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A one step enhanced extraction and encapsulation system of cornelian cherry (*Cornus mas* L.) polyphenols and iridoids with β-cyclodextrin

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ABSTRACT

The objective of this study was simultaneous extraction and encapsulation of cornelian cherry active principles. As an encapsulating agent, β-cyclodextrin (β-CD) was used to enhance the ultrasound-assisted extraction of cornelian cherry polyphenols and iridoids. Lyophilized cornelian cherry fruit was extracted by four different solvents: pure water, 50% aqueous ethanol (conventional system), 1.5% β-CD water solution and 1.5% β-CD aqueous ethanol solution. The highest enhancement of the extraction efficiency was observed for flavonoids and anthocyanins, especially for cyaniding 3-galactoside and pelargonidin 3-galactoside. Water-ethanolic extract was used to form inclusion complexes between β-CD and cornelian cherry bioactives in the solid form. The encapsulation efficiency of cornelian cherry polyphenols in β-CD was 65.62%. Due to the polyphenol encapsulation within β-CD, the extract showed better solubility in water, higher antioxidant power (for 40.61%), and the release of anthocyanins from the dried powder was prolonged for 50% in the first 2 h making it suitable for diverse applications in food and pharmaceutical industries.

1. Introduction

Cornus mas L. (Cornaceae family) is a European and Asiatic shrub known as cornelian cherry whose fruits have been used in traditional medicine in various countries. Fruits from several Cornus spp. have been characterized as antibacterial, antihistamine, anti-allergic, antimicrobial, antimalarial, and antidiabetic agents, with potential to be used in treatment of some medical conditions such as gastrointestinal disorders and diarrhea, and to improve liver and kidney functions (Blagojević et al., 2021; Hosseinpour-Jaghdani, Shomali, Gholipour-Shahraki, Rahimi-Madiseh, & Rafieian-Kopaei, 2017; Popović, Štajner, Kevrešan, & Bijelić, 2012). Medicinal benefits from cornelian cherry fruits are attributed to the polyphenols and iridoids among which loganic acid and cornuside as well as anthocyanins of cyanidin, delphinidin and pelargonidin are the most dominant (Blagojević et al., 2021). Apart from health benefits, antioxidant properties of polyphenol and iridoid compounds potentiate the usage of cornelian cherry fruits in the food industry as a source of protective food agents or as anthocyanin-based natural dyes, as well (Blagojević et al., 2021; Blagojević, Četojević-Simin, Parisi, Lazzara, & Popović, 2020).

Conventional methods for the extraction of bioactives from plant material such as Soxhlet extraction and maceration have been intensively used despite certain disadvantages such as excessive consumption of time, energy and polluting organic solvents (Ameer, Shahbaz, & Kwon, 2017). The objective of "the green chemistry approach" is to obtain higher extraction efficiency with reduced extraction time, quantity of solvent, global energy consumption, economical costs and quantity of generated waste. Pure water is considered to be the greenest solvent because it is non-toxic, non-corrosive, non-flammable, environmentally friendly and widely available at low cost. The only disadvantage of water is its limitation to extract less-polar compounds. The extraction by water can be enhanced using microwave irradiation (MW) or ultrasound (ultrasound assisted extraction UAE). Also, the ability of water to extract a wide range of compounds can be enhanced by using different co-solvents, such as ethanol and glycerol as well as "green additives" like sugars (El Kantar et al., 2019). According to Albahari

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et al. (2018), β-cyclodextrin (β-CD) can be used as a solubility enhancer of polyphenols in water.

The extraction method combined with the encapsulation of polyphenols using β-CD was applied in extraction of resveratrol and polydatin from the roots of P. cuspidatum (Mantegna et al., 2012). Cyclodextrins are cyclic oligosaccharides capable of molecular encapsulation and controlled release by reversible binding of hydrophobic molecules in their hydrophobic pockets (Reineccius, Reineccius, & Peppard, 2003). Bioactives encapsulated into cyclodextrins can be incorporated as additives for their controlled release of bioactive components (Plackett, Ghanbari-Siahkali, & Szente, 2007), prolonging food shelf-life by inhibition of the oxidation (Kagami et al., 2003) and enhancement of nutraceutical delivery into the cancer cells (Yallapu, Jaggi, & Chauhan, 2010). Inclusion complexes of plant-derived bioactive agents with cyclodextrins protects them from thermal oxidation and other degradation factors, such as pH or UV-light (Kalogeropoulos, Yannakopoulou, Gioxari, Chiou, & Makris, 2010; Pinho, Grootveld, Soares, & Henriques, 2014). Because of this, encapsulation of cornelian cherry bioactives with β-cyclodextrin is possible strategy for their prolonged storage stability.

This aim of this study was to perform the one-step extraction and encapsulation of cornelian cherry polyphenols and iridoids in β-cyclodextrin. This was done using β-CD (1.5% w/w) in water and aqueous ethanol solution. The extraction of individual polyphenols and iridoids were compared with the extraction by pure water as a control and 50% aqueous ethanol as a conventional system. Furthermore, the controlled release of the main cornelian cherry constituents (loganic acid, cornuside, cyanidin 3-galactoside, and pelargonidin 3-galactoside) was measured. The cytotoxic profile of β-cyclodextrin, cornelian cherry ethanolic extract and β-cyclodextrin encapsulate was evaluated using *in vitro* cell based assays in MCF7, HT-29 and MRC-5 cell lines.

