Pigment from *Aronia melanocarpa* var. Nero and optimum extraction conditions

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SUMMARY. *Aronia melanocarpa* var. Nero (black chokeberry) is shown to be a good source of an anthocyanin colorant. HPLC analysis of anthocyanins reveals the ratio between the four components (cyanidin-3-0-galactoside, cyanidin-3-0-arabinoside, cyanidin-3-0-glucoside and cyanidin-3-0-xyloside) to be almost constant during processing. Investigations about changes in the anthocyanins, polyphenols, acids and sugars content of chokeberry during the ripening process allow to find out the optimal status of maturity for anthocyanin extraction. In order to optimise the extraction process, variables such as ethanol concentration in extraction solvent, extraction temperature, size of extracted particles, kind of acid used, pH-value and SO_2-concentration were investigated. Extraction kinetics were recorded and optimal extraction conditions for practical purposes defined.

Black chokeberry *Aronia melanocarpa* (Michx.) Elliot is an ideal source for a natural red colorant production. Originated in North America, it came to Slovakia from the former USSR about 70 years ago [1,2]. Today, the clone Nero is cultivated in Slovakia in plantations of altogether about 90 ha, mainly in North and East of the country. As an unpretentious species, it may be grown also in higher sites under fairly rough climates. The data about composition of *Aronia* were already published [3,4].

Pigments in *Aronia melanocarpa* belong to carotenoids and anthocyanins. Whereas the carotenoid content (mainly ß-carotene and ß-cryptoxantene) is not greater than 50 mg per kg of berries [5], the anthocyanins make up

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between 4 and 10 $g.kg^{-1}$, depending on climate conditions and the status of maturity at the harvest time [6,7]. As about three quarters of the anthocyanins in *Aronia* are retained in fruit skins [6], their winning process comprises first separation of juice, then extraction of pomace with acidified water, methanol or ethanol in two or three steps and following concentration by evaporation of the solvent. In food technology ethanol is preferred due to tocixity of methanol [8,9,10]. For economical reasons, the anthocyanin extraction should be possibly quick, with the minimum amount of solvent necessary, quantitative, and, if possible, selective for anthocyanins, leading to products with a high colour strength.

Materials and Methods

1. Raw Material for Ripening Experiments

Different plantations with varying climatic conditions at different harvest times were chosen for this study. Berries were gained from plantations in Sabinov (East Slovakia, 430 m above sea level), Blhovce (Southern Slovakia, 235 m), and Vyšný Kubín (Orava, 550 m) at three different dates between August and October. Samples of about 1 kg were taken statistically and homogenised with a blender. Anthocyanins, acids, reducing sugars and the sum of polyphenols were determined as described [3,4].

2. HPLC of Anthocyanins

Anthocyanins were measured after a modified method of Koswig [11]. A Waters Miilipore Chromatography LC-Modul I with Millenium 2010 software was used. Detection was performed at 518 nm. Fractionation was carried out using an analytical RP-18 (5 μm particles) 250*4 mm I.D. column from Merck, protected with a guard cartridge of the same package. Elution was carried out at room temperature using a mixture of two eluents, eluent A containing water/formic acid/acetonitrile 87:10:30 and eluent B water/formic acid/acetonitrile 40:10:50 (v:v:v), with a pH value of 1.9. The increasing gradient was run as follows: 0-1 min 88 % A and 12 % B; 1-25 min 100 % B, 25-30 min 100 % B and 30-38 min 88 % A and 12 % B, equilibrium time 5 min. The flow rate was 1 $ml.min^{-1}$, the injection volume 10 μl. *Aronia* juice was diluted 1:6, *Aronia* concentrate was diluted to the same absorption as the juice. After adding the internal standard (cyanidinchloride, Rothe 4545.1 for HPLC) the samples were filtrated with a millipore filter (0.22 μm) and injected directly without any purification step.

3. Extraction experiments

Experiments were carried out in both, a laboratory and in a pilot scale. To optimise extraction conditions, central parameters of the process varied in the following range:

ethanol: 40-80 %
temperature: 20; 30; 40 °C
particle size
kind of acidifier: acetic, citric, formic acid
pH: 3.0; 3.5; 4.0; 4.5
concentration of SO_2 in the solvent: 0; 150; 300; 500; 1000, 2000 ppm.

Single extraction experiments were arranged in a Greco Latin Square [12] experimental design.

