Optimization of chokeberry (*Aronia melanocarpa* (michx.) Elliott) extraction, microencapsulation of extract by electrostatic extrusion and spray drying methods

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Chokeberry (*Aronia melanocarpa* (Michx.) Elliott) represents one of the richest sources of polyphenols. The aim of this research were: optimization of the polyphenols extraction process from chokeberry dried fruit, microencapsulation of obtained extract in order to improve the stability and bioavailability of polyphenols and examination of biological activity of extract in the model of experimental hypertension. Extract with the greatest amount of active principles, total phenols (27.7 mg GAE/g dry weight) and anthocyanins (0.27%), was obtained by maceration method using 50% ethanol as a solvent, 1:20 solid-solvent ratio, 0.75 mm particle size, during 60 minutes. Dominant anthocyanins in the extract were cyanidin-3-Ogalactoside, cyanidin-3-O-arabinoside and cyanidin-3-O-glucoside, respectively, while among flavonoids predominantly were presented rutin, hyperoside and isoquercetin, respectively. The extract with the highest content of polyphenols was encapsulated in suitable carriers by two microencapsulation methods, electrostatic extrusion and spray drying. In a third part of study, a four week term usage of the lyophilized extract in spontaneously hypertensive rats significantly reduced the systolic blood pressure (SHR-K: 205.1 ± 20.5mmHg for the control group, compared with the treated one, SHR-A: 184.5 ± 10.9mmHg) and pulse pressure ($p<0.05$).

Key words: *chokeberry, polyphenols, anthocyanins, optimization, extraction, microincapsulation, extrusion, spray drying, hypertension, oxidative stress*

NOTE: Results from this dissertation have been published previously in:

Ćujić, N., Trifković, K., Bugarski, B., Ibrić, S., Pljevljakušić, D., Šavikin, K. (2016). Chokeberry (*Aronia melanocarpa* L.) extract loaded in alginate and alginate/inulin system, *Industrial Crops and Products*, 86, pp. 120-131.

Ćujić N., Šavikin K., Janković T., Pljevljakušić D., Zdunić G., Ibrić S. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional tehnique. Food Chemistry, 194, pp. 135-142.

1. INTRODUCTION

In recent past years, there is a growing interest for application and investigation of alternative natural substances, antioxidants which could achieve protective effects on human health. Examination of antioxidants, especially ones from natural origin nowadays represents the main focus in pharmaceutical research. Among numerous plants, dark blue or purple berry fruits, especially chokeberry have the highest polyphenols content and antioxidant capacity. Growing interest in berry fruits, peculiarly in chokeberry is in increasing in the recent past years because high intake of chokeberry may have beneficial effects on cardiovascular risks, cancer and various degenerative diseases reducing. Chokeberry (*Aronia melanocarpa* (Michx.) Elliott) belongs to the family of Rosacea and it is one of the richest sources of polyphenols, particular in anthocyanins, proanthocyanidins, phenolic acids, flavanols, and this active principles are representing one of the most potent natural antioxidants. Therefore, the isolation and identification of chokeberry biologically active compounds, their further application in terms of enrichment the different products with these compounds, the possibility for their use as a