2. Materials and methods

2.1. Fruit material, extraction and encapsulation procedure

For the experiment, cornelian cherry (*Cornus mas* L.) fruits were picked at the stage of full maturity. Fruits were from Svetlyachok cultivar grown in the orchard at the experimental field of the Faculty of Agriculture, the University of Novi Sad at Rimski Šančevi. For the freeze-drying process, fresh, depitted-fruits were frozen at −70 °C (deep freezer VF360-86, Snijders Labs, Tilburg, Holand) for 4 h. After the main drying process (performed at p = 0.01 mbar and temperatures from −40 °C to 20 °C for 59.5 h), the final drying lasted 5.5 h at p = 0.005 mbar and temperatures from 20 °C to 30 °C. Freeze drying was performed in Martin Crist Alpha 1–2 LD plus lyophilizator (Osterode, Germany).

The extraction of cornelian cherry bioactives in β-CD were performed according to (Mantegna et al., 2012).

Four different extracts were prepared from lyophilized fruit (LF). The solvent/plant ratio was 50 mL/g. Solvents used for the extraction were: a) water, b) conventional (50% ethanol in water) extract, c) β-CD/water (1.5% w/w) d) β-CD/50% ethanolic (1.5% w/w). All four extracts were prepared by ultrasound-assisted extraction with following experimental conditions: 20 min at 30 °C under 550 W (Elmasonic S 100 H, Elma Schmidbauer, GmbH, Germany). The suspensions were centrifuged at 3500 rpm for obtaining clear supernatant. Water and 50% ethanol solutions were directly used for further analysis while β-CD extract in 50% ethanol was concentrated under vacuum to 15 mL and then lyophilized to dryness by above described procedure to obtain encapsulate in powdered form.

β-CD powder encapsulate of 50% EtOH/water extract was used for thermogravimetric analysis (TGA), scanning electron microscopy (SEM), encapsulation efficiency determination, controlled release measurement and cell assays. For spectrophotometric and HPLC determination powder encapsulate was dissolved in a solvent containing ethanol:water:acetic acid (50:42:8) in ultrasound bath (20 min, at 30 °C under 550 W) to dissolve all encapsulated polyphenols and to precipitate β-CD. After precipitation of β-CD, the supernatant was used for further analysis of the extracts and comparison with water and 50% ethanolic extracts.

2.2. Characterization of cornelian cherry encapsulate by thermogravimetric analysis (TGA) analysis and scanning electron microscopy (SEM)

TGA experiments were done using the Q5000 IR (TA Instruments, Milan, Italy) under nitrogen flow (25 $cm^3\ min^{-1}$) by heating the samples from room temperature to 800 °C. The sample (each ca. 8 mg) was placed in a platinum pan and heated with a ramp β = 20 °C·min^{-1}. Composition of the encapsulated composite was calculated according to the procedure reported in the literature for CD based composites and other materials (Meo, Lazzara, Liotta, Riela, & Noto, 2014).

The SEM images were obtained using a JSM 6460 LV scanning electron microscope (JEOL, Tokyo, Japan) at custom magnification.

2.3. Encapsulation efficiency

Encapsulation efficiency (EE) presents the ratio of encapsulated phenolic content (core phenolic content-CPC) and total phenol content (TPC), where CPC presents the difference between total phenol content and surface phenolic content (SPC) (Robert et al., 2010). For the determination of TPC, 100 mg of sample was treated with 1 mL methanol:acetic acid:water (50:8:42). After vortexing for 1 min, the suspension was vortexed and ultrasonicated twice for 20 min (20 min, at 30 °C under 550 W). TPC was determined in the supernatant after centrifugation at 9500×*g* for 5 min (Boeco U-320 R, Hmburg, Germany). For the surface phenolic content (SPC) the same amount of sample was suspended in 1 mL of ethanol and methanol (1:1) mixture. After vortexing for 1 min, and centrifugation for 5 min, the supernatant was separated. The TPC and SPC were determined by Folin–Ciocalteu method and results were expressed as mg of gallic acid equivalents per 100 g of encapsulate (mg GAE/100 g enc.). The encapsulating efficiency was calculated according to Eq. (1):

$$EE(\%) = \frac{(TPC - SPC)}{TPC} \times 100(\%) \quad (1)$$

2.4. Spectrophotometric determinations

Total phenolic content. TPC of cornelian cherry was determined according to Folin & Ciocalteu assay (Ainsworth & Gillespie, 2007). An aliquot of fruit extract (250 μl) was mixed with 4.0 mL distilled water and 250 μl of prepared Folin & Ciocalteau reagent. In order to produce basic conditions, an aliquot of saturated Na_2CO_3 solution (500 μl) was added. The mixture was diluted to 10 mL with distilled water. The absorbance was read at 760 nm. The same procedure was applied for six standard solutions of gallic acid (0–600 mg/100 mL). Final results were expressed as mg gallic acid equivalents per 1 g lyophilized fruit (LF).

