Concentration of chokeberry (*Aronia melanocarpa*) juice by nanofiltration

KRISTINA POPOVIĆ – ANDRIJA POZDEROVIĆ – LIDIJA JAKOBEK – JOSIPA RUKAVINA – ANITA PICHLER

Summary
Chokeberries are among the healthiest berries due to their high nutritional value. They are rich in antioxidants, dietary fibre, phytonutrients, folate and other interesting compounds. Because of astringent taste, they are usually processed to various food products like juices and jams. In the present study, chokeberry juice was concentrated by nanofiltration membrane process at three different pressures (4.5 MPa, 5.0 MPa and 5.5 MPa) and two different temperature regimes (with cooling and without cooling of the retentate). The maximum achievable total soluble solids content was 26.9%, which corresponded to the volume reduction factor of 2.39. The aromatic components in initial juice and concentrates were identified by gas chromatography-mass spectrometry and individual phenolics by high performance liquid chromatography. The permeate flux highly depended upon processing parameters. The highest recorded (19.43–9.61 $l \cdot m^{-2} h^{-1}$) was in the process at 5.5 MPa without cooling. The best retention of total phenolics was also observed in that process, while aromatic components were mostly retained at 5.5 MPa with cooling. The collected permeates had 1.0% of total soluble solids content and contained small amounts of phenolics and aromatic components that passed through the membrane.

Keywords
nanofiltration; chokeberry; permeate flux; antioxidant activity; phenolic compounds; aroma compounds

Chokeberry (*Aronia melanocarpa*) fruits have been increasing in popularity among health-conscious consumers, who are looking to add more "superfoods" to their diets. These fruits have one of the highest contents of phenolic compounds among small berry fruits like blackberries, raspberries or blueberries [1]. Anthocyanins, proanthocyanins, phenolic acids and flavonols are the most abundant [2, 3]. Numerous health-promoting effects of chokeberry phenolics were reported. Anti-inflammatory [4], antiproliferative [5], gastroprotective [6], and chemoprotective activity against colon cancer [7] are some of them. Due to their astringent, bitter-almond taste, chokeberries are mostly used in the production of juices, nectars, jams, wines, cordials and as a natural colourant for food products [8–10].

For more than three decades, membrane processes have been studied and applied to replace, or partially replace, evaporation in concentration processes [11]. Fruit juices are usually concentrated by multi-stage evaporation. However, that process results in a loss of fresh juice flavours, colour degradation, and a "cooked" taste, recognized as off-flavours, due to thermal effects. Numerous studies were conducted with reverse osmosis and nanofiltration (NF) membranes for fruit juice concentration [12, 13]. The advantages of reverse osmosis and NF over conventional concentration techniques reside in the low thermal damage of the product, reduction of energy consumption, and lower capital investments, as the process is carried out at low temperatures and does not involve phase change for water re-

Kristina Popović, **Andrija Pozderović**, **Anita Pichler**, Department of Food Technologies, Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 20, 31 000 Osijek, Croatia.
Lidija Jakobek, Department of Applied Chemistry and Ecology, Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 20, 31 000 Osijek, Croatia.
Josipa Rukavina, graduate student, Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 20, 31 000 Osijek, Croatia.

Correspondence author:
Kristina Popović, e-mail: kristina.popovic@ptfos.hr

moval [14]. NF is a recently developed membrane separation technology. It can be operated at a lower pressure than reverse osmosis and can be considered an intermediate technique between ultrafiltration and reverse osmosis. The NF membranes have a looser structure and partially allow ions and small molecules (up to 500 Da) to pass.

The advantages of NF over reverse osmosis are lower operating pressure, higher flux, high retention of multivalent ions and organic molecules, low maintenance and investment cost, and energy consumption lower by about 21% [13, 15]. Also, replacing reverse osmosis by NF can improve efficiency of the process, because the high pressure required in reverse osmosis can damage the sensitive but very valuable components in juice, like anthocyanins [13]. Although membrane separation is a very good alternative in food technology, limiting flux phenomena (fouling and concentration polarization) may confine its applications. MANTTARI et al. [16] investigated the problem of limiting flux phenomena in NF process. They concluded that the factors such as hydrophobicity, charge and roughness of the membrane have a decisive influence on fouling. Osmotic pressure and viscosity of the juice increase rapidly with the increase in sugar concentration, so the final concentration cannot be higher than 28–35 °Brix [17]. For these limitations, reverse osmosis and NF can be considered as advantageous techniques for preconcentration of juices but, to achieve concentrations higher than 60–70 °Brix, they need to be integrated with other processes like evaporation or osmotic distillation [18]. According to MERRY [19], the usage of membrane processes as a pre-concentration step before evaporation reduces the energy costs by approx. 3 euro per tonne.

The objective of this study was to evaluate the potential of NF for concentration of chokeberry juice on the basis of characterization of the influence of temperature and pressure on the permeate flux, antioxidant activity and retention of aromatic compounds, namely, anthocyanins, phenolic acids and flavonols in concentrates. The composition of permeates was also studied.

MATERIALS AND METHODS

Fruit harvesting and preparation of chokeberry juice

Chokeberries (*Aronia melanocarpa)*, cultivar *Nero* were harvested in Croatia, region Slavonia, in 2014. Immediately after harvesting, fruits were transported and pressed in a fruit and vegetable processing plant. Fresh compressed juice was pasteurized at 85 °C for 10 min and stored in glass bottles, in a dark place at 4 °C until analysis. The pasteurized juice had total soluble solids content (*TSSC*) of 13.0% and pH was 3.62. This juice (initial juice) was used in all NF processes. Concentrates (retentates) gained by NF processes were diluted to an initial juice *TSSC* and then total polyphenol concentration (*TPC*), monomeric anthocyanin concentration (*MAC*), total flavonoid concentration (*TFC*), antioxidant activity, polymeric colour (*PC*), aroma and individual phenolic compounds were determined. Permeates were analysed undiluted. All spectrophotometric measurements were done in Jenway 6300 spectrophotometer (Bibby Scientific, Stone, United Kingdom).

Nanofiltration concentration procedure

NF experiments were conducted in a plate and frame module, DDS LabUnit M20 (De Danske Sukkerfabrikker, Nakskov, Denmark). Six composite membranes type Alfa Laval NF (DSS, A Tetra Pak, Silkeborg, Denmark) were used, with the following main characteristics: polyamide thin-film composite with pH range 3–10, maximum pressure 5.5 MPa, maximum temperature 60 °C and sodium chloride rejection of > 98%. Total filtration surface was 0.1736 m^2. Initial volume of feed juice in all experiments was 4 l and the juice was concentrated to maximum *TSSC* (26.9%). The permeate flux and *TSSC* were measured every 12 min. *TSSC* was determined in an Abbe refractometer (Carl Zeiss, Jena, Germany). Initial temperature of the feed juice in all experiments was 10 °C. Experiments were conducted in recirculation mode and with cooling of the retentate via heat exchanger (propylene glycol was used as a refrigerant) or without cooling. The transmembrane pressures utilized were 4.5 MPa, 5.0 MPa and 5.5 MPa.

Chemicals

Potassium chloride, hydrochloric acid, sodium bisulphite, sodium hydroxide, sodium chloride, sodium nitrite, sodium acetate, sodium carbonate and Folin-Ciocalteu reagent were purchased from Kemika (Zagreb, Croatia); gallic acid monohydrate, aluminium chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), chlorogenic acid (3-*O*-caffeoylquinic acid), rutin hydrate (quercetin-3-rutinoside hydrate), (+)-catechin and quercetin dihydrate from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Missouri, USA); high performance liquid chromatography (HPLC)-grade methanol from Merck (Darmstadt, Germany);

cyanidin-3-*O*-galactoside chloride and cyanidin-3-*O*-glucoside chloride (kuromanin chloride) from Extrasynthese (Genay, France).