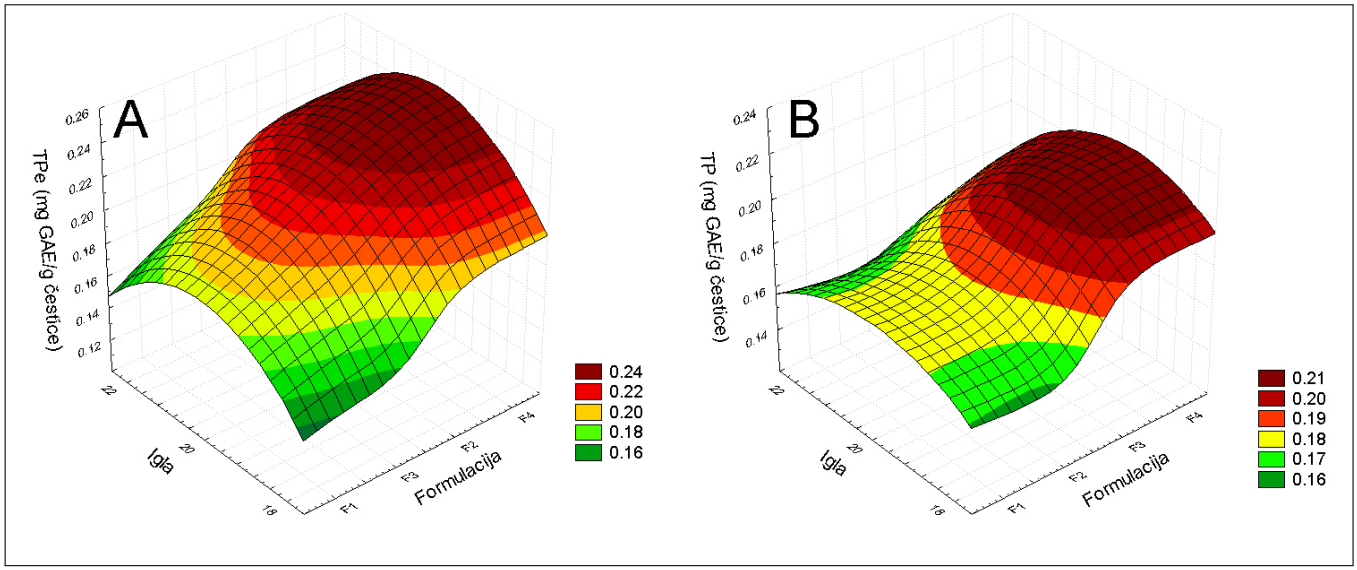

Fig. 1. Response surface plot showing the influence of varied factors on a a) polyphenolics load (encapsulation efficiency) b) release of polyphenols of hydrogel microbeads

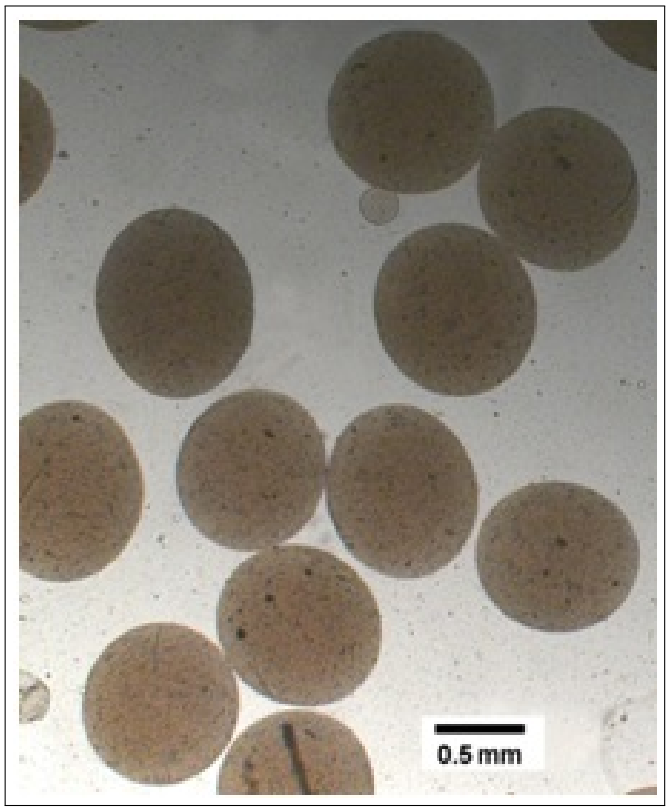

Fig. 2. Micrograph of hydrogel alginate beads encapsulating chokeberry extract, 2.5×10 (bar 500 µm)