Total tannin content. TTC was determined by Folin-Ciocalteu procedure as above, after removal of tannins by their adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Insoluble, polyvinylpyrrolidone (PVPP) was weighed into test tubes and 1.0 mL of fruit extracts were added. After 15 min at 4 °C, the tubes were vortexed and centrifuged for 10 min at 5000 g. Aliquots of supernatant (200 μl) were transferred into test tubes and nonabsorbed phenolics determined as described in previous section. Calculated values were subtracted from total phenolic contents and total tannin contents expressed as mg gallic acid equivalents per 1 g lyophilized fruit (LF).

The total flavonoid content. TFC was estimated by the method of (Quettier-Deleu et al., 2000). The reaction mixture contained 0.5 mL $AlCl_3$ in methanol (20 mg mL^{-1}) and 0.5 mL of extracts solutions in methanol (1 mg mL^{-1}). The absorbance was measured at 415 nm after 1

h of incubation at room temperature. Results were expressed as milligrams of quercetin equivalents per gram of lyophilized fruit.

Total anthocyanin content. TAC of cornelian cherry was determined by pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol L^{-1}) and sodium acetate buffer, pH 4.5 (0.4 M) (Lako et al., 2007). Briefly, 0.4 mL of fruit sample was mixed with 3.6 mL of corresponding buffers and read against water as a blank at 510 and 700 nm. Total anthocyanin content of samples was expressed as mg cyanidin-3-glucoside equivalents per 1 g lyophilized fruit (LF).

FRAP. Total antioxidant capacity was estimated according to the FRAP (Ferric Reducing Antioxidant Power) assay (Benzie & Strain, 1996), (Štajner, Popović, Ćalić-Dragosavac, Malenčić, & Zdravković-Korać, 2011). FRAP reagent was prepared by mixing: acetate buffer (300 mM pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) reagent (10 mmol L^{-1} in 40 mmol L^{-1} HCl) and $FeCl_3 \cdot 6H_2O$ (20 mmol L^{-1}) in ratio 3:1:1. Sample (100 μl) was mixed with 3 mL of working FRAP reagent and absorbance (593 nm) was measured at 4 min after vortexing. FRAP value was calculated by comparison with ascorbic acid as standard and expressed by ascorbic acid equivalents per 1 g lyophilized fruit (LF).

2.5. HPLC analysis of polyphenol and iridoid profile

HPLC analysis was performed by using a liquid chromatograph (Shimadzu Nexera X2), equipped with a photodiode array detector (PDA), LabSolution Software (Shimadzu, Tokyo, Japan), on a Luna C18 (2), 3 μm, 2 × 150 mm column, with a C18 guard column, 2 × 4 mm (both from Phenomenex, Torrance, CA, USA), at a flow rate of 0.25 mL min^{-1}. There was followed a procedure for determination of cornelian cherry bioactives described by (Blagojević et al., 2021). The solvent gradient was performed by changing the proportion of solvent A (1% formic acid in water (v/v)) to solvent B (1% formic acid in methanol (v/v)) as follows: initial 5% B; 10 min, 20% B; 13 min, 20% B; 30 min, 25% B; 35 min, 30% B; 45 min, 70% B; 55 min, 70% B. The total running time and post-running time were 60 and 10 min, respectively. The column temperature was 40 °C. The injected volume of samples and standards was 3 μL, and it was done automatically using an autosampler. The spectra were acquired in the range 200–650 nm with a bandwidth of 4 nm, and with reference wavelength/bandwidth of 620/20 nm. Chromatograms were plotted at 280 (for hydroxybenzoic acids), 320 (hydroxycinnamic acids), 350 (flavonols), 520 nm (anthocyanins) and 245 (iridoids).

2.6. Controlled release measurement

Controlled release measurement was conducted according to Massaro et al. (2018) with modifications. For the controlled release study, 0.5 g of encapsulate was placed in molecularporous membrane, with 6–8 kD molecular weight cut off (MWCO; Spectra/Por, Spectrum Laboratories, Inc., CA, USA). At both ends, the membrane was closed with weighted closures and covered with 25 mL of distilled water pH 7. The system was stirred at 60 rpm and 37 °C. Each hour, 200 μ-L of release medium was taken out for HPLC analysis and replaced with the same volume of water to maintain the volume.

2.7. In vitro cell based assays

Human cell lines MCF7 (breast adenocarcinoma; ECACC No. 86012803), HT-29 (colon adenocarcinoma; ECACC No. 91072201) and MRC-5 (fetal lung fibroblasts; ECACC No. 84101801) were grown in Dulbecco's modified Eagle's medium (DMEM; PAA Laboratories GmbH, Pashing, Austria) with 4.5 g 100 mL^{-1} glucose, supplemented with 10 g 100 mL^{-1} heat-inactivated fetal calf serum (FCS; PAA Laboratories GmbH, Pashing, Austria), 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (Galenika, Belgrade, Serbia). They were cultured in 25 cm^2 flasks (Corning, New York, USA) at 37 °C in an atmosphere of 5% CO_2, high humidity and subcultured twice a week. Single-cell suspension was obtained using 100 mg 100 mL^{-1} trypsin (Serva, UK) with 40 mg 100 mL^{-1} EDTA.

Cytotoxicity assay was performed for pure β-CD, dry fruit extract obtained by conventional extraction (50% ethanol in water), and β-CD encapsulate obtained after lyophilization of β-CD extract (in water and 50% ethanol). Samples used in cell based were dissolved and diluted in DMSO to obtain the required final concentrations from 0.03125 to 2.5 mg mL^{-1}, whereas the final concentration of DMSO in the samples was ≤0.05% (v/v).