Solid phase microextraction of volatiles

A volume of 5 ml of sample was introduced in a vial with 1 g of sodium chloride and a small magnetic stir bar, and sealed with a crimp cap lined with polytetrafluoroethylene (PTFE)/silicone. Samples were pre-heated for 5 min at 40 °C and volatiles were collected by solid phase microextraction (SPME) fibre for 20 min at 40 °C. A 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre (Supelco, Bellefonte, Pennsylvania, USA) was used.

Determination of aromatic components by GC-MS

Samples extracted by SPME were analysed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5890B gas chromatograph equipped with a mass detector Agilent 5977A (Agilent, Santa Clara, California, USA). The capillary column was CP-WAX 52CB (Agilent; 60 m × 250 μm × 0.25 μm). Helium 5.0 (purity 99.9%; Messer Austria, Gumpoldskirchen, Austria) was used as a carrier gas. Working conditions were as follows: injector temperature 250 °C; mass spectrometric detector interface temperature 250 °C; oven temperature programmed from 40 °C (2 min hold) to 230 °C (5 min hold) at 6 °C·min^{-1}; carrier gas (He) at a flow rate of 1 ml·min^{-1} (average velocity 25.502 cm·s^{-1}); injection port operated in splitless mode. Compounds were identified by comparing their mass spectra with the spectral library Wiley 9 (John Wiley and Sons, Hoboken, New Jersey, USA) and NIST 0.8 (NIST, Gaithersburg, Maryland, USA) and expressed as peak area. Three replicate measurements were performed for each sample.

Total polyphenol concentration determination

TPC was measured spectrophotometrically using Folin-Ciocalteu reagent according to the method of Ough et al. [20]. Briefly, 1.8 ml of distilled water was added in the test tube with 0.2 ml of juice sample followed by 10 ml of Folin-Ciocalteu reagent (1:10). The reaction solution was left to stand for 5 min. After that time, 8 ml of 7.5% sodium carbonate solution was added. After 2 h, the absorbance of blue colouration was measured at 765 nm against the blank sample. Measurements were performed in triplicate and expressed as grams per litre of gallic acid equivalent (GAE).

Total flavonoid concentration determination

TFC assay was carried out according to Zhishen et al. [21] with slight modifications. Briefly, diluted sample was mixed with 4 ml of distilled water in a 10 ml test tube and 0.3 ml of 5% sodium nitrite was added. After 5 min, 1.5 ml of 2% aluminium chloride and, after further 5 min, 2 ml of 1.0 mol·l^{-1} sodium hydroxide were added sequentially. Finally, the volume was brought up to 10 ml by adding more distilled water. The absorbance was measured at 510 nm. Measurements were conducted in triplicate and TFC was reported as grams per litre of catechin equivalents (CE).

Monomeric anthocyanin concentration and polymeric colour determination

The pH differential method [22] applicable to *MAC* determination, expressed in fruit as cyanidin-3-glucoside, was used. The method is suitable to determine *MAC* based on structural changes in the anthocyanin chromophore between pH 1.0 and 4.5. Monomeric anthocyanins undergo a reversible structural transformation as a function of pH. Two dilutions of samples were prepared, one with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5). After 15 min of incubation at room temperature, absorbance was measured simultaneously at 512 nm and 700 nm. The concentration of monomeric anthocyanins was expressed as milligrams per litre of cyanidin-3-glucoside equivalents (CGE) using a molar extinction coefficient (e) of cyanidin-3-glucoside of 26 900 1·mol^{-1}cm^{-1} and molar weight of 449.2 g·mol^{-1}.

Bisulphite bleaching method described by Gusti et al. [22] was used to determine colour density (*CD*) and polymeric colour (*PC*). For the assay, 0.2 ml of sodium bisulphite was added to 2.8 ml diluted sample, and 0.2 ml of distilled water was added to 2.8 ml diluted sample. After 15 min, absorbance of the samples was measured at 420 nm, 512 nm and 700 nm. *CD* was calculated using the control sample according to the following formula:

$$CD = [(A_{420} - A_{700}) + (A_{512} - A_{700})] \times DF \quad (1)$$

where A_{420}, A_{700} and A_{512} are absorbance values of sample with bisulphite, and *DF* is dilution factor.

Polymeric colour (*PC*) was determined using the bisulphite-bleached sample using the following formula:

$$PC = [(A_{420} - A_{700}) + (A_{512} - A_{700})] \times DF \quad (2)$$

Percentage of *PC* (*PCP*) was expressed as and calculated using the formula:

$$PCP = \frac{PC}{CD} \times 100 \quad (3)$$

All measurements were done in triplicate.

Assay of •DPPH radical-scavenging activity

Free radical-scavenging activity was measured by DPPH according to the method of SHIMADA et al. [23] with slight modifications. Briefly, 0.2 ml of the sample was diluted with methanol and 1 ml of DPPH solution (0.5 mmol·l^{-1}) was added. The absorbance was measured at 517 nm after 15 min. Measurements were done in triplicate and results expressed as millimoles of Trolox equivalents per litre of sample.

Determination of individual phenolic compounds by HPLC

Identification and quantification of phenolic compounds were performed by using Varian HPLC system consisting of ProStar 230 solvent delivery module, ProStar 330 photodiode array detector (Varian, Palo Alto, California, USA), OmniSpher C18 column (Agilent; 250 mm × 4.6 mm inner diameter, particle size 5 μm) and ChromSep guard column (Agilent, 10 mm × 3 mm). Anthocyanins were separated using 0.5% water solution of phosphoric acid as solvent A and 100% HPLC-grade methanol as solvent B. Elution conditions were: 0–38 min from 3% to 65% B; 38–45 min 65% B; flow rate 1 ml·min^{-1}; injection volume 20 μl). For phenolic acid and flavonol separation, 0.1% water solution of phosphoric acid was used as a solvent A and 100% HPLC-grade methanol as a solvent B. Elution conditions were: 0–30 min from 5% to 80% B; 30–33 min 80% B; 33–35 min from 80% to 5% B; flow rate 0.8 ml·min^{-1}; injection volume 20 μl). Validation of used methods was conducted in a previous study by JAKOBEK et al. [2]. Prior to injection into the HPLC system, concentrates were diluted to the initial juice *TSSC* and filtered trough syringe filter Chromafil Xtra (PTFE, pore size 0.45 μm, diameter 25 mm; Macherey-Nagel, Düren, Germany). Identification of phenolic acids and flavonols was conducted by comparing retention times and spectra with those of standard compounds. Quantification was made by using calibration curves of standards. Some compounds were tentatively identified (cyanidin-3-arabinoside, cyanidin-3-xyloside and neochlorogenic acid) based on literature data [2, 24, 25]. Anthocyanins were quantified by using cyanidin-3-glucoside and cyanidin-3-galactoside calibration curves, phenolic acids by using chlorogenic acid calibration curve and flavonols by using quercetin-3-rutinoside calibration curve. Standard calibration curves were constructed by diluting stock standards in HPLC methanol to yield 10–240 mg·l^{-1} (cyanidin-3-glucoside), 10–242.50 mg·l^{-1} (cyanidin-3-galactoside), 1–500 mg·l^{-1} (quercetin-3-rutinoside) and 10–500 mg·l^{-1} (chlorogenic acid).