dietary supplements are currently one of the most scientific and research topics. Chokeberry is available on the market in the form of fresh and dry fruits, juice, jams, but it is not enough present in the form of phytopreparates, extracts or dietary supplements, and there is growing interest for obtaining chokeberry extract. However, the use of extracts rich in polyphenols has a number of limitation, extract instability due to the effects of oxygen, light, moisture and other adverse factors. Among all chokeberry polyphenols, anthocyanins are the most sensitive. One of the methods for preservation the biologically active compounds from chokeberry extract, which can reduce their instability is microencapsulation technology. This method could preserve the stability of the active principles, could extend their shelf life, protect from the negative impact of the external environment, control release of polyphenols, cover the polyphenols bitter taste and prevent the negative effects of the gastrointestinal tract. Among many microencapsulation methods, electrostatic extrusion and spray drying are pointed out as a simple, precise and cost-effective methods for production of microparticles, with suitable organoleptic and biopharmaceutical characteristics, which can later be applied in the industry.

Among many beneficial effects on human health which chokeberry active principles are demonstrating, antihypertensive, antioxidative and hypolipemic are dominant. Some of these chokeberry extract effects on blood pressure lowering were examined for the first time through in vivo monitoring of systemic and regional hemodynamics and biochemical parameters in the model of essential hypertension.

The aim of this research in this doctoral dissertation were: optimization of the polyphenols extraction process from chokeberry dried fruit, microencapsulation of extract in order to improve the stability and bioavailability of polyphenols and examination of biological activity of extract in the model of experimental hypertension.

2. MATERIAL AND METHODS

Experimental work was carried out through three phases: 1) The first one, optimization of the extraction process was conducted and extract with the highest amount of polyphenols was obtained. Four different parameters were varied (5 particles sizes of dried chokeberry fruit, 4 different solvents, 3 solid-solvent ratios, 4 extraction times) and examined as independent variables. Optimal conditions were determined and based on the content of total phenols and total anthocyanins (by spectrophotometric method) and all of the results were based on experimental design. Quantitative analysis of individual polyphenolic compounds was performed using HPLC methods (high performance liquid chromatography) from selected extracts. In order to determine the effect of ultrasound on the extraction of polyphenolics and to make comparison with classical maceration method, it was examined the influence of ultrasonic extraction using the previously selected optimum solvent, solid-solvent ratio, particle size and extraction time. After that, the selected extract with the highest