Cell lines were harvested and plated into 96-well microtiter plates (Sarstedt, Newton, NC, USA) at seeding density of 4–8 x 10^3 cells per well, in a volume of 199 μ-L, and preincubated in complete medium supplemented with 5 g 100 mL^{-1} FCS, at 37 °C for 24 h. Serial dilutions of samples or solvent (1 μL per well) were added to the test and control wells, respectively. Microplates were then incubated at 37 °C for an additional 48 h.

Sulforhodamine B (SRB) assay. Cell growth was evaluated by the colorimetric SRB assay according to (Skehan et al., 1990). Cells were fixed with 50% TCA (1 h, + 4 °C), washed with distilled water (Wellwash 4; Labsystems; Helsinki, Finland) and stained with 0.4% SRB (30 min, room temperature). The plates were then washed with 1% acetic acid to remove the unbound dye. Protein-bound dye was extracted with 10 mM Tris base. Absorbance was measured on a microplate reader (Multiscan Ascent, Labsystems, New Jersey, USA) at 540 / 620 nm. Cytotoxic activity, based on the effect on cell growth, was expressed as a percent of cytotoxicity and calculated as % Cytotoxicity = 1−(At/Ac)· 100 [%], where At is the absorbance of the test sample and Ac is the absorbance of the control.

2.8. Statistical analysis

Results were expressed as the mean ± standard error. All determinations were done in triplicate. Statistical comparisons between samples were performed with Duncan's multiple range tests for independent observations using STATISTICA 13.3. Differences were considered significant at $P < 0.01$.

3. Results and discussion

3.1. Polyphenol parameters and antioxidant activity in different extracts of cornelian cherry

Polyphenol parameters and antioxidant activity in different cornelian cherry extracts are presented in Table 1. Water extracts were regarded as a control (100%). Contents of polyphenols (extraction yields) obtained by other extraction systems were expressed compared with the control value, as a percent of decrease (−) or increase (+) of the control.

The lowest levels of total phenolic content, total flavonoids, total anthocyanins, total tannins and FRAP were detected in water extracts, followed by CD-water extracts, CD-EtOH/H_2O extracts and the highest level of all these parameters were obtained by 50% ethanolic water. When comparing extraction with pure water and CD-water extracts, cyclodextrin significantly enhanced extraction and increased values of all parameters except tannins. The extraction efficiency of EtOH/H_2O and CD-EtOH/H_2O was not significantly different except in the case of tannins, for which 50% ethanolic water gave significantly higher extraction yield.

An observed impact on the improved extractability of cornelian cherry polyphenols is due to inclusion complex formation. Many reports have been published regarding the encapsulation of polyphenolic compounds by CDs, for food and drug delivery proposes (Albahari et al., 2018; Kalogeropoulos et al., 2010; Lu, Cheng, Hu, Zhang, & Zou, 2009; Pinho et al., 2014). The process of inclusion of the 'guest' into the CD is a substitution of water molecules from the central cavity, by the lipophilic

Table 1
Polyphenol parameters and ferric reducing antioxidant capacity (FRAP) in different extracts of cornelian cherry.

Compound	Calculated as	H_2O	% (con-trol)	EtOH/H_2O	(±) % control	β-CD-H_2O	(±) % control	β-CD-EtOH/H_2O	(+/−-) % control
Total phenolics	mg GAE/g LF	8.72 ± 0.10^{c}	100	19.29 ± 0.27^{a}	+121.10	10.73 ± 0.35^{b}	+23.03	18.62 ± 0.39^{a}	+113.37
Total flavonoids	mg QE/g LF	0.10 ± 0.03^{d}	100	0.47 ± 0.11^{a}	+355.56	0.38 ± 0.03^{c}	+261.11	0.43 ± 0.15^{a}	+311.11
Total anthocyanins	mg Cy3gluE/g LF	1.02 ± 0.05^{c}	100	2.28 ± 0.13^{a}	+122.83	2.03 ± 0.07^{b}	+98.10	2.22 ± 0.01^{a}	+117.12
Total tannins	mg GAE/g LF	5.28 ± 0.43^{c}	100	16.37 ± 0.56^{a}	+210.32	6.35 ± 0.40^{c}	+20.39	14.31 ± 0.56^{b}	+171.25
FRAP	mg AAE/g LF	7.82 ± 0.23^{c}	100	18.52 ± 0.19^{a}	+136.92	10.99 ± 0.50^{b}	+40.61	18.26 ± 0.70^{a}	+133.55

*Different letters indicate statistically significant differences at ($P < 0.01$) according to Duncan test.
*Abbreviations for different parameters are: β-CD – β-cyclodextrin; LF – liophylized fruit; GAE – gallic acid equivalents; QE – quercetin equivalents; Cy3gluE – cyanidin 3-glucoside equivalents; AAE – ascorbic acid equivalents.

molecules with noncovalent bonds (Pinho et al., 2014; Sengupta, Bhattacharjee, Chakraborty, & Bhowmik, 2018). Formation of CD inclusion complexes contributes to the increased solubility of cornelian cherry polyphenols in water but also increases their chemical stability during extraction process by ultrasound which may trigger their degradation and/or oxidation (Albahari et al., 2018). According to Pinho et al. (2014) β-CD improve solubility numerous polyphenols in water including phenolic acids, flavonoids and anthocyanins as well as antioxidant capacity of the extract.