Data calculations

The permeate flux (J) was measured and expressed as litre per square meter per hour

$$J = \frac{V_\mathrm{p}}{A \cdot t} \tag{4}$$

where V_p is the permeate volume (in litres), A is the membrane effective area (in square metres) and t the time (in hours) necessary for the production of V_p.

Volume reduction factor (F) was calculated from the following equation:

$$F = \frac{V_\mathrm{f}}{V_\mathrm{r}} \tag{5}$$

where V_f is the volume of the initial feed solution (in litres), V_r is the volume of the retentate (in litres).

The equation for retentate concentration index (I_rt) was:

$$I_\mathrm{rt} = \frac{A_\mathrm{rt}}{A_\mathrm{fc}} \tag{6}$$

where A_{rt} is *TSSC* in retentate, and A_fc *TSSC* of the initial juice (13%).

Retention of specific compound (R) expressed in percent was calculated from the following equation:

$$R = \left(1 - \frac{c_\mathrm{pt}}{c_\mathrm{fc}}\right) \times 100 \tag{7}$$

where c_pt is the concentration of specific compound (in milligrams per litre) or *TSSC* in the permeate, and c_fc is the concentration of specific compound (in milligrams per litre) or *TSSC* in initial chokeberry juice.

All measurements were made in duplicate or triplicate and data were expressed as average values. Statistical analysis and data calculations of average, standard deviation and level of significance were performed using MS Excel 2013 (Microsoft, Redmond, Washington, USA) and Statistica 12.0 (StatSoft, Tulsa, Oklahoma, USA). Statistical analysis of the obtained results was performed using one-way analysis of variance (ANOVA), differences between samples were evaluated by Fisher's test ($p < 0.05$).

RESULTS AND DISCUSSION

Influence of processing parameters on permeate flux

Factors affecting NF membrane separations include: feed variables such as solute concentration, temperature, pH and pretreatment require-

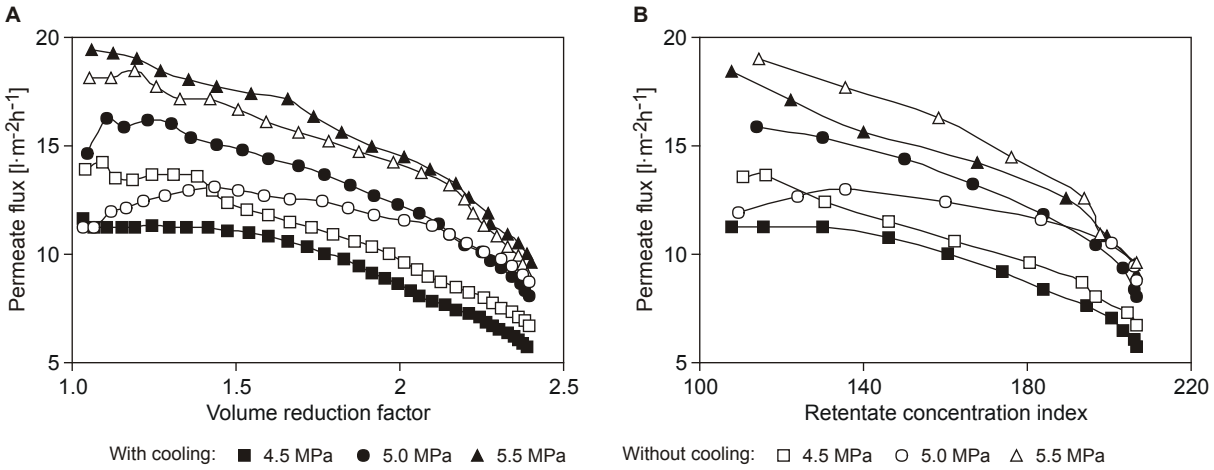

Fig. 1. Influence of volume reduction factor and retentate concentration index on permeate flux in nanofiltration concentration of chokeberry juice at different processing conditions.

ments; membrane variables such as polymer type, module geometry and module arrangement; and process variables such as feed flow rate, operating pressure, operating time and water recovery [26]. The influence of transmembrane pressure (4.5 MPa, 5.0 MPa and 5.5 MPa) and temperature (with cooling and without cooling of the retentate) on the permeate flux were examined during NF of chokeberry juice. Maximum concentration that could be achieved in all concentration processes was 26.9% (*TSSC*), which was consistent with literature data [17, 27]. The initial volume of the feed juice was 4 l. After NF concentration, 1.67 l of concentrate (retentate) and 2.33 l of permeate were obtained in all processes. The volume reduction factor, defined as the relationship between the volume of initial feed juice and the volume of the fraction retained by the membrane (concentrated juice or retentate), was 2.39, which corresponded to *TSSC* of 26.9%.

Fig. 1 shows the influence of volume reduction factor and retentate concentration index on permeate flux. From this diagrams it can be concluded that with an increase of the volume reduction factor and the retentate concentration index, the permeate flux proportionally decreased. This was in agreement with other authors [28, 29]. The highest permeate flux (19.00–9.61 $l \cdot m^{-2}h^{-1}$) was obtained at 5.5 MPa without cooling, and the lowest (11.23–5.74 $l \cdot m^{-2}h^{-1}$) at 4.5 MPa with cooling. VINCZE et al. [30] obtained a permeate flux of sea buckthorn juice varying from 14 $l \cdot m^{-2}h^{-1}$ to 4.6 $l \cdot m^{-2}h^{-1}$, while FERRARINI et al. [31] recorded variation of the permeate flux from 7.4 $l \cdot m^{-2}h^{-1}$ to 5.4 $l \cdot m^{-2}h^{-1}$ for grape juice.

Fig. 2 shows the influence of retentate concentration index on volume reduction factor in NF processes with cooling. The ratio was almost linear and indicated that an increase in volume reduction factor led to a significant increase in retentate concentration index. With an increase of the pressure from 4.5 MPa to 5.5 MPa at the same temperature regime, the permeate flux significantly increased. At all studied pressures (4.5 MPa, 5.0 MPa and 5.5 MPa), the permeate flux was slightly lower in the process with cooling. A decrease in the permeate flux along the processes was observed. This phenomenon can be explained by accumulation of material on membrane surface (fouling and polarization of concentration), and by increase in the osmotic pressure and viscosity of the juice due to *TSSC* increase (Fig. 3). At the same pressure, the process with cooling lasted longer. An increase in

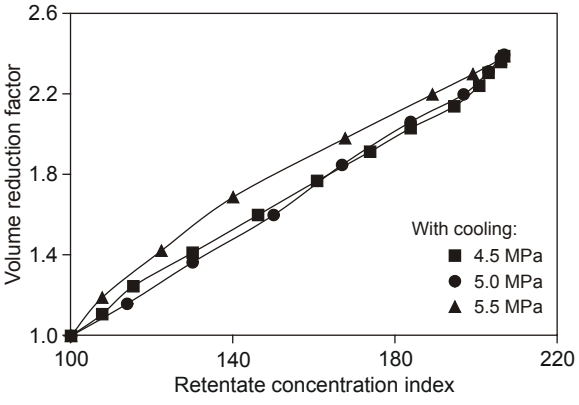

Fig. 2. Influence of retentate concentration index on volume reduction factor in nanofiltration concentration of chokeberry juice at different processing conditions with cooling.

