanat@tex.tuiasi.ro

Corresponding author: Bucișcanu, Ingrid, E-mail: ingrid-ioana.buciscanu@academic.tuiasi.ro

Abstract: *Gaining sustainability in the textile finishing industry deals among others with the replacement of synthetic dyes with non-toxic and biodegradable natural dyes. Lately, increasing preoccupation has been noticed with the reassessment of the North America and Europe flora, as potential resources for extraction of natural colorants. Within the wide range of natural colorants, anthocyanins - found in fruits and vegetables, dye natural fibers in beautiful pink and purple shades. Chokeberry (*Aronia melanocarpa*) fruit, ordinarily used as a nutraceutical, has one of the highest contents of anthocyanins in the plant kingdom. This paper deals with the extraction of anthocyanins from dried chokeberry fruits and pomace, using a conventional solvent extraction with acidulated acetone-water mixture and a non-conventional aqueous two-phase extraction based on salt-alcohol mixtures. The anthocyanins extraction yield from chokeberry fruits was very low and had comparable values for both extractants, of around 70 mg/100 g dried fruit. Pomace had a much higher anthocyanins content, namely 876.37 mg/100 g dry pomace for the acetone extractant and 1287.2 mg/100 g dry pomace for the two-phase extractant. Anthocyanins' color and stability showed an expected variation when the solution pH shifted from acidic to alkaline. Aronia juice extraction leftovers can be turned from a potential waste into a valuable and easily available source of natural dyes.*

Key words: *vegetable dye, plant pigments, two-phase extraction, solvent extraction, eco-dyeing, chokeberry*

1. INTRODUCTION

Chemical finishing is a critical stage of the textile value chain, in terms of environmental impact derived from water and toxic chemicals, extensively used in the manufacturing processes; synthetic dyes raise the main pollution issues, due to toxicity and low biodegradability. In recent years, the textile industry's duty to comply with sustainability commandments revived the interest in natural dyeing and in finding new renewable resources for the extraction of natural dyes [1, 2].

Natural dyes with feasible applications in textile finishing cover a wide range of chemical structures: anthraquinone, indigoid, carotenoid, flavonoid, dihydropyran, etc. [3]. Anthocyanins (ANs) are considered the main water-soluble plant pigments from the flavonoid/polyphenolic class, which render vivid red, purple, or blue colors to different fruits, seeds, and vegetables [4]. Dyeing of cotton, wool, and silk with ANs extracted from vegetal sources has been reported in literature [5, 6].

Anthocyanins are conveniently extracted with methanol, ethanol, or acetone aqueous solutions [7], preferably slightly acidulated to avoid degradation. Extraction with aqueous two-phase

systems (ATPSs) made up of a lower alcohol and a highly soluble salt has lately attracted attention due to several advantages over organic solvent extraction – no use of toxic solvents, convenient recycling of extraction agents, low sugar content of the anthocyanin-rich phase [8,9].

Aronia is a species of shrub native to Eastern North America, but it was brought to Europe, including Romania, where it is cultivated in several plantations and traded as a nutraceutical, due to its antioxidant properties. Amongst berries in general, chokeberry has the highest total anthocyanin concentration, with a maximum of about 1480 mg/100 g of fresh fruit weight (FW) [10].

In this paper, conventional solvent and ATPS extraction are assessed as possible techniques for the extraction of anthocyanins from chokeberry fruits and pomace, in terms of extraction yield and optimal plant material: solvent ratio. Color change and stability of the aqueous extracts as a function of pH were also evaluated.

2. MATERIALS AND METHODS

The dehydrated whole fruits and powdered pomace of *Aronia melanocarpa* were purchased from SC Aronia Charlottenburg SRL, Romania. Grounded fruits and pomace were sieved at 100 mesh and stored hermetically at room temperature. All reagents: acetone, ethanol (EtOH), citric acid, ammonium sulfate, monosodium phosphate, and chemicals for buffers preparation, were of analytical grade.

The water content of the vegetal materials was determined gravimetrically by air-drying at 105°C till constant weight.