Extraction yields of different phenolic acids, flavonoids, ellagic acid, anthocyanins and iridoids in cornelian cherry obtained by different extraction systems are presented in Table 2. Extraction yields obtained by water were considered as a control (100%) and yields obtained by other extraction systems were expressed compared with the control value (±%).

The most abundant phenolic acid in cornelian cherry water extract is gallic acid (8.16 mg/100 g LF), followed by chlorogenic acid (4.76 mg/100 g LF). Ethanolic water (conventional system) was more efficient than water for the extraction of all phenolic acids except gallic acid (−20.76). The presence of cyclodextrin enhanced the extraction of all phenol acids except gallic acid (−50.52%) and coumaric acid derivative (3), (−15.21%). The highest increase of the extraction yield was detected for the caffeic acid derivative (+33.83%) which is extracted better compared to 50% ethanolic water as an extractant. The addition of cyclodextrin in 50% EtOH/H_2O suppressed the extraction of gallic acid and coumaric acid derivative (3) and increased the extraction yield of caffeic acid derivative comparing with pure ethanolic water.

The highest extraction yield among all investigated compounds in water extract was achieved for the iridoid compound – loganic acid (636.81 mg/100 g LF) followed by anthocyanin cyanidin 3-galactoside (111.55 mg/100 g LF). Anthocyanin pelargonidin 3-galactoside and iridoid cornuside had smaller yields, 45.90 and 46.32 mg/100 g LF respectively. The smallest extraction yield was achieved for ellagic acid (7.29 mg/100 g LF) and flavonoid kaempferol 3-galactoside (2.83 mg/100 g LF). The addition of cyclodextrin significantly increased the extraction of kaempferol 3-galactoside (+54.20) elevating it to the level achieved with 50% EtOH/H_2O. β-CD assisted water extraction also increased the level of cyanidin 3-galactoside (+93.19), pelargonidin 3-galactoside (+86.00) and cornuside (+53.43). However, for anthocyanins cyanidin and pelargonidin derivatives, 50% EtOH/H_2O was a more efficient extractant (for +140.32 and + 145.56 compared to water extraction). Ethanolic water (conventional solvent) was also the most

Table 2
Extraction yields of polyphenols and iridoids in cornelian cherry obtained by different extractants.

N^{O}	Compound	Calculated as	tR (min)	λ_{max} (nm)	H_2O (mg/100 g LF)	% (con-trol)	EtOH/H_2O (mg/100 g LF)	(±) % control	β-CD-H_2O (mg/100 g LF)	(±) % control	β-CD-EtOH/H_2O (mg/100 g LF)	(±) % control
1	Gallic acid	standard	4.36	270	8.16 ± 0.08^{a}	100	6.47 ± 0.26^{b}	−20.76	4.04 ± 0.16^{c}	−50.52	3.37 ± 0.12^{d}	−58.70
2	Caffeic acid derivate	CA	10.07	327	1.82 ± 0.07^{c}	100	2.15 ± 0.02^{b}	+17.77	2.44 ± 0.02^{a}	+33.83	2.47 ± 0.09^{a}	+35.64
3	Coumaric acid derivate	*p*-CoA	10.33	311	1.36 ± 0.05^{b}	100	1.62 ± 0.02^{a}	+19.56	1.15 ± 0.04^{c}	−15.21	1.09 ± 0.04^{c}	−19.99
4	Coumaric acid derivate	*p*-CoA	12.97	313	3.75 ± 0.04^{b}	100	4.74 ± 0.17^{a}	+26.46	4.53 ± 0.16^{a}	+20.84	4.54 ± 0.04^{a}	+21.07
5	Chlorogenic acid	standard	13.37	327	4.76 ± 0.19^{a}	100	4.91 ± 0.18^{a}	+3.19	4.95 ± 0.05^{a}	+4.06	4.83 ± 0.17^{a}	+1.56
6	Coumaric acid derivate	*p*-CoA	16.04	309	0.97 ± 0.01^{b}	100	1.22 ± 0.05^{a}	+25.01	1.12 ± 0.04^{a}	+15.46	0.96 ± 0.03^{b}	−1.89
7	Coumaric acid derivate	*p*-CoA	17.51	313	2.65 ± 0.11^{b}	100	2.95 ± 0.03^{a}	+11.13	3.00 ± 0.03^{a}	+13.24	$2.75 \pm 0,03^{b}$	+3.56
8	Kaempferol 3-galactoside	Q3glu	41.00	349	2.83 ± 0.03^{b}	100	4.43 ± 0.04^{a}	+56.51	4.36 ± 0.17^{a}	+54.20	4.39 ± 0.04^{a}	+55.31
9	Ellagic acid	standard	37.97	254	7.29 ± 0.26^{c}	100	10.67 ± 0.38^{a}	+46.42	7.19 ± 0.07^{c}	−1.35	8.82 ± 0.09^{b}	+20.98
10	Cyanidin 3-galactoside	Cy3glu	15.70	517	111.55 ± 1.09^{c}	100	268.09 ± 9.67^{a}	+140.32	215.51 ± 8.62^{b}	+93.19	229.29 ± 9.17^{b}	+105.54
11	Pelargonidin 3-galactoside	Pg3glu	18.30	505	45.90 ± 1.84^{d}	100	112.71 ± 1.10^{a}	+145.56	85.37 ± 3.08^{c}	+86.00	93.56 ± 0.91^{b}	+103.83
12	Loganic acid	standard	13.40	245	636.81 ± 22.96^{a}	100	656.21 ± 26.25^{a}	+3.05	677.35 ± 6.57^{a}	+6.37	662.54 ± 23.89^{a}	+4.04
13	Cornuside	standard	41.88	245	46.32 ± 0.45^{b}	100	70.90 ± 0.69^{a}	+53.05	71.07 ± 2.84^{a}	+53.43	72.11 ± 2.60^{a}	+55.66