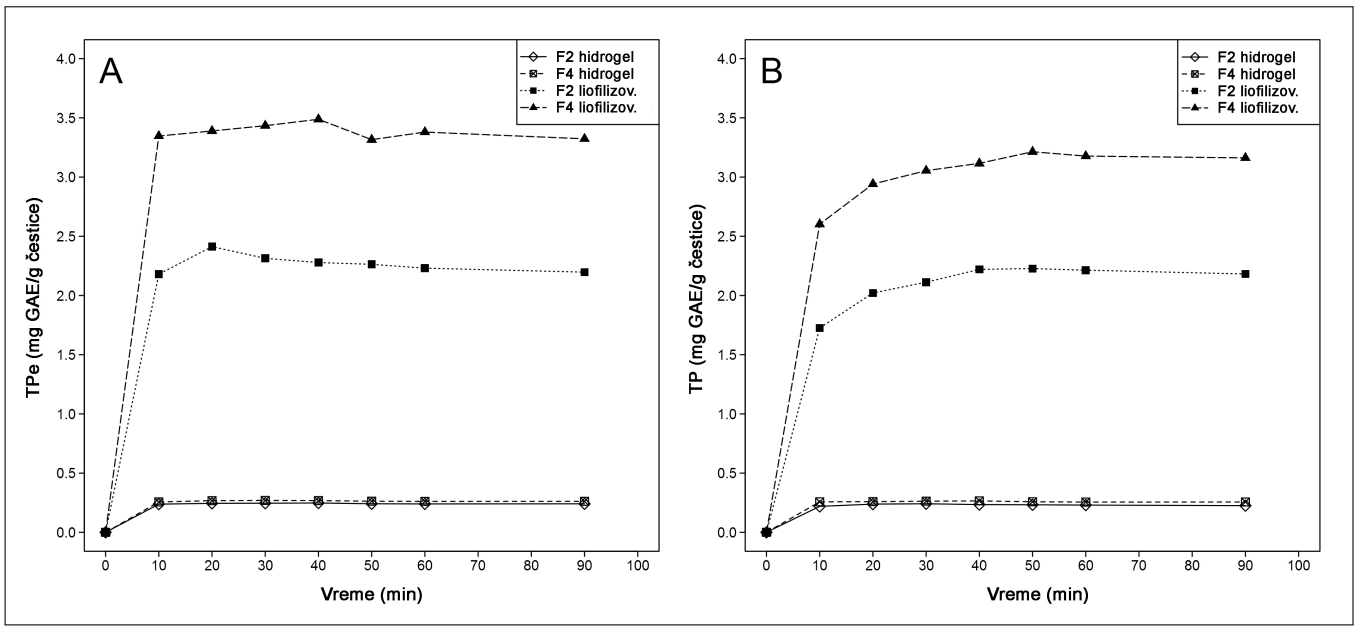

Fig. 3. Release of polyphenols from hydrogel and freeze dried particles in a) water b) in acidic environment

content of active principles was used in the following stages of the examination.

In the second part of examinations, the extract with the highest content of polyphenols obtained by optimizing the extraction process was encapsulated in suitable carriers by two microencapsulation methods, electrostatic extrusion and spray drying. During the microencapsulation by electrostatic extrusion were varied 4 factors which could affect on the encapsulation process (4 different types of carriers-low and medium viscosity alginates, with or without addition of inulin, as well as 3 different needle sizes-18, 20, 22). Obtained microparticles were physicochemical and biopharmaceutical characterized on encapsulation efficiency (spectrophotometric determination of total phenols in the particles disintegrated in sodium citrate) and in vitro assessment of efficiency by testing the release rate of encapsulated polyphenols in precisely defined time intervals (spectrophotometrically), FTIR analysis of microparticles (Fourier transmission infrared spectroscopy) and Scanning electron microscope analysis (SEM). The particle sizes of the hydrogel and lyophilized particles, both forms were determined using an optical microscope.

Extract obtained by optimization of extraction process was encapsulated also by spray drying method with different carriers (milk powder, maltodextrin, gum arabic) in order to compare this microencapsulation technique with the previously examined electrostatic extrusion method. Obtained microbeads were physicochemical and biopharmaceutical characterized: in vitro dissolution of total phenolics and anthocyanins in appropriate dissolution media (spectrophotometrically), FTIR analysis of microparticles (Fourier transmission infrared spectroscopy) and determining the microbeads size by Mastersizer.

In the third phase of the study, the extract with the highest content of polyphenols obtained by optimization of extraction process was lyophilized and used to test the antihypertensive effect on a model of essential hypertension in the spontaneously hypertensive rats (SHR). For the purpose of in vivo study male rats with congenital hypertension were used. The animals were distributed in two experimental groups, control and treated ones. In experimental groups were determined the systemic (systolic, diastolic, mean arterial pressure, heart rate, pulse pressure and cardiac output) and regional hemodynamic parameters (flow rates and resistances in the carotid and renal arteries and aorta). Biochemical parameters were also determined on the total cholesterol, HDL, LDL and triglycerides, glucose level, minerals, lipid peroxidation by TBARS method in plasma and erythrocytes. Activities of antioxidative enzymes in erythrocytes were also determined, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.

3. RESULTS

Extract with the greatest amount of active principles, total phenols (27.7 mg GAE/g dry weight) and anthocyanins (0.27%), was obtained by maceration method using 50% ethanol as a solvent, 1:20 solid-solvent ratio, 0.75 mm particle size, during 60 minutes. HPLC analysis confirmed that with the same, selected extraction conditions was achieved the highest yield of polyphenolics. Dominant anthocyanins in the extract were cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside and cyanidin-3-O-glucoside, respectively, while among flavonoids predominantly were presented rutin, hyperoside and isoquercetin, respectively. The use of ultrasound extraction method for the dried chokeberry fruit extraction did not show higher yields of extracted active principles. The results showed that maceration was effective and simple method for polyphenolics extraction from dried chokeberry.