Laboratory Scale

Frozen berries were defrosted over night in a fridge. In each case, 150 g were mixed in a blender (Krups ProMix 170 metal) and centrifuged (Hermle Lab-centrifuge, at 40 000 rpm for 30 min) to separate pomace and juice. With each sample, three extractions were carried out by varying the parameters of temperature, SO_2-concentration ($Na_2S_2O_5$), kind of acid, pH, and particle size. The latter was varied by different times of mixing with the blender and determined using standardised sieves (type 3D Retsch, Germany) and filters. In the extracts, the anthocyanin content as well as sugars and sorbitol were determined, the latter by HPLC [13]. Anthocyanins were measured in a buffer solution of pH 1 according to the single pH method according to Wrolstadt [8]. Kinetics was recorded by taking samples at defined intervals.

Pilot Scale

Fresh berries - about 500 kg of each - were mashed in a grape mill, pressed to separate the juice, and extracted in a discontinual extractor while the pomace:ethanol ratio was about 1:1. Parameters under investigation were the ethanol concentration, the extraction temperature, and the particle size. Finer particles were obtained by cutting the skins in a cutter. Analyses were carried out according to [3]; Anthocyanins were determined according to Fuleki [14].

Results and Discussion

1. Ripening experiments

The influence of *Aronia* maturity on the anthocyanin concentration is shown for the year 1995. Climate based variation of raw material composition is unavoidable. The influence of the ripening time is significant.

As shown in Table 1., the anthocyanin content is still rising during advanced maturity, although the berries appeared deeply black already at the earlier harvest dates. The sugar content in juice is increasing as well, while the acids are decreasing (Tab. 2.).

TABLE 1. Development of anthocyanin content during ripening.
TABUĽKA 1. Zmena obsahu antokyánov počas zrenia plodov.

Plantation [1]	Date of harvest [2]	Anthocyanins [3] [g.kg^{-1} berries] [4]	Dry material [5] [g.100 g^{-1}]
Sabinov	14.08.1995	2.7	27.4
	25.08.1995	3.2	26.8
	20.09.1995	8.6	25.5
Blhovce	25.08.1995	3.4	27.8
	13.09.1995	7.04	28.0
	24.10.1995	17.9	30.0
Vyšný Kubín	14.08.1995	1.9	21.2
	25.08.1995	2.8	20.6
	05.09.1995	4.7	18.2

1 - plantáž, 2 - dátum zberu, 3 - antokyány, 4 - g.kg^{-1} plodov, 5 - sušina.

Similar results were found by Kaack [7]. The content of total polyphenols showed no clear tendency in the investigated samples. Regarding the ratio of accompanying compounds (sugars, sorbitol, acids and polyphenols altogether) to anthocyanins as a parameter of raw material quality, it can clearly be seen that this ratio gets also more favourable at later dates of harvest. Consequently, the berries should be harvested at the latest time possible. Losses by birds should, however, be kept in mind.

TABLE 2. Content of anthycyanins, sugars and acids in aronia juice.
TABUĽKA 2. Obsah antokyánov, cukrov a kyselín v arónievej šťave.

Plantation [1]	Date of harvest [2]	Anthocyanin [3] [g.l^{-1}]	Sugars [4] [g.l^{-1}]	Acids [5] [g.l^{-1}]
Sabinov	14.08.1995	1.25	83.6	17.7
	25.08.1995	1.53	91.0	15.2
	20.09.1995	1.88	92.6	13.3
Blhovce	25.08.1995	1.24	85.4	13.2
	13.09.1995	1.90	92.9	11.9
	24.10.1995	2.50	90.8	9.0
Vyšný Kubín	14.08.1995	0.39	62.8	15.5
	25.08.1995	1.05	75.2	15.1
	05.09.1995	1.25	74.4	12.4

1 - plantáž, 2 - dátum zberu, 3 - antokyán, 4 - cukry, 5 - kyseliny.

2. HPLC of Anthocyanins

Using this method a suitable separation of the four anthocyanins found in *Aronia melanocarpa* can be made. The ratio of the peak area of cyanidin-3-0-galactoside, cyanidin-3-0-glucoside, cyanidin-3-0-arabinoside, and cyanidin-3-0-xyloside is almost constant with 64.5 : 2.6 : 29.2 : 3.5. No significant differences at fresh juice, processing or ageing of the concentrates, regarding to the peak areas, can be seen. The amount of cyanidin-3-0-galactoside and cyanidin-3-0-arabinoside is always more than 93 % [15,16]. Broad peaks at the end of the chromatogram may be caused by polymerised anthocyanins.