*Different letters indicate statistically significant differences at ($P < 0.01$) according to Duncan test.
*Abbreviations for different parameters are: β-CD – β-cyclodextrin; LF – lyophilized fruit; CA – caffeic acid; *p*-CoA – *p*-coumaric acid, Q3glu – quercetin 3-glucoside; Cy3glu – cyanidin 3-glucoside; Pg3glu – pelargonidin 3-glucoside.

efficient extractant for ellagic acid (+46.42 compared to water extraction). The addition of cyclodextrin in 50% EtOH/H_2O affected just ellagic acid and anthocyanins which extraction was suppressed in a certain extent compared to water extraction (Table 2).

The extraction of gallic acid and coumaric acid derivative (3) was negatively affected by CD in water, while ellagic acid, chlorogenic acid and loganic acid were not significantly affected. Taking into account the polarity of these compounds and their solubility in water, this result is expected. The increased extractability of flavonoid kaempferol 3-galactoside, anthocyanins cyanidin 3-galactoside and pelargonidin 3-galactoside, as well as cornuside, is due to their lower polarity and solubility in water, contrary to phenolic acids (Mantegna et al., 2012; Müller, Bednář, Barták, Lemr, & Ševčík, 2005; Rothwell, Day, & Morgan, 2005).

Kaempferol is a flavonol with great interest in the pharmaceutical field due to its huge bioactive potential. Its antioxidant potential in the aqueous environment had been improved by complexation with CDs as encapsulating agents (Kim, Choi, & Jung, 2009). Encapsulation of other polyphenols including phenolic acids and anthocyanins is also a good choice to protect them from degradation by UV-light, pH, temperature and oxidation. Caffeic and chlorogenic acids and their derivatives are known as antibacterial and antioxidant phenolic acids, but their sensibility to oxidation and lower solubility are limiting factors, thus, some authors have described its encapsulation with β-CD to overcome these issues (Górnas, Neunert, Baczyński, & Polewski, 2009). The results obtained by Mourtzinos et al. (2008) indicated that the presence of β-CD can improve the thermal stability of hibiscus extract rich with two anthocyanins based on cyanidin and delphinidin, both in solution and in solid-state. That research showed that anthocyanins form inclusion complexes with β-CD by NMR and differential scanning calorimetry (Mourtzinos et al., 2008). The conventional method for polyphenol extraction based on ethanol and water showed the best yields for most of polyphenol and iridoid compounds (Popović et al., 2020). However, the addition of cyclodextrin slightly suppressed the extraction of major constituents, probably consuming the solvent molecules for its solvation.

3.2. Cornelian cherry encapsulate in β-cyclodextrin – encapsulation efficiency and thermal properties

The freeze-dried encapsulates of cornelian cherry water/ethanol extract in β-CD were hygroscopic and developed a sticky and rubber-like appearance shortly after the exposure to the atmosphere. Extracts containing cyclodextrins were in the form of micro-flakes glued together with nonencapsulated surface cornelian cherry extract. The SEM images, presented in Fig. 1, showed pure β-CD powder (a) and cyclodextrin encapsulate (b). Encapsulation efficiency, measured spectrophotometrically, was 65.62 ± 1.31%.

Fig. 2 shows the degradation curves of cornelian cherry water/ethanol extract, β-CD and cornelian cherry/β-CD encapsulates normalized for mass at 100 °C to avoid the contribute of adsorbed water. A single degradation step is observed for β-CD. The degradation starts at ca. 320 °C and it has the maximum rate at 340 °C (Fig. 2). The degradation of cornelian cherry dry extract is more complex, namely a multi step degradation pattern occurs at ca. 157, 219 and 324 °C. The most relevant mass loss is at 219 °C. The cornelian cherry-extract weight ratio in the encapsulate sample can be calculated by comparing the mass losses at 350 °C being the CD degradation negligible at this temperature and using the rule of mixtures that provides 51%. Furthermore, to the cornelian cherry/β-CD encapsulates showed the characteristic degradations of both components. In addition, there is a shift in the degradation step from 219 °C (free cornelian cherry) to 225 °C (cornelian cherry/β-CD).

It is known that the formation of inclusion complexes with CDs can enhance the thermal stability due to the interaction with the CD cavity (Abarca, Rodríguez, Guarda, Galotto, & Bruna, 2016; Lazzara & Milioto, 2008). The enhancement of the cornelian cherry polyphenol and iridoid extraction performances could be explained by the formation of inclusion complexes with the CD.