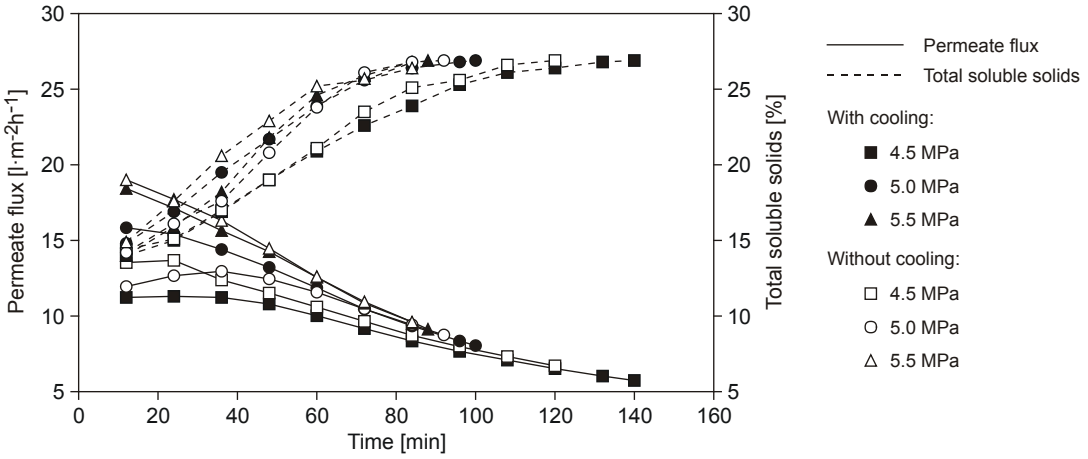

Fig. 3. Permeate flux and total soluble solids content of chokeberry juice in nanofiltration processes at different processing conditions.

the pressure from 4.5 MPa to 5.5 MPa led to the reduction of the process duration at the same temperature regime (with or without cooling). Process at 5.5 MPa without cooling was significantly shorter (84 min) than process at 4.5 MPa without cooling (120 min).

NF membranes have a larger pore size (1–10 nm) than the reverse osmosis membranes, so some molecules and ions pass through it and so permeate is not pure water. Fig. 4 presents the data on permeability and retention during all studied processes. Inversely proportional, an increase in permeability led to a decrease in retention. The greatest permeability (3.7%) was observed at 4.5 MPa at both temperature regimes. At the end of all other processes, permeability was 3.4%, which was in accordance with retention of 96.7%. This small increase at 4.5 MPa was probably due to longer duration of processes at this pressure. Longer processing at lower pressure was necessary to obtain the same *TSSC*, but longer processing led to development of a greater amount of heat. At higher temperature, the membrane permeability coefficient is higher, the diffusivity coefficient in the solution increases and the viscosity coefficient decreases [32]. Higher temperature can also damage the membrane and lead to degradation of sensitive valuable components in the juice, such as anthocyanins. Also, aroma compounds can be degraded at a higher temperature.

The feed juice temperature in all studied processes was 10 °C. The chokeberry juice heated during NF concentration. The temperatures at the end of the processes without cooling were

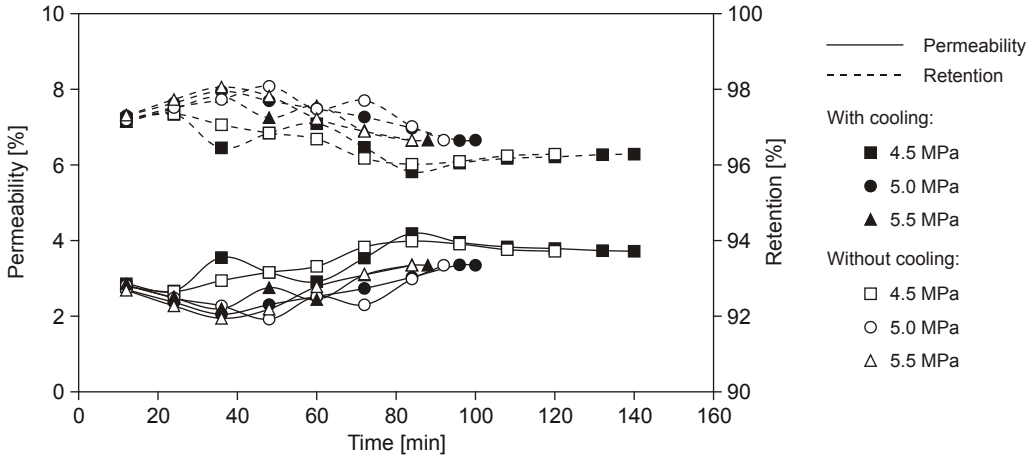

Fig. 4. Influence of nanofiltration processing time on permeability and retention at different processing conditions.

Abbreviations: P – permeability, R – retention, C – cooling, WC – without cooling.

remarkably higher (53–57 °C) compared to the processes with cooling (35–44 °C) at all tested pressures. The temperature influence on permeate flux is given in Fig. 5. Water flux increased linearly with applied pressure. It also increased with temperature, since water diffusivity in the membrane increased and water viscosity in the membrane decreased with temperature. The increase in water flux can usually be described by an Arrhenius temperature dependence of water permeability constant or by water viscosity changes [33]. Water flux is greater at higher feed flow rates (high feed velocities over the membrane surface) since this minimizes concentration polarization. Water flux decreases with increasing feed solute concentration since the higher concentrations result in greater osmotic pressures and also in a smaller driving force across the membrane. This behaviour is predicted by most of the transport models. Water flux can also gradually decrease over operating time because of compaction (mechanical compression) or other physical or chemical changes in membrane structure [26]. At a fundamental level, NF is a very complex process. Several models have been developed, which can be divided into two main types: irreversible thermodynamics models and transport mechanism models. The fundamental models derived from irreversible thermodynamics are the Kedem-Katchalsky model and the Spiegler-Kedem model. They were employed in predicting transport through NF membranes for single and binary solute systems and, most recently, for multiple systems [34].

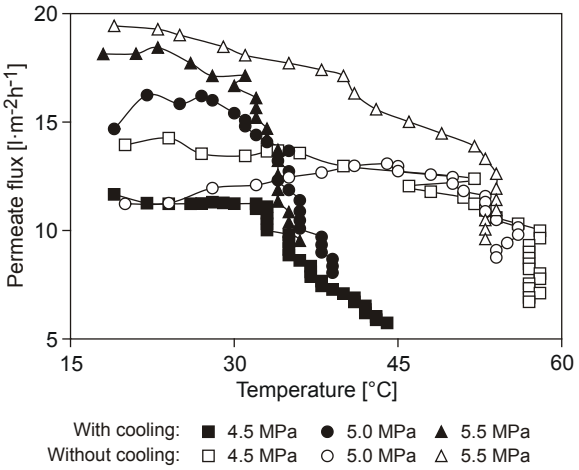

Fig. 5. Influence of temperature on permeate flux in nanofiltration concentration of chokeberry juice at different processing conditions.

Influence of processing parameters on aroma retention

SPME, a solventless extraction technique, was used to assess the volatile components in chokeberry juice, as well as in NF concentrates and permeates. The aroma profile by this technique may be influenced by the fibre, by the matrix and by competition between compounds in terms of adsorption to fibres [35]. After extraction, volatile compounds were assessed by GC-MS.

Overall, 24 volatile compounds were identified in initial chokeberry juice and in NF concentrates. Tab. 1 shows the individual volatile compounds and their retention times. The identified volatile compounds are divided into five groups: alcohols (isoamyl alcohol (3-methyl-1-butanol), 3-hexenol, 2-hexenol, 2-ethylhexanol, benzene methanol and benzene ethanol), acids (hexanoic acid, octanoic acid, nonanoic acid, decanoic acid and dodecanoic acid), carbonyl compounds (2-hexenal, 6-methyl-5-hepten-2-one, nonanal, benzaldehyde, vitispirane and β-damascenone), esters (*n*-hexyl

Tab. 1. Volatiles identified in chokeberry juice, nanofiltration concentrates and nanofiltration permeates.