Conventional ANs extraction was performed with acidified acetone solution (acetone:water 70:30 v/v, and 0.2% citric acid w/w), at a solvent:solid ratio of 40:1 v/w. Extraction proceeded in 4 stages 30 min each, at 40°C, under magnetic stirring at 200 rpm. Acetone was removed from the crude extract by vacuum rotary evaporation at 30°C, and fine solid particles were separated by centrifugation at 1600 RCP for 20 min. The supernatant was collected and kept at 4°C till characterization.

Two-phase extraction was performed with two ATPSs, namely $(NH_4)_2SO_4$- EtOH, and NaH_2PO_4- EtOH, with identical salt:alcohol:water ratio, equal to 20:30:50 w/w/w. ATP extraction experiments were performed with batches of 20 mL ATP and plant matter mass of 0,2 to 0.7 g. First, salt was dissolved in water, then the plant solid, fruit or pomace, was dispersed in the salt solution. After ethanol addition, extraction was performed for 15 min at 40°C under magnetic stirring at 600 rpm. The mixture was left still for phase separation at 30°C for 2 h. The upper ethanol-rich phase with higher ANs content and the lower salt-rich phase were separated, and the solid was discarded.

Anthocyanin content in extracts was determined by the pH differential method, as described in [11]. Briefly, two standard buffer solutions were prepared: a KCl/HCl buffer solution of pH 1.0 and a CH_3COONa/HCl buffer solution of pH 4.5 Equal volumes of Aronia extract were introduced in two test tubes and completed to 10 mL with solutions of each buffer. The solutions were left still at dark for 6 h. The absorbance of each solution was measured at 500 nm and 700 nm against distilled water, on a HACH DR/2010 spectrophotometer. The estimate of the anthocyanin content was expressed as cyanidin-3-O-glucoside (Cy-3-glc) equivalents and calculated by eq (1):

$$ANC\,(mg/L) = \frac{A \times MW \times DF}{\varepsilon \times L} \times 1000 \quad \textbf{(1)}$$

where: $A = (A_{500} - A_{700})_{pH1.0} - (A_{500} - A_{700})_{pH4.5}$ is the turbidity corrected absorption; MW = 449,2 g/mol is the Cy-3-glc molar weight, DF – the dilution factor; ε – extinction coefficient (ε = 26,900 L/cm×mol), and L – optical path (L = 1.36 cm).

Color change and stability of pomace extracts were assessed at pH values of 2, 4, 6, 8, and 10, in appropriate buffer solutions.

3. RESULTS AND DISCUSSION

3.1. Influence of ATPS content and plant material dosage on the extraction efficiency

Different ATPSs are used for anthocyanin extraction, of which the $(NH_4)_2SO_4$- EtOH is the most popular; optimal ranges of salt and alcohol concentrations have been established for several plant sources [10]. The NaH_2PO_4- EtOH ATP also works well in anthocyanin extraction, even with a superior yield to that of the $(NH_4)_2SO_4$- EtOH ATP for certain plant sources [9].

Anthocyanin concentration in the upper phase resulted from $(NH_4)_2SO_4$-EtOH and NaH_2PO_4- EtOH extraction from Aronia fruits and pomace at different plant solid dosages are given in **Fig. 1** and **Fig. 2**.

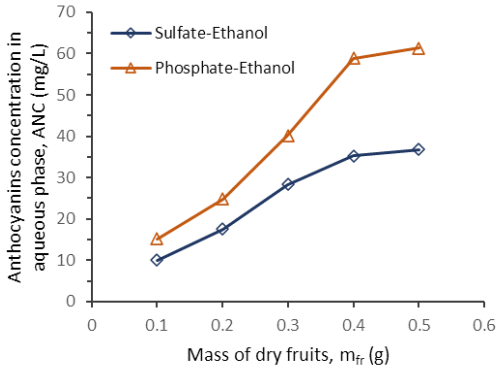


Fig. 1: Anthocyanin concentration in the ethanol-rich phase from ATP extraction from chokeberry fruit, as a function of vegetal material dosage per 20 mL ATPS.