3.3. Release profiles of polyphenols from cornelian cherry encapsulate in β-cyclodextrin

The release profiles of major constituents of cornelian cherry – anthocyanins (cyanidin 3-galactoside and pelargonidin 3-galactoside) and iridoids (loganic acid and cornuside) in distilled water (pH 7) are presented in Fig. 3.

The current analysis evaluated the release mechanism in water solution, the most common solvent in food industries. The β-CD microcapsules demonstrated the gradual release of active components over 10 h. As shown in Fig. 3., loganic acids and cyanidin 3-galactoside had higher release than cornuside and pelargonidin 3-galactoside. This behavior is explained by the chemical uniqueness of the mentioned compounds, where loganic acid is the most polar compound with the highest water solubility.

During the first 2 h, the highest release (71.56%) was observed for more polar loganic acid, followed by cornuside (54.72%), cyanidin 3-galactoside (53.64%) and pelargonidin 3-galactoside (48.70%). After 5 h, loganic acid is released for 87.51%, cornuside 81.17%, Cy 3-gal for 81.35% and Pg 3-gal for 74.71%. *In vitro* dissolution/release test demonstrated that β-CD can retain polyphenols, especially during the first 2 h of release and can be attributed to their slow dissolution rate (Lauro et al., 2015).

3.4. Cytotoxic profile of cornelian cherry encapsulate in β-cyclodextrin

Cytotoxic profile of β-cyclodextrin, cornelian cherry water/ethanol extract and β-cyclodextrin encapsulate of 50% EtOH/water extract on MRC-5, MCF7 and HT-29 cell lines were presented in Fig. 4.

Cytotoxic profiles of β-cyclodextrin, cornelian cherry extract and β-cyclodextrin encapsulate were evaluated in 312.5–2500 μg mL^{-1}

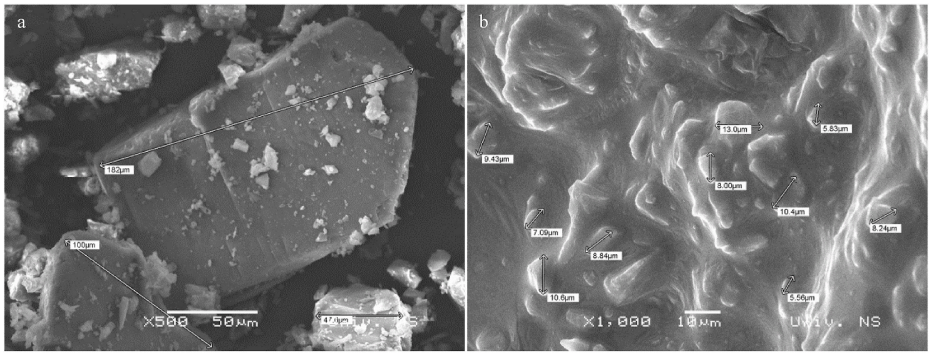

Fig. 1. SEM micrograph of a) β-cyclodextrin and b) cornelian cherry β-cyclodextrin encapsulate (encapsulated 50% EtOH/water extract).

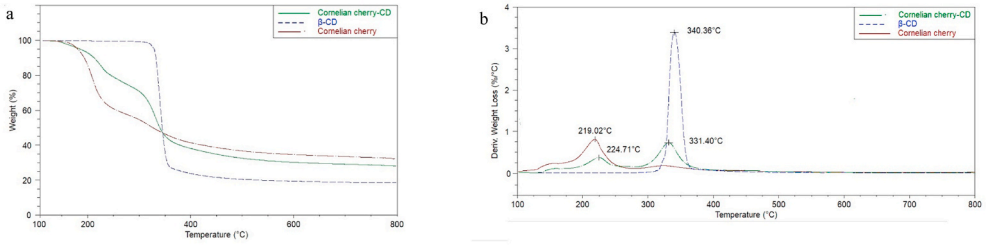

Fig. 2. a) Thermogravimetric (TG) curves of cornelian cherry (red), β-CD (blue) and cornelian cherry/β-CD encapsulates (encapsulated 50% EtOH/water extract); b) First derivative of the TG curves of cornelian cherry (red), pristine β-CD (blue) and cornelian cherry/β-CD encapsulates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