Volatile compound	Retention time [min]
Alcohols	
Isoamyl alcohol (3-methyl-1-butanol)	13.54
3-Hexenol	17.79
2-Hexenol	18.25
2-Ethylhexanol	20.04
Benzene methanol	27.95
Benzene ethanol	28.58
Acids	
Hexanoic acid	27.34
Octanoic acid	30.94
Nonanoic acid	32.62
Decanoic acid	34.23
Dodecanoic acid	37.97
Esters	
n-Hexyl acetate	15.17
Ethyloctanoate	18.89
Terpenoids	
DL-Limonene	13.34
1,8-Cinole	13.73
p-cymene	15.27
Linalool oxide	19.16
Geraniol	27.12
Carbonyl compounds	
2-Hexenal	14.03
6-Methyl-5-hepten-2-one	16.78
Nonanal	18.09
Benzaldehyde	21.22
Vitispirane	21.28
β-damascenone	26.94

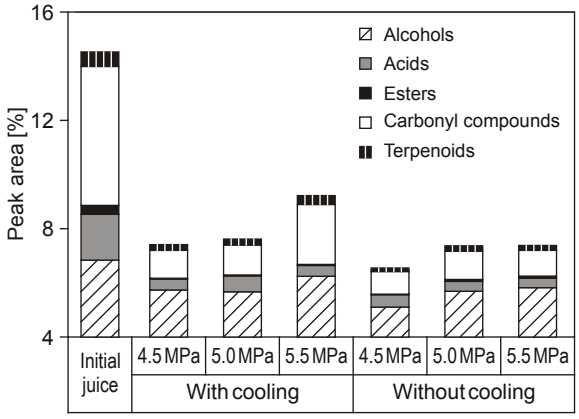

Fig. 6. Retention of groups of aromatic compounds in initial chokeberry juice and in concentrates obtained in nanofiltration processes at different processing conditions.

acetate and ethyloctanoate) and terpenoids (DL-limonene, 1,8-cinole, *p*-cymene, linalool oxide and geraniol). Most of them were previously identified in other studies [10, 36] and are characteristic for chokeberry. Fig. 6 presents data on aroma (expressed by peak area) retention in NF concentrates with regards to the initial juice. The initial chokeberry juice contained 48.6% carbonyl compounds, 26.2% alcohols, 16.0% acids, 6.5% terpenoids and 2.6% esters. The analysed concentrates contained approximately 42.9–53.9% alcohols, 28.1–32.7% carbonyl compounds, 7.7–17.8% acids, 5.1–6.3% terpenoids and 0.7–2.5% esters. The processes with cooling retained more of the volatile compounds than those without cooling at the same examined pressure. Also, slightly better retention was obtained at higher pressures. Due to highest retention of alcohols, carbonyl compounds and terpenoids, the concentrate gained at 5.5 MPa with cooling was the best. It retained 78.9% alcohols, 23.8% acids, 11.3% esters, 43.1% carbonyl compounds and 61.2% terpenoids with regards to the initial juice. This means that 49.6% of all aroma compounds was retained.

Influence of processing parameters on retention of phenolic compounds

Chemical composition (*TPC, MAC, PC, TFC* and antioxidant activity) of initial juice, as well as of NF concentrates and permeates, is presented in Tab. 2. The results demonstrate a significant decrease of *TPC, MAC* and *TFC* in all prepared concentrates. An increase of the process pressure from 4.5 MPa to 5.5 MPa led to greater retention of polyphenols, anthocyanins and flavonoids in concentrates. Processes without cooling proved to be slightly better in terms of higher retention. The process at 5.5 MPa without cooling was the best. The concentrate gained in that process contained 3.77 $g \cdot l^{-1}$ polyphenols (expressed as GAE), 330.32 $mg \cdot l^{-1}$ monomeric anthocyanins (expressed as CGE) and 2.27 $g \cdot l^{-1}$ flavonoids (expressed as CE). This means that 94.3% of polyphenols, 89.4% of anthocyanins and 90.8% of flavonoids were retained with regard to the initial juice. The decrease of *MAC* led to the increase of *PC*. *PC* corresponds to the percentage of colour represented by polymerized material formed by anthocyanins degradation [37]. Due to their high reactivity, anthocyanins easily convert to colourless or undesirable brown degradation compounds. Temperature, pH, light, oxygen, enzymes, ascorbic acid, saccharides and hydrogen peroxide have a significant effect on stability of anthocyanins [38]. There was no statistically significant difference in antioxidant activity between all examined concentrates. The values for concentrates (6.90–7.10 $mmol \cdot l^{-1}$) were slightly lower than for the initial chokeberry juice (8.20 $mmol \cdot l^{-1}$).

Small amounts of polyphenols (from 0.05 $g \cdot l^{-1}$ to 0.08 $g \cdot l^{-1}$, expressed as GAE) and flavonoids (0.01–0.02 $mg \cdot ml^{-1}$, expressed as CE) were found in permeates. In concordance with this, low antioxidant activity was determined for all permeates. *MAC* could not be quantified by using the pH-differential method.

The concentrations of individual anthocyanins, phenolic acids and flavonols in initial chokeberry juice and concentrates obtained at different processing conditions in NF processes are shown in Tab. 3. Chokeberry juice and concentrates contained a mixture of four different cyanidin-glycosides: 3-galactoside, 3-glucoside, 3-arabinoside and 3-xyloside of cyanidin. This was in accordance with data of other authors [2, 39, 40]. Cyanidin-3-galactoside and cyanidin-3-arabinoside were found in high concentrations, whereas the concentrations of cyanidin-3-glucoside and cyanidin-3-xyloside were significantly lower. Concentrations of individual anthocyanins in all concentrates were lower than in initial juice. During juice processing, some degradation and losses of anthocyanins occurred, and also a small amount passed through membrane and was identified in permeate. Better retention of individual anthocyanins in concentrates was observed at higher pressures (5.0 MPa and 5.5 MPa) and in processes without cooling of the retentate. Cyanidin-3-galactoside (238.69 $mg \cdot l^{-1}$) and cyanidin-3-arabinoside (64.55 $mg \cdot l^{-1}$) were found at highest concentrations in the concentrate prepared at 5.0 MPa without cooling, whereas cyanidin-3-glucoside (7.52 $mg \cdot l^{-1}$) and cyanidin-3-xy-

Tab. 2. Phenolic compounds of initial chokeberry juice and nanofiltration concentrates (retentates) and permeates and their antioxidant activity.