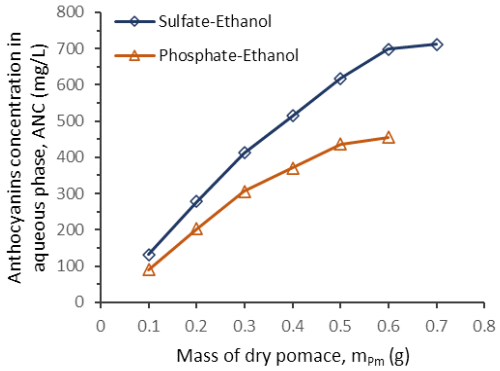


Fig. 2: Anthocyanin concentration in the ethanol-rich phase from ATP extraction from chokeberry pomace, as a function of vegetal material dosage per 20 mL ATPS.

No matter the ATPS composition, the extraction yield of ANs from dried fruits, given as anthocyanin concentration in liquid extracts (ANC), is significantly lower than that corresponding to pomace powder: for the sulfate-EtOH ATPS, the pomace yield: fruit yield ratio is about 17, while for the phosphate-EtOH system, the pomace yield: fruit yield ratio is about 7. This behavior can be

related to the ANs sensitivity to water and processing temperature. The highest anthocyanin content is found in fresh fruits and is lowered by temperature and humidity [12]. Fresh chokeberry fruits have a high water content, of about 75 – 83% [13] while the pomace resulting from juice extraction has a much lower humidity content. In the fabrication process, both fruits and pomace are air-dried in mild conditions at 40°C, but the drying time for pomace is lower, due to the lower water content and comminuted physical state. For whole fruits, dehydration time is longer, enough to produce severe anthocyanin degradation. Moreover, it is stated that ANs are found mostly in the pulp and peels remained in pomace after juice extraction [9], which can explain the experimental findings. When fruits were used as extraction materials, the phosphate-EtOH provided a higher extraction yield than the sulphate-EtOH system.

For fruits, the extraction yield increased linearly with dosage increase, up to a maximum of 0.4 g, and then recorded a negligible raise. Identical evolution was recorded for pomace in sulfate-EtOH up to 0.6 g, but, at this dosage, the phase separation was hindered by the high volume of the solid phase. In the phosphate-EtOH, a maximum dosage of 0.5 g was recorded. Anyway, the amount of ANs dissolved in the upper ethanol-rich phase (ATP technique) gets to a saturation point, beyond which the increase of the solid mass dosage becomes non-feasible. Further extraction experiments were performed on samples with a dosage of 0.5 g plant material for 20 mL ATPS. which are very convenient to manipulate in the extraction experiments and correspond to a solvent:solid ratio of 40:1 v/w, identical to that used in acetone extraction.

3.2. Influence of the extraction agent on the extraction yield

Comparative studies of ANs extraction using acetone, methanol, and water, showed that acetone renders efficient and more reproducible extraction, avoids problems with pectins, and requires a much lower temperature for concentration [14].

The values of ANs extraction yield from the acetone: water and the sulfate-EtOH extractants, given in mg/L extract and in mg/100 g dry vegetal matter, are given in **Fig. 3**. As with the $(NH_4)_2SO_4$-EtOH ATP, extraction yield in acetone was very low for fruits and significantly higher for pomace. The AN extracted from pomace, reported to the dry matter, was about 13 times higher than that extracted from fruits:876.37 mg/g DW for pomace vs 66.32 mg/g DW for fruit.