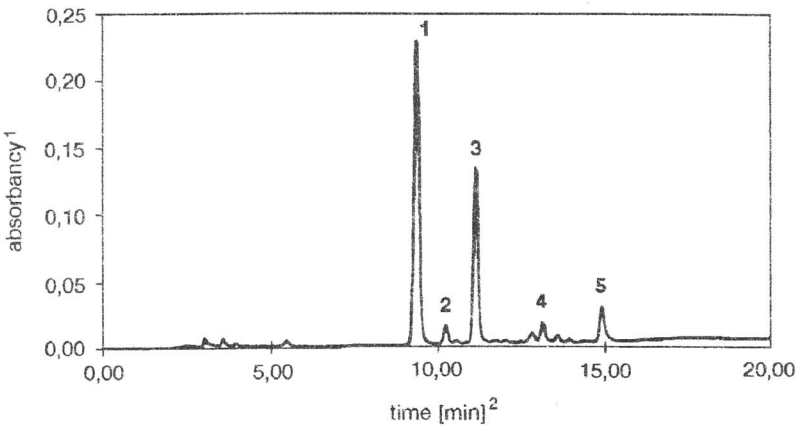

FIG. 1. HPLC chromatogram of *Aronia* anthocyanins (1) cyanidin-3-0-galactoside, (2) cyanidin-3-0-glucoside, (3) cyanidin-3-0-arabinoside, (4) cyanidin-3-0-xyloside, (5) cyanidinchloride.
OBR. 1. HPLC chromatogram antokyánov arónie (1) kyanidín-3-0-galaktozid, (2) kyanidín-3-0-glukozid, (3) kyanidín-3-0-arabinozid, (4) kyanidín-3-0-xylozid, (5) kyanidínchlorid.

1 - absorbancia, 2 - čas (min).

3. Extraction experiments

About 80 % of total anthocyanins is being extracted during the first extraction step.

With torn pomace, the absorption maximum (which is directly correlated to the anthocyanin content) was already reached after 40 to 60 minutes, the 2nd and 3rd extraction being even more quickly finished in lab scale. In pilot scale, however, the extraction took 4 to 5 hours.

The influence of the alcohol concentration in the extraction solvent [6], was tested with 500 kg of pomace that was only mashed, but not cut or torn apart. As shown in Fig. 3., there is a clear improvement in extraction kinetics as well as in the final colour yield when rising the ethanol concentration from 40 to 80 %. There is still an increase in extraction kinetics between 70 and 80 %

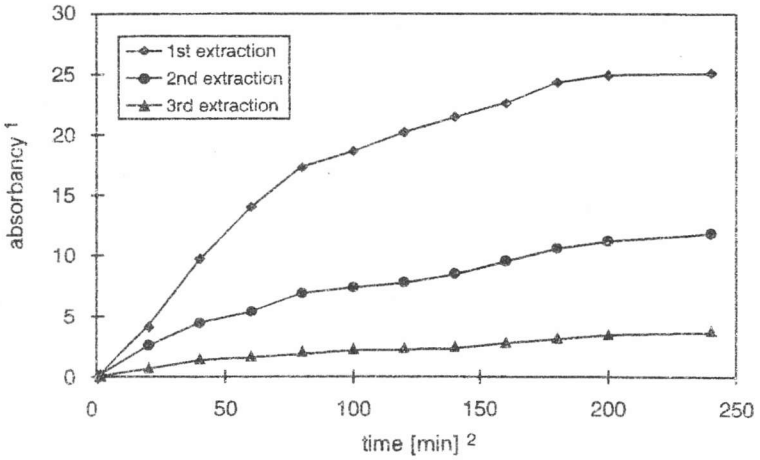

FIG. 2. Kinetics of the 1st, 2nd and 3rd extraction of *Aronia* pomace in 70 % ethanol, laboratory scale.
OBR. 2. Kinetika prvej, druhej a tretej extrakcie arónových výliskov 70 % etanolom, laboratórny pokus.
1 - absorbancia, 2 - čas (min).

ethanol at 20 °C (Fig. 3.). At 40 °C the kinetics at 70 and 80 % ethanol is almost the same (Fig. 4.).