	Initial juice		Nanofiltration concentration procedure					
			With cooling			Without cooling		
			4.5 MPa	5.0 MPa	5.5 MPa	4.5 MPa	5.0 MPa	5.5 MPa
Total phenolic concentration [g·l^{-1}]	4.00 ± 0.04^{a}	Retentate	3.38 ± 0.03^{e}	3.59 ± 0.03^{d}	3.59 ± 0.04^{d}	3.63 ± 0.03^{cd}	3.68 ± 0.03^{c}	3.77 ± 0.02^{b}
		Permeate	0.08 ± 0.01^{a}	0.05 ± 0.01^{e}	0.06 ± 0.01^{d}	0.08 ± 0.01^{b}	0.07 ± 0.01^{c}	0.05 ± 0.01^{d}
Monomeric anthocyanin concentration [mg·l^{-1}]	369.47 ± 0.91^{a}	Retentate	271.35 ± 7.11^{f}	315.83 ± 2.94^{c}	307.08 ± 1.42^{d}	290.56 ± 1.26^{e}	318.95 ± 2.08^{c}	330.32 ± 4.06^{b}
Polymeric colour [%]	18.7 ± 1.4^{e}	Retentate	37.2 ± 1.2^{a}	30.1 ± 1.4^{bc}	32.8 ± 1.3^{b}	36.1 ± 1.4^{a}	29.7 ± 2.2^{cd}	27.1 ± 1.9^{d}
Total flavonoid concentration [g·l^{-1}]	2.50 ± 0.04^{a}	Retentate	2.06 ± 0.02^{c}	2.01 ± 0.04^{d}	2.04 ± 0.01^{cd}	2.25 ± 0.02^{b}	2.27 ± 0.03^{b}	2.27 ± 0.05^{b}
		Permeate	0.02 ± 0.01^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}
Antioxidant activity [mmol·l^{-1}]	8.20 ± 0.01^{a}	Retentate	7.00 ± 0.10^{b}	6.90 ± 0.10^{b}	7.00 ± 0.10^{b}	7.10 ± 0.20^{b}	7.00 ± 0.10^{b}	7.10 ± 0.20^{b}
		Permeate	0.60 ± 0.10^{ab}	0.50 ± 0.10^{ab}	0.50 ± 0.10^{ab}	0.60 ± 0.10^{a}	0.50 ± 0.10^{ab}	0.40 ± 0.10^{b}

Values represent the mean of 3 replicates ± standard deviation. Different lowercase letters in superscript in the same row indicate significant differences ($p < 0.05$) by Fisher's test.
Total phenolic concentration is expressed as grams of gallic acid equivalents. Monomeric anthocyanin concentration is expressed as milligrams of cyanidin-3-glucoside equivalents. Total flavonoid concentration is expressed as grams of catechin equivalents. Antioxidant activity is expressed as millimoles of Trolox.

Tab. 3. Concentration of individual phenolic compounds in the initial chokeberry juice and the nanofiltration concentrates.

Phenolic compounds [mg·l^{-1}]		Initial juice	Nanofiltration concentration procedure					
			With cooling			Without cooling		
			4.5 MPa	5.0 MPa	5.5 MPa	4.5 MPa	5.0 MPa	5.5 MPa
Anthocyanins	Cyanidin-3-galactoside	278.43 ± 25.03^{a}	182.32 ± 17.07^{d}	228.14 ± 2.76^{bc}	217.93 ± 11.59^{bc}	210.89 ± 9.17^{c}	238.69 ± 1.56^{ab}	237.77 ± 4.91^{ab}
	Cyanidin-3-glucoside	9.28 ± 0.96^{a}	6.52 ± 0.44^{c}	7.23 ± 0.40^{bc}	7.50 ± 0.46^{ab}	7.05 ± 0.08^{bc}	7.42 ± 0.14^{bc}	7.52 ± 0.57^{ab}
	Cyanidin-3-arabinoside	78.47 ± 4.27^{a}	45.19 ± 4.67^{e}	56.81 ± 1.10^{cd}	57.19 ± 3.74^{bcd}	52.31 ± 3.66^{de}	64.55 ± 0.11^{b}	62.67 ± 2.33^{bc}
	Cyanidin-3-xyloside	6.88 ± 0.51^{a}	4.32 ± 0.41^{d}	5.66 ± 0.07^{bc}	5.75 ± 0.51^{abc}	5.09 ± 0.08^{cd}	6.36 ± 0.11^{ab}	6.43 ± 0.37^{ab}
	Total	373.06 ± 30.77^{a}	238.35 ± 22.59^{d}	297.84 ± 4.33^{bc}	288.37 ± 16.30^{bc}	275.34 ± 12.99^{c}	317.02 ± 1.92^{ab}	314.39 ± 8.18^{ab}
Phenolic acids and flavonols	Neochlorogenic acid	426.57 ± 0.98^{a}	375.01 ± 11.64^{bc}	355.51 ± 6.40^{c}	377.49 ± 1.42^{bc}	400.98 ± 10.34^{ab}	377.42 ± 27.62^{bc}	402.08 ± 8.07^{ab}
	Chlorogenic acid	370.06 ± 3.29^{a}	343.53 ± 18.17^{ab}	310.59 ± 3.25^{c}	348.45 ± 17.58^{ab}	354.07 ± 6.07^{ab}	323.95 ± 12.66^{bc}	351.41 ± 9.01^{ab}
	Quercetin-3-rutinoside	107.13 ± 5.08^{a}	91.73 ± 8.33^{abc}	79.95 ± 5.08^{c}	93.93 ± 4.66^{ab}	100.21 ± 5.71^{a}	86.01 ± 4.12^{bc}	94.89 ± 4.86^{ab}
	Quercetin	0.27 ± 0.23^{a}	nd	nd	nd	nd	nd	nd
	Total	904.03 ± 9.58^{a}	810.27 ± 38.14^{bcd}	746.05 ± 14.73^{d}	819.87 ± 23.66^{abc}	855.26 ± 22.12^{ab}	787.38 ± 44.40^{cd}	848.38 ± 21.94^{abc}
Total		1277.09 ± 40.35^{a}	1048.62 ± 60.73^{c}	1043.89 ± 19.06^{c}	1108.24 ± 49.96^{bc}	1130.60 ± 35.11^{bc}	1104.40 ± 46.32^{bc}	1162.77 ± 30.12^{ab}

Values represent the mean of 2 replicates ± standard deviation. Different lowercase letters in superscript in the same row indicate significant difference ($p < 0.05$) by Fisher's test.
nd - not detected.

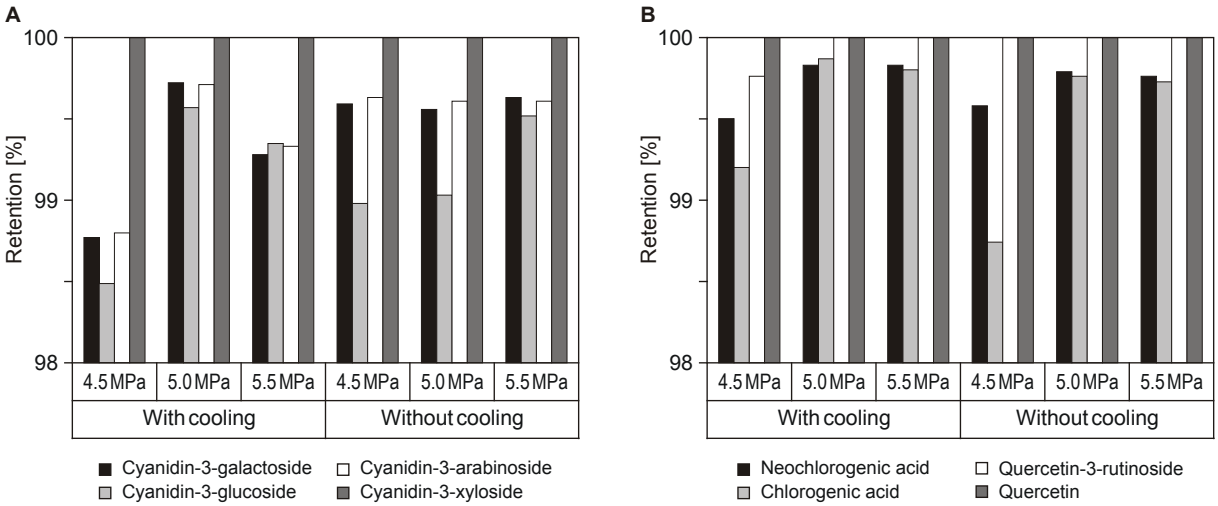

Fig. 7. Retention of individual phenolic compounds in chokeberry concentrates obtained by nanofiltration at different processing conditions.