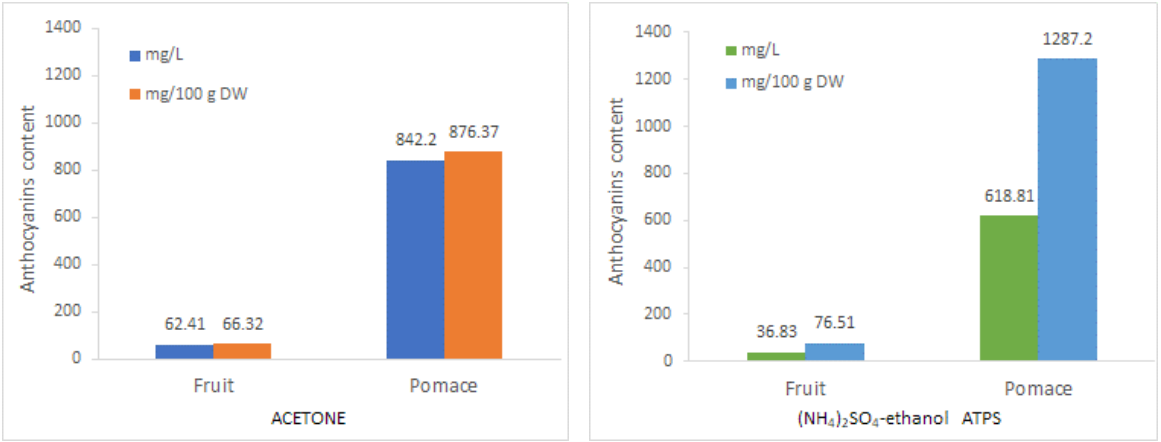


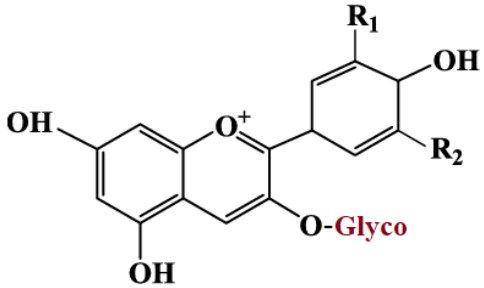
Fig. 3: Dependence of anthocyanins extraction yield on the extraction agent.

The concentration of ANs extracted in the ethanol-rich phase of the $(NH_4)_2SO_4$-EtOH ATP was lower than that obtained with acetone, both for fruit and pomace, because the volume of the upper phase was higher than the extract resulted from acetone extraction, but the AN yield reported to the dry plant was superior. For fruit, yield values were quite comparable: (66.32 mg/100 g fruit and 76.51 mg/100 g fruit) due to the low AN content in the chokeberry fruits, and this may be the maximum attainable concentration. The AN extracted from fruits are close to the actual AN content in the dry chokeberry fruits. As regards the pomace, the yield of the two-phase extraction is about 1.5 higher than that of acetone extraction. Taking into account that the air-dried pomace suffered inevitable ANs degradation, an extraction yield of 1287.2 g AN/100 g dry pomace can be considered satisfactory.

3.3. Dependence of anthocyanins color and stability on solution pH

When pH varies from acidic to alkaline, structural transformations of AN pigments occur, which result in obvious color changes. It is generally admitted that in acidic medium, anthocyanins appear red, have a purple hue in neutral pH, and the color changes to blue in alkaline conditions.

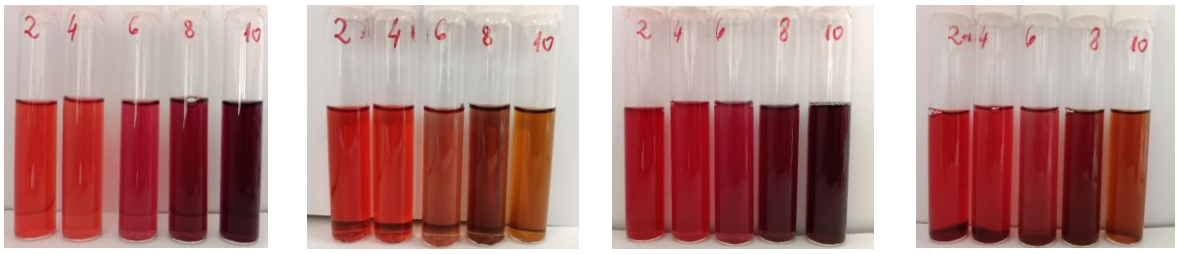
There are six main types of anthocyanins that commonly occur in nature, namely Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, and Petunidin. They derive from a common polyphenol skeleton and differentiate themselves by the substituent groups (see **Fig. 4**) In nature, they derive from anthocyanidins - the sugar-free counterparts of anthocyanins, after their combination with different glycosides, such as glucoside, arabinoside, rutinoside, etc. Anthocyanins from Chokeberries mainly consist of cyanidin (up to 98%), followed by delphinidin, and the other types in very small amounts [15].



Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Peonidin	OCH_3	H
Pelargonidin	H	H
Petunidin	OCH_3	OH

Fig. 4: Chemical structures of main naturally occurring anthocyanins, named after their sugar-free anthocyanidin counterparts

Colors of extracted ANs at different pH values and at identical concentration, of 56 mg/L in the buffer solutions, are given in **Fig. 5.** Antocyanins color ranges from red at acidic pH, light magenta at pH 6 and reddish purple at alkaline pH, with higher intensity at pH 10 than at pH 8. Acetone extracts are transparent, while the ATPS extracts are slightly turbid at acidic pH and show darker shades.