Apparently, the anthocyanins are dissolved more quickly and more quantitatively in ethanol of higher concentrations. As a consequence, the amount of by-products, especially sugars, found in the extracts in relation to the antho-

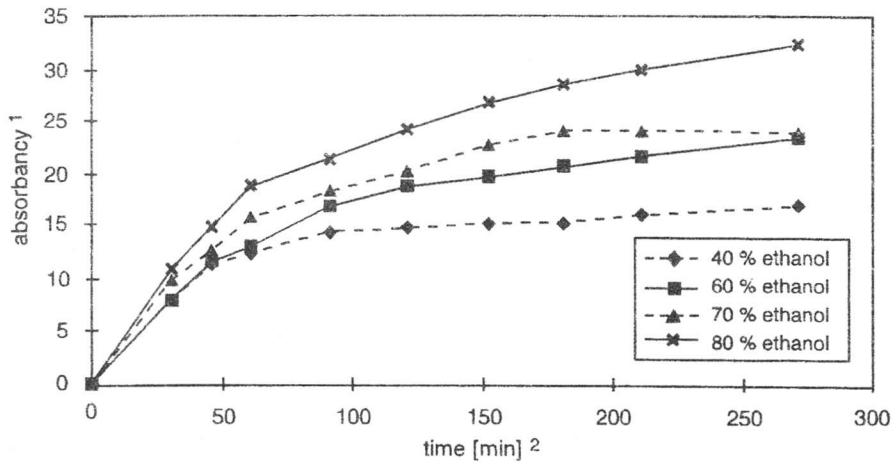

FIG. 3. Influence of alcohol concentration on extraction kinetics at 20 °C, the 1st extraction, pilot scale.
OBR. 3. Vplyv koncentrácie etanolu na kinetiku extrakcie pri 20 °C, prvá extrakcia, poloprevádzkový pokus.
1 - absorbancia, 2 - čas (min).

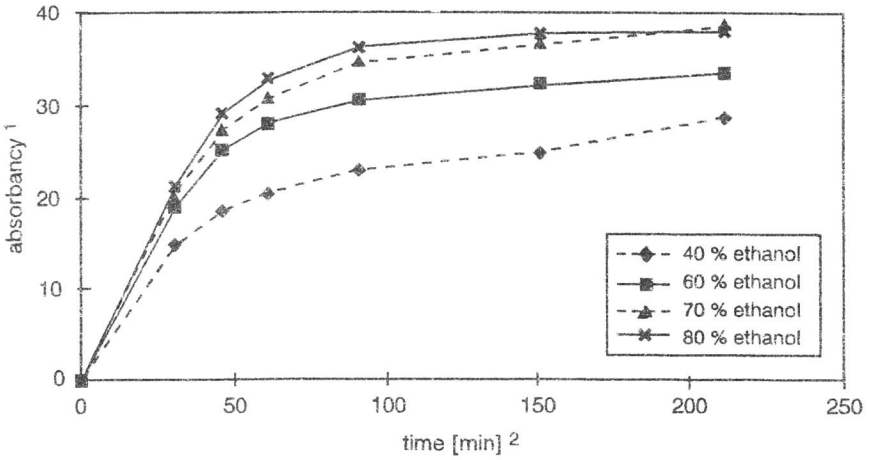

FIG. 4. Influence of alcohol concentration on extraction kinetics at 40 °C, the 1st extraction, pilot scale.
OBR. 4. Vplyv koncentrácie etanolu na kinetiku extrakcie pri 40 °C, prvá extrakcia, poloprevádzkový pokus.
1 - absorbancia, 2 - čas (min).

cyanins gets lower with higher concentrations of ethanol. We found that in a three hours' extraction at 20 °C, there were 13 g reducing sugars per 1 g of anthocyanin in the extract when using 40 % ethanol as extraction solvent, while only 7 g sugars per 1 g anthocyanin were found in extracts with 70 % ethanol. From the technological view point, higher concentrations of ethanol are favourable as they can be removed by evaporation more easily, with a smaller input of energy and at lower temperatures. The temperatures above 40 °C should be avoided because of anthocyanin instability. Consequently, we decided to choose 70 % of ethanol as a standard concentration for further extraction experiments. The influence of temperature in the range of 20 to 40 °C was examined in lab as well as at pilot scale. In the bigger scale with uncut pomace, a temperature rise caused improvement of extraction kinetics, while in lab scale with finely grinded particles of pomace, no difference could be observed. Even after 24 hours at 40 °C the colour losses remained negligible. In laboratory as well as in pilot scale experiments, particle size turned out to be an important parameter of kinetics, also determining the final colour yield. The smaller the particle size and the bigger the surface area accessible to the solvent, the faster and more quantitatively the anthocyanins may be extracted. Probably, increased disintegration of cells of grinded fruit skins makes possible the release of anthocyanins, which can not be otherwise extracted with the solvent. For practical purpose too tiny particles are unfavourable because of their slow separation from the liquid and corresponding difficulties at filtration (Fig. 5.).