The extract obtained by extraction optimization with the highest yield of polyphenols was used for both microencapsulation process, electrostatic extrusion and spray drying method. Microparticles obtained by electrostatic extrusion, using a medium viscosity alginate as a carrier (1.5%), with the addition of inulin as filler (5%) and using medium needle diameter (20) indicated the best encapsulation efficiency and amount of in vitro released polyphenols (Figure 1). Hydrogel particles obtained by electrostatic extrusion had regular shape, size ranged between 800 to 1340 μm (Figure 2). After lyophilization the particle size was reduced by 18-24%.

The hydrogel particles contained 0.24 mg GAE/g of the encapsulated polyphenols and achieved sustained release of 10 min, while freeze dried particles contained 3.57 mg GAE/g of the encapsulated polyphenols with prolonged release of 40 min (Figure 3). SEM micrographs confirmed that the particles obtained by electrostatic extrusion had uniform shape, without destructions, while the addition of inulin as filler contributed to the better performances of microbeads (Figure 4). FTIR

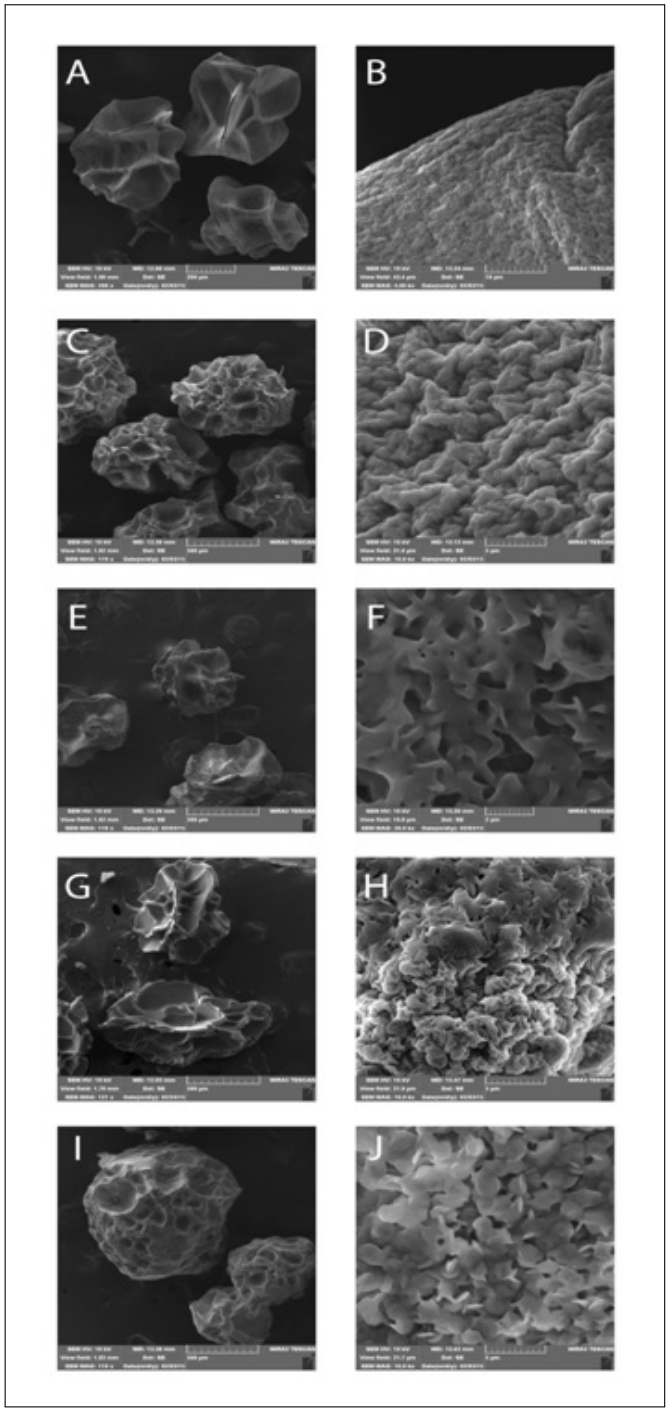

Fig. 4. SEM micrographs of (a) (b) blank alginate beads, 200× and 4990×; (c) (d) alginate beads encapsulating chokeberry extract, 119x and 10.000×; (e) (f) (g) blank alginate/inulin beads, 119×, 20.000× and 121×, respectively; (h) (i) (j) alginate/inulin beads encapsulating chokeberry extract, 10.000×, 119× and 10000×, respectively.