(a) (b) (c) (d)

Fig. 5: Anthocyanins color at different values of pH, from acidic to alkaline: a) acetone; b) acetone after 24 h; c) $(NH_4)_2SO_4$-ethanol; d) $(NH_4)_2SO_4$-ethanol after 24 h

It is accepted that in plants, cyanidin pigment has a reddish-purple (magenta) hue, while delphinidin appears as a blue-reddish pigment. In acidic aqueous media pH (pH <4) cyanidin has a red color, which shifts to purple at pH 6-7 and turns to blue at higher pH values (pH =8-9). The blue color can persist even at higher pH values. Changes in the chemical structure of cyanidin along with pH, which accounts for its color variation, are depicted in **Fig. 6**. In strongly acidic solutions (pH < 2), the flavylium cation predominates in the structures of anthocyanin, conferring them bright, stable red hues and high solubility in water [16]. With pH increase over 4, the quinoidal species start to emerge and determine an apparent color change starting with pH 6.

Delphinidin turns from purple to blue only in higher pH conditions. [16]. Due to its low concentration in Aronia ANs, delphinidin's influence on color change is very weak. The experimental findings on color change of Aronia ANs as a function of pH are consistent with previous literature data [6].

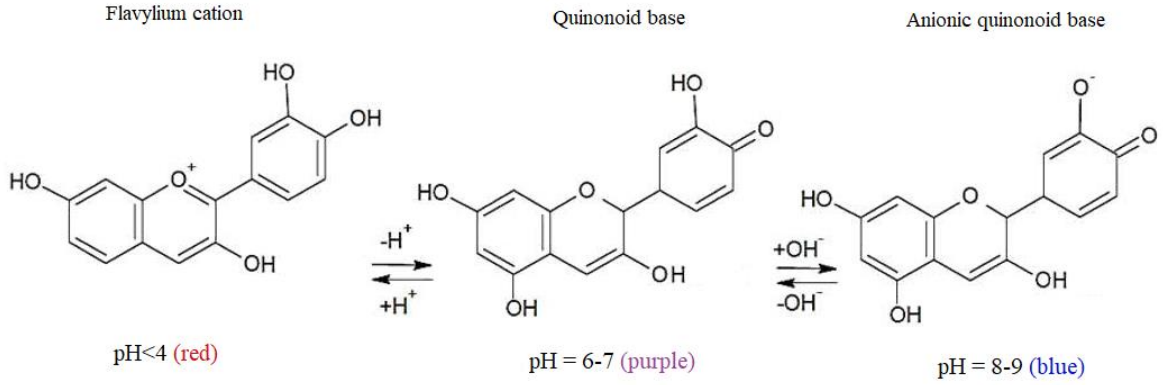


Fig. 6: Structural and color changes of cyanidin with pH

Color stability in time at acidic pH values of 2 and 4 is easily noticeable. At pH 6, ANs extracted with acetone showed a color shift to reddish-brown, while ANs extracted in ATPS were still red-pink. In alkaline pH, both AN extracts underwent an obvious color fading and shifting to brown shades after 24 h, due to the instability of quinoidal molecular structures of cyanidin. Exposure of alkaline samples to daylight may be also responsible for color alteration [17].

5. CONCLUSION

Acetone extraction of anthocyanins from Aronia provided a good extraction yield and a straightforward extract concentration process.

The aqueous two-phase extraction with $(NH_4)_2SO_4$-EtOH rendered a superior extraction yield than the acetone extraction and worked well with the plant material in solid form.

Anthocyanins concentration in dried fruits was considerably lower than that in pomace, which proves that the water content and the physical state of the vegetal material, together with the parameters of the drying process have a major influence on anthocyanins stability.

Anthocyanins extracted from Aronia exhibited high stability at acidic pH and rapid degradation at alkaline pH, which is common for this flavonoid class.

Aronia pomace, a potential waste coming from an indigenous plantation, can be turned into a valuable source for anthocyanin colorant extraction.

Further studies on anthocyanins extracted from chokeberry are needed, to assess their tinctorial properties and use for textile dyeing.

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