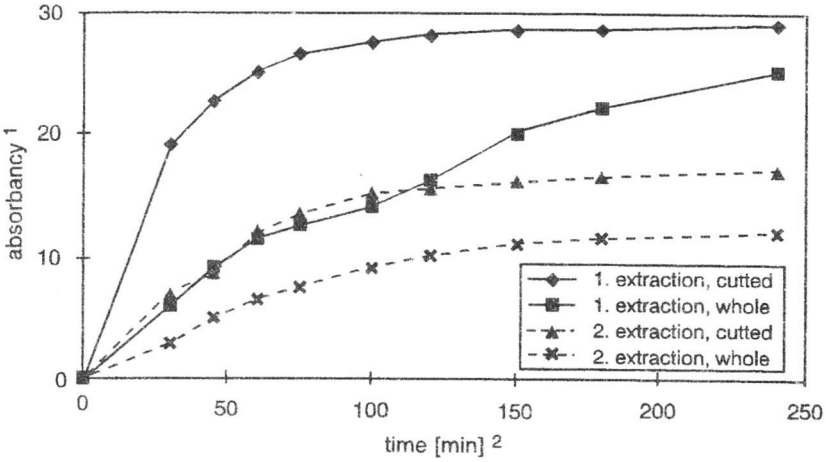

FIG. 5. Influence of particle size on kinetics of the 1st and 2nd extraction, pilot scale, whole and cut pomace.
OBR. 5. Vplyv veľkosti častíc na kinetiku prvej a druhej extrakcie, poloprevádzkový pokus, celé a narezané výlisky.
1 - absorbancia, 2 - čas (min), 3 - 1. extrakcia, narezaná, 4 - 1. extrakcia, celá, 5 - 2. extrakcia, narezaná, 6 - 2. extrakcia, celá.

The other parameters under investigation, the pH-value, the kind of applied acid and the SO_2-concentration, showed no influence on extraction kinetics. The acid used should be harmless or easy removable and it should not interfere with the following processing steps. It is also possible, that the kind of acidifier as well as the pH have distinct influence on anthocyanin stability and on the shelf life of the final products. HCl, for instance, has corrosive properties; additionally, it may catalyse the acidic hydrolysis of sugar moieties from the anthocyanin to release the aglycone which is much less stable. Citric acid, in contrast, may act as a protective agent as it chelates metallic ions. SO_2 is expected to have a protective effect. It acts as an antioxidant inhibiting enzymes such as polyphenoloxidases, but also diminishes microbial growth [17]. As a consequence of our investigations, extractions are carried out at outside temperatures at harvest time (20-30 °C), with 70 % of ethanol, acidified with citric acid to a pH value under 4, adding about 200 ppm SO_2, with intermediate particle sizes of the pomace.

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Farbivo z arónie čiernoplodej (*Aronia melanocarpa* var. Nero) a optimálne podmienky jeho extrakcie

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SÚHRN. *Aronia melanocarpa* var. Nero (arónia čiernoplodá) je dobrý zdroj antokyánových farbív. HPLC analýzou sa zistila prítomnosť štyroch antokyánov (kyanidín-3-0-galaktozid,

kyanidín-3-O-arabinozid, kyanidín-3-O-glukozid, kyanidín-3-O-xylozid). Sledovanie zmien antokyánov, polyfenolov, kyselín a cukrov v arónii počas zrenia umožňuje určiť stav optimálnej zrelosti pre extrakciu farbív. Optimalizoval sa postup extrakcie farbív - sledoval sa vplyv rôznej koncentrácie etanolu, extrakčnej teploty, veľkosti častíc, druhu použitej kyseliny, hodnoty pH a koncentrácie SO_2. Definovali sa optimálne extrakčné podmienky, ktoré boli overené v poloprevádzkových podmienkach.