analysis showed a number of relevant peaks in the spectra of the systems which were encapsulated with extract, without visible incompatibility and matrix vs. extract interactions. Due to the extended release of polyphenols, microbeads obtained by electrostatic extrusion method have been demonstrated as efficient systems for the prolonged polyphenols delivery and maintenance their stability.

From microparticles obtained by another microencapsulation method, spray drying, the active principles released immediately. The greatest amount of active principles, total phenols (2.168 mg GAE/g) and total anthocyanins (0.04%) released from the gum arabic as a carrier. Sizes of the obtained mi-

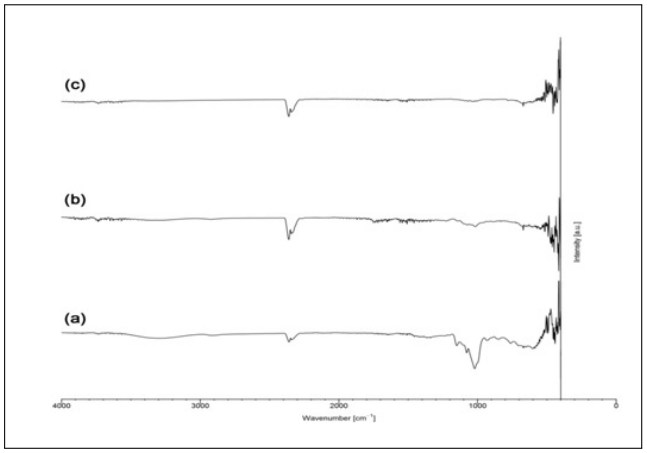

Fig. 5. FTIR spectra of chokeberry extract encapsulated by spray drying method in (a) maltodextrin; (b) arabic gum; (c) summed milk.

croparticles were in range between 8.5 to 15.87 μm which confirmed that spray drying is suitable technique for small and uniform particles production, which can be used for oral administration. FTIR analysis confirmed that the extract could be successfully incorporate into the microbeads by spray drying method (Figure 5). The obtained results showed that the encapsulation in a spiral jet air mill was also suitable technique for the production of microparticles.

In a third part of study in this dissertation, a four week term usage of the lyophilized extract in spontaneously hypertensive rats significantly reduced the systolic blood pressure (SHR-K: 205.1 ± 20.5 mm Hg for the control group, compared with the treated one, SHR-A: 184.5 ± 10.9 mm Hg) and pulse pressure ($p<0.05$). A significant reduction of systolic blood pressure was associated with increased diuresis. Chokeberry extract administration significantly decreased plasma and erythrocytes TBARS ($p<0.001$), increased lipid peroxidation which is consequence of increased oxidative stress. Plasma iron level was increased, which significantly increased the activity of plasma FRAP activity ($p<0.01$). Chokeberry extract significantly decreased SOD activity in the treated group compared to control one ($p<0.01$), with no changes in GPx and GR activities, while the CAT activity was increased for 33.27%.

CONCLUSION

The results of this doctoral thesis demonstrated that the optimized chokeberry extract obtained by maceration as extraction technique is suitable for production of extract, rich in natural antioxidants, with a maximum amount of active ingredients, which can be used as a mild antihypertensive agent in the early stages of disease or as a supplement to conventional antihypertensive therapy. Microencapsulation methods have been proven to be effective in preserving the stability of the extracted compounds. Both microencapsulation techniques have a number of advantages. The biological effects of chokeberry extract showed that its chronic use could have a positive impact on systolic blood pressure and oxidative status. All this results pointed to the potential application of chokeberry extract and its products in mild stage of hypertension, metabolic syndrome but also in prevention inhealthy subjects.