A – Anthocyanins. B – Phenolic acids and flavonols.

loside (6.43 mg·l^{-1}) were found at highest concentrations in the concentrate prepared at 5.5 MPa without cooling. Overall, the highest concentration of total anthocyanins (317.02 mg·l^{-1}), identified by HPLC, was obtained at 5.0 MPa without cooling.

The main phenolic acids in initial juice and NF concentrates were chlorogenic and neochlorogenic acid. The concentrations of neochlorogenic acid were slightly higher than those of chlorogenic acid in initial juice and in all concentrates. The highest retention of chlorogenic acid (354.07 mg·l^{-1}) was observed in concentrate gained at 4.5 MPa without cooling, and the highest retention of neochlorogenic (402.08 mg·l^{-1}) at 5.5 MPa without cooling. There were no statistically significant differences in their concentrations between these two pressures.

Quercetin and quercetin-3-rutinoside are main flavonols found in chokeberry juice. In all concentrates, quercetin was found below the quantification threshold. The highest retention of quercetin-3-rutinoside (100.21 mg·l^{-1}) was observed in concentrate gained at 4.5 MPa without cooling. So, generally, the highest retention of total phenolic acids and flavonols was obtained in concentrate gained at 4.5 MPa without cooling. This was slightly different than retention of total anthocyanins. It can be concluded that, at higher pressures regardless of temperature regime, degradation of phenolic acids and flavonols is more pronounced than at lower pressures. The highest retention of total phenolic compounds was achieved at 5.5 MPa without cooling (Fig. 7). Processes without cooling lasted significantly shorter than those with cooling, so degradation and permeation of individual components trough membrane pores were lower. Also, the increased retention could be explained by the solution-diffusion mechanism, which means that increase in pressure led to increase in water adsorption, due to largely stronger interaction of water with the hydrophilic membrane than of solute molecules, through hydrogen bonding [41].

CONCLUSIONS

Concentration of fruit juices by evaporation leads to great loss of their nutritional value and highly valuable sensitive compounds like anthocyanins. Therefore, membrane processes (reverse osmosis and NF) are widely investigated to partially replace it. In our study, NF of chokeberry juice was investigated at different pressures and temperature regimes. The chosen processing parameters significantly influenced the permeate flux, as well as retention of aromatic and phenolic compounds. Permeate flux was the greatest at the highest applied pressure (5.5 MPa) without cooling of the retentate. That process was significantly shorter than the others and gave the concentrate with the highest concentration of phenolic compounds. Aroma loss was observed in all concentrates, with the lowest one at 5.5 MPa with cooling. Overall, it is concluded that processes using NF membranes are capable to concentrate chokeberry juice to a certain level and have a potential to partially replace the conventional evaporation process.

REFERENCES

1. Jakobek, L. – Šeruga, M. – Novak, I. – Medvidović-Kosanović, M.: Anthocyanin content and antioxidant activity of various red fruit juices. Deutsche Lebensmittel Rundschau, *103*, 2007, pp. 58–64. ISSN: 0012-0413.
2. Jakobek, L. – Drenjančević, M. – Jukić, V. – Šeruga, M.: Phenolic acids, flavonols, anthocyanins and antiradical activity of Nero, Viking, Galicianka and wild chokeberries. Scientia Horticulturae, *147*, 2012, pp. 56–63. DOI: 10.1016/j.scienta.2012.09.006.
3. Kulling, S. E. – Rawel, H. M.: Chokeberry (*Aronia melanocarpa*) – A review on the characteristic components and potential health effects. Planta Medica, *74*, 2008, pp. 1625–1634. DOI: 10.1055/s-0028-1088306.
4. Shama, V. J. – Edirisinghe, I. – Burton-Freeman, B. M.: Berries: anti-inflammatory effects in humans. Journal of Agricultural and Food Chemistry, *62,* 2014, pp. 3886–3903. DOI: 10.1021/jf4044056.
5. Stanisavljević, N. – Samardžić, J. – Janković, T. – Šavikin, K. – Mojsin, M. – Topalović, V. – Stevanović M.: Antioxidant and antiproliferative activity of chokeberry juice phenolics during in vitro simulated digestion in the presence of food matrix. Food Chemistry, *175*, 2015, pp. 516–522. DOI: 10.1016/j.foodchem.2014.12.009.
6. Matsumoto, M. – Hara, H. – Chiji, H. – Kasai, T.: Gastroprotective effect of red pigments in black chokeberry fruit (*Aronia melanocarpa* Elliot) on acute gastric hemorrhagic lesions in rats. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 2226–2229. DOI: 10.1021/jf034818q.
7. Zhao, C. – Giusti, M. M. – Malik, M. – Moyer, M. P. – Magnuson, B. A.: Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 6122–6128. DOI: 10.1021/jf049517a.
8. Espin, J. C. – Soler-Rivas, C. – Wichers, H. J. – Garcia-Viguera, C.: Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. Journal of Agricultural and Food Chemistry, *48,* 2000, pp. 1588–1592. DOI: 10.1021/jf9911390.
9. Ochmian, I. – Grajkowski, J. – Smolik, M.: Comparison of some morphological features, quality and chemical content of four cultivars of chokeberry fruits (*Aronia melanocarpa*). Notulae Botanicae Horti Agrobotanici, *40*, 2012, pp. 253–260. ISSN (print): 0255-965X; ISSN (electronic): 1842-4309. <http://www.notulaebotanicae.ro/index.php/nbha/article/view/7181/6818>
10. Kraujalyte, V. – Leitner, E. – Venskutonis, P. R.: Characterization of *Aronia melanocarpa* volatiles by headspace-solid-phase microextraction (HS-SPME), simultaneous distillation/extraction (SDE), and gas chromatography-olfactometry (GC-O) methods. Journal of Agricultural and Food Chemistry, *61*, 2013, pp. 4728–4736. DOI: 10.1021/jf400152x.
11. Pan, B. – Yan, P. – Zhu, L. – Li, X.: Concentration of coffee extract using nanofiltration membranes. Desalination, *317,* 2013, pp. 127–131. DOI: 10.1016/j.desal.2013.03.004.
12. Vivekanand, V. – Iyer, M. – Ajlouni, S.: Clarification and stability enhancement of pear juice using loose nanofiltration. Journal of Food Process Technology, *3*, 2012, Article No. 1000162. DOI: 10.4172/2157-7110.1000162.
13. Warczok, J. – Ferrando, M. – Lopez, F. – Guell, C.: Concentration of apple and pear juices by nanofiltration at low pressures. Journal of Food Engineering, *63*, 2004, pp. 63–70. DOI: 10.1016/S0260-8774(03)00283-8.
14. Medina, B. G. – Garcia, A.: Concentration of orange juice by reverse osmosis. Journal of Food Process Engineering, *10*, 1988, pp. 217–230. DOI: 10.1111/j.1745-4530.1988.tb00014.x.
15. Lu, X. – Bian, X. – Shi, L.: Preparation and characterization of NF composite membrane. Journal of Membrane Science, *210*, 2002, pp. 3–11. DOI: 10.1016/S0376-7388(02)00120-5.
16. Mänttäri, M. – Puro, L. – Nuortila-Joniken, J. – Nyström, M.: Fouling effects of polysaccharides and humic acid in nanofitration. Journal of Membrane Science, *165*, 2000, pp. 1–17. DOI: 10.1016/S0376-7388(99)00215-X.
17. Jiao, B. – Cassano, A. – Drioli, E.: Recent advances on membrane processes for the concentration of fruit juices: a review. Journal of Food Engineering, *63*, 2004, pp. 303–324. DOI: 10.1016/j.jfoodeng.2003.08.003.
18. Cassano, A. – Drioli, E. – Galaverna, G. – Marchelli, R. – Di Silvestro, G. – Cagnasso, P.: Clarification and concentration of citrus and carrots juice by integrated membrane process. Journal of Food Engineering, *57*, 2003, pp. 153–163. DOI: 10.1016/S0260-8774(02)00293-5.
19. Merry A.: Membrane processes in fruit juice processing. In: Chui, Z. F. – Muralidhara, H. S. (Ed.): Membrane technology. A practical guide to membrane technology and applications in food and bioprocessing. Oxford : Elsevier, 2010, pp. 33–43. DOI: 10.1016/B978-1-85617-632-3.00003-3.
20. Ough, C. S. – Amerine, M. A.: Phenolic compounds. In: Ough, C. S. – Amerine, M. A. (Ed.): Methods for analysis of musts and wines. 2nd edition. New York: John Wiley and Sons, 1988, pp. 196–221. ISBN: 978-0-471-62757-9.
21. Zhishen, J. – Mengcheng, T. – Jianming, J.: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, *64*, 1999, pp. 555–559. DOI: 10.1016/S0308-8146(98)00102-2.
22. Gusti, M. M. – Wrolstad, R. E.: Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad, R. E. (Ed.): Current protocols in food Analytical Chemistry. New York: John Wiley and Sons, 2001, pp. F1.2.1–1.2.13. ISBN: 9780471142911.
23. Shimada, K. – Fujikawa, K. – Yahara, K. – Nakamura, T.: Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry,