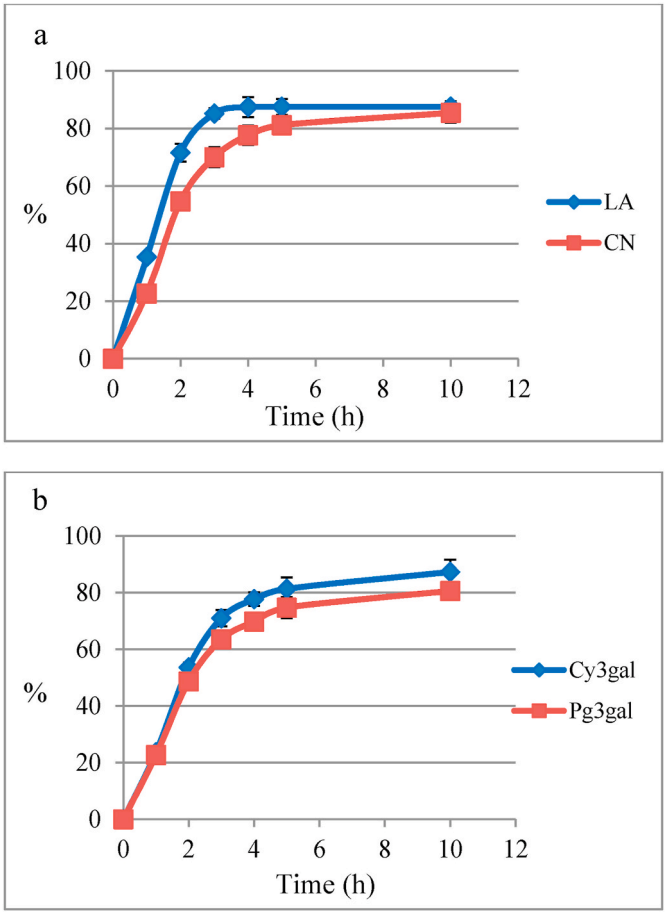

Fig. 3. Controlled release graphs of major a) iridoids and b) anthocyanins from β-cyclodextrin encapsulate (encapsulated 50% EtOH/water extract) during 10h Abbreviations: LA (loganic acid); CN (cornuside); Cy3gal (cyanidin 3-galactoside); Pg3gal (pelargonidin 3-galactoside).

concentration range. At lowest evaluated concentrations cytotoxicity was mild for all samples, with values under 10% - indicating low bioactivity (Fig. 4a–c). IC_{50} value was achieved only for fruit extract in MCF7 cell line (Fig. 4b), but at high concentration ($IC_{50}^{MCF7} = 1604$ μg mL^{-1}). β-Cyclodextrin induced stimulation of cell growth of estrogen receptor positive MCF7 cell line. β-Cyclodextrin encapsulate induced uniform cytotoxic response in all cell lines and low dose-dependent increase of cytotoxic activity in evaluated concentration range (Fig. 4a–c).

Cyclodextrin cytotoxicity is explained by the hypothesis of the destruction of membranes by the removal of basic membrane components (Leroy-Lechat, Wouessidjewe, Andreux, Puisieux, & Duchêne, 1994). Šavikin et al. (2009) also showed that methanol extract of cornelian cherry possesses cytotoxicity against certain cell lines. Natural CDs are more resistant towards nonenzymatic hydrolysis than the linear oligosaccharides and there are not hydrolyzed by human salivary and pancreatic amylases (Leroy-Lechat et al., 1994). Therefore, CD-drug conjugates remain intact until they reach the colon (Shahiwala, 2020). Many polyphenols and other natural bioactives have poor solubility and bioavailability (Pinho et al., 2014). Therefore, cyclodextrin conjugation with these molecules could be successfully utilized to enhance their bioavailability.

4. Conclusions

Cyclodextrin-enhanced ultrasound-assisted extraction was found to be a useful and sustainable approach for obtaining cornelian cherry extract rich in bioactive polyphenolic compounds and simultaneous encapsulation of its constituents in β-cyclodextrin. The β-CD-assisted extraction significantly enhanced the extraction of hydroxycinnamic acids, flavonoids, anthocyanins and iridoids from water. However, the extraction efficiency of cornelian cherry polyphenols and iridoids did not exceed the efficiency of the conventional method by ethanolic water. The encapsulation efficiency of cornelian cherry active principles in β-CD was found to be 65.62%. Liquid β-CD extract could be either used as raw material for the development of pharmaceutical preparations or could be converted into a solid form. It was found that the release of anthocyanins from solidified encapsulate retained the release of anthocyanins for about 50% in the first 2 h β-cyclodextrin encapsulate

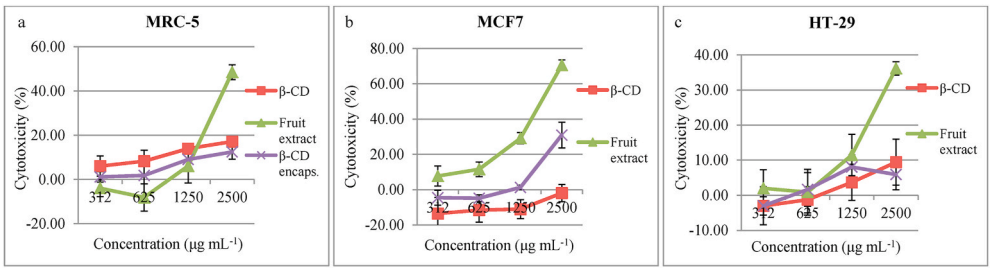

Fig. 4. Cytotoxic profiles of β-cyclodextrin, cornelian cherry extract and β-cyclodextrin encapsulate (encapsulated 50% EtOH/water extract) in a) MRC-5, b) MCF7 and c) HT-29 cell lines.

induced uniform cytotoxic response and low dose-dependent increase of cytotoxic activity in evaluated concentration range. Therefore it could be starting material for the development of added value food products offering higher stability and enhanced antioxidant activity.

CRediT authorship contribution statement

Boris M. Popović: Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision. **Bojana Blagojević:** Formal analysis, Investigation, Data curation. **Dragana Latković:** Resources, Funding acquisition. **Dragana Četojević-Simin:** Formal analysis, Investigation, Validation, Resources, Writing - review & editing. **Alicja Z. Kucharska:** Investigation, Validation. **Filippo Parisi:** Investigation, Data curation. **Giuseppe Lazzara:** Resources, Validation, Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.110884.

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