40, 1992, pp. 945–948. DOI: 10.1021/jf00018a005.
24. Määttä-Riihinen, K. R. – Kamal-Eldin, A. – Mattila, P. H. – González-Paramás, A. M. – Törrönen, A. R.: Distribution and contents of phenolic compounds in eighteen scandinavian berry species. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 4477–4486. DOI: 10.1021/jf049595y.
25. Slimestad, R. – Torskangerpoll, K. – Nateland, H. S. – Johannessen, T. – Gisked, N. H.: Flavonoids from black chokeberries, *Aronia melanocarpa*. Journal of Food Composition and Analysis, *18*, 2005, pp. 61–68. DOI: 10.1016/j.jfca.2003.12.003.
26. Reverse osmosis. In: Ho, W. – Sirkar, K. (Ed.): Membrane handbook. New York: Van Nostrand Reinhold, 1992, pp. 263–390. ISBN: 978-1-4613-6575-4 (print), 978-1-4615-3548-5 (online). DOI: 10.1007/978-1-4615-3548-5.
27. Vincze, I. – Stefanovits-Banyai, E. – Vatai, G.: Using nanofiltration and reverse osmosis for the concentration of seabuckthorn (*Hippophae rhamnoides* L.) juice. Desalination, *200*, 2006, pp. 528–530. DOI: 10.1016/j.desal.2006.03.423.
28. Díaz-Reinoso, B. – Moure, A. – Domínguez, H. – Parajó, J. C.: Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace. Journal of Food Engineering, *91,* 2009, pp. 587–593. DOI: 10.1016/j.jfoodeng.2008.10.007.
29. Aguiar Prudêncio, A. P. – Schwinden Prudêncio, E. – Castanho Amboni, R. D. M. – Negrão Murakami, A. N. – Maraschin, M. – Cunha Petrus, J. C. – Ogliari, P. J. – Leite R. S.: Phenolic composition and antioxidant activity of the aqueous extract of bark from residues from mate tree (*Ilex paraguariensis* St. Hil.) bark harvesting concentrated by nanofiltration. Food and Bioproducts Processing, *90*, 2012, pp. 399–405. DOI: 10.1016/j.fbp.2011.12.003.
30. Vincze, I. – Bányai-Stefanovits, E. – Vatai, G.: Concentration of sea buckthorn (*Hippophae rhamnoides L.*) juice with membrane separation. Separation and Purification Technology, *57*, 2007, pp. 455–460. DOI: 10.1016/j.seppur.2006.06.020.
31. Ferrarini, R. – Versari, A. – Galassi, S.: A preliminary comparison between nanofiltration and reverse osmosis membranes for grape juice treatment. Journal of Food Engineering, *50*, 2001, pp. 113–116. DOI: 10.1016/S0260-8774(00)00199-0.
32. Álvarez, S. – Riera, F. A. – Álvarez, R. – Coca, J.: Permeation of apple aroma compounds in reverse osmosis. Separation and Purification Technology, *14*, 1998, pp. 209–220. DOI: 10.1016/S1383-5866(98)00076-8.
33. Mehdizadeh, H. – Dickson, J.: Modelling of temperature effects on the performance of reverse osmosis membranes. Chemical Engineering Communications, *103*, 1991, pp. 99–117. DOI: 10.1080/00986449108910865.
34. Sabbaghi, S. – Maleki, R. – Shariaty-Niassar, M. – Zerafat, M. M.: Modeling chloride ion removal from gas condensates by nanofiltration membrane separation. International Journal of Chemical and Environmental Engineering, *3*, 2012, pp. 30–33. ISSN: 2078-0737.
35. Sostaric, T. – Boyce, M. C. – Spickett, E. E.: Analysis of the volatile compounds in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. Journal of Agricultural and Food Chemistry, *48*, 2000, pp. 5802–5807. DOI: 10.1021/jf000515+.
36. Hirvi, T. – Honkanen, E.: Analysis of the volatile constituents of black chokeberry (*Aronia melanocarpa* Ell.). Journal of the Science of Food and Agriculture, *36*, 1985, pp. 808–810. DOI: 10.1002/jsfa.2740360908.
37. Poiana, M. A. – Munteanu, M. F. – Bordean, D. M. – Gligor, R. – Alexa, E.: Assessing the effects of different pectins addition on color quality and antioxidant properties of blackberry jam. Chemistry Central Journal, *121*, 2013, pp. 1–13. DOI: 10.1186/1752-153X-7-121.
38. Moldovan, B. – David, L. – Chişbora, C. – Cimpoiu, C.: Degradation kinetics of anthocyanins from european cranberrybush (*Viburnum opulus* L.) fruit extracts. Effects of temperature, pH and storage solvent. Molecules, *17*, 2012, pp. 11655–11666. DOI: 10.3390/molecules171011655.
39. Oszmiański, J. – Wojdyło, A.: *Aronia melanocarpa* phenolics and their antioxidant activity. European Food Research and Technology, *221,* 2005, pp. 809–813. DOI: 10.1007/s00217-005-0002-5.
40. Mayer-Miebach, E. – Adamiuk M. – Behsnilian, D.: Stability of chokeberry bioactive polyphenols during juice processing and stabilization of a polyphenol-rich material from the by-product. Agriculture, *2*, 2012, pp. 244–258. DOI: 10.3390/agriculture2030244.
41. Murthy, G. – Sridhar, S. – Shyam Sunder, M. – Shankaraiah, B. – Ramakrishna, M.: Concentration of xylose reaction liquor by nanofiltration for the production of xylitol sugar alcohol. Separation and Purification Technology, *44,* 2005, pp. 221–228. DOI: 10.1016/j.seppur.2005.01.009.

Received 12 January 2016; 1st revised 29 February 2016; 2nd revised 7 April 2016; accepted 11 April 2016; published online 6 May 2016.