forests

MDPI

Article

Composition of Phenolic Compounds, Cyanogenic Glycosides, Organic Acids and Sugars in Fruits of Black Cherry (*Prunus serotina* Ehrh.)

Jakub Brozdowski [1,*], Boguslawa Waliszewska [1], Jakub Loffler [2], Metka Hudina [3], Robert Veberic [3] and Maja Mikulic-Petkovsek [3]

[1] Department of Chemical Wood Technology, Poznan University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznań, Poland; boguslawa.waliszewska@up.poznan.pl
[2] Department of Forest Sites and Ecology, Poznan University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznań, Poland; jakub.loffler@up.poznan.pl
[3] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; metka.hudina@bf.uni-lj.si (M.H.); robert.veberic@bf.uni-lj.si (R.V.); maja.mikulic-petkovsek@bf.uni-lj.si (M.M.-P.)
* Correspondence: jakub.brozdowski@up.poznan.pl

Abstract: The forest understory is rich in fruit-bearing trees and shrubs. They include both native species and invasive alien plant species (IAPS). One of the most spreading IAPS is black cherry (*Prunus serotina* E.). Problems with the invasiveness of *P. serotina* is well known in many European countries. However, there are very few studies related to this IAPS. This article aims to fill the gap in research on the bioactive compounds of black cherry fruits and thus to start a discussion on the potential use of this species on an industrial or semi-industrial scale, which may lead to the reduction of this species presence in forests. Fruits were collected in a forest of Northern Poland. Contents of sugars, phenolic compounds as well as cyanogenic glycosides were determined. Pphenolic compounds and cyanogenic glycoside were assayed using HPLC-DAD coupled with MS^n. Sugars and organic acids were determined with the use of HPLC-DAD. Fruits of *P. serotina* can be considered as rich in anthocyanins, even at a lower level of total phenolics than in their plantation-grown couterparts, fruits of black cherry are still a good source of dietary phenolics of natural, forest-grown origin. When stones are not crushed during processing the level of GCC is within a safe, admissible range.

Keywords: *Prunus serotina*; HPLC-DAD-MS; phenolics; organic acids; sugars; cyanogenic glycosides; invasive alien plant species

Citation: Brozdowski, J.; Waliszewska, B.; Loffler, J.; Hudina, M.; Veberic, R.; Mikulic-Petkovsek, M. Composition of Phenolic Compounds, Cyanogenic Glycosides, Organic Acids and Sugars in Fruits of Black Cherry (*Prunus serotina* Ehrh.). *Forests* **2021**, *12*, 762. https://doi.org/10.3390/f12060762

Academic Editor: Maks Merela

Received: 12 May 2021
Accepted: 8 June 2021
Published: 10 June 2021

1. Introduction

One of the fruit-producing plants growing abundantly in European forests is the black cherry, *Prunus serotina* (Ehrh.). This is an invasive alien plant species (IAPS) for many European countries, such as e.g., Germany, the Netherlands, France, Hungary, Italy, Norway and Belgium [1–7]. As an invasive species *P. serotina* has spread to areas previously occupied by the European bird cherry (*Prunus padus* Mill.). Fruits of black cherry are globe-shaped and very dark [8]. Their dark color suggests a high level of anthocyanins which are well known for their colorant effect as well as antioxidant and health-promoting properties [9–11]. Results of a study [12] show a correlation between the color and content of phytochemicals. The darker the fruit, the higher the content of phenolics and other phytochemicals. Several studies have been carried out on phenolic compounds of black cherry [13–16]. These compounds play an important role in the antioxidant activity of fruits and various fruit-based products. If the high content of phenolics, including anthocyanins, is confirmed it may indicate considerable potential for

the use of *P. serotina* fruits in the food industry as a natural colorant or, thanks to their antioxidant activity, as a functional additive.

Prunus serotina was introduced in Poland in 1813 to fertilize weak soils. It did not take long for it to move from weak soils to fertile ones, where it spread rapidly and widely. Since its introduction black cherry has become a common understory species [17]. Now it is treated as an invasive plant species. Researchers from many European countries are now focused on determining factors that limit or promote the spread of black cherry [18–20]. Very few studies have been conducted on the characterization of chemical constituents in black cherry fruits growing in Poland [21].

The aim of this study was to fill the gap in research on the compounds in fruits of the genus *Prunus* by analyzing fruits of *P. serotina*, which contain many valuable bioactive substances such as sugars, organic acids and phenolic compounds as well as cyanogenic glycosides, which can be toxic to humans.

2. Materials and Methods

2.1. Chemicals

For the determination of sugars and organic acids the following standards were used: fructose, glucose, sorbitol and sucrose; citric and malic acids from Fluka Chemie (Buchs, Switzerland) and fumaric and shikimic acids from Sigma-Aldrich Chemie (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: kaempferol-3-glucoside, chlorogenic acid (5-caffeoylquinic acid), and eriodictyol from Sigma-Aldrich Chemie; ferulic and caffeic acids, (+)-catechin from Roth (Karlsruhe, Germany), *p*-coumaric acid, quercetin-3-glucoside, (−)-epicatechin, quercetin-3-galactoside, quercetin-3-rutinoside, procyanidin B1 and cyanidin-3-galactoside from Fluka Chemie; quercetin-3-arabinofuranoside, quercetin-3-xyloside, quercetin-3-arabinopyranoside, and isorhamnetin-3-glucoside and peonidin-3-glucoside from Extrasynthese (Genay, France). The chemicals for sample extractions and for the mobile phases were HPLC-MS grade methanol and acetonitrile and formic acid from Fluka Chemie. Water for the mobile phase was double distilled and purified with the Milli-Q system (Millipore, Bedford, MA, USA). For cyanogenic glycosides two standards were used: amygdalin and prunasin from Sigma-Aldrich Chemie.

2.2. Plant Materials

Fruits of *Prunus serotina* for this study were collected from the area of the Dabrowa Forest District of the Polish State Forests (location: latitude 53°29′45.74″ N, longitude 18°31′0.72″ E, 79 m altitude). Fully ripe fruits were collected on 20 August 2019. The collected fully ripe fruits were immediately frozen and stored at −20 °C until further analysis.

2.3. Preparation of Phenolic and Cyanogenic Glycoside Extracts

In total, 2 g of fresh fruits with intact stones were placed in a 12 mL screw-top tube with 5 mL of the extraction solution (60% $MeOH_{(aq)}$ and 3% of formic acid). The share of methanol with water in the extraction solution and the mass of added fruit material was experimentally optimized by testing samples using HPLC. The solution concentration was optimized based on chromatography peaks. Extraction was conducted in an ice-cooled ultrasonic bath. After 45 min the extraction screw-top tube with extracts were centrifuged for 9 min, 12.857 g (Eppendorf centrifuge with rotor F-34-6-38) at 4 °C and then filtered through a polyamide 0.2 µm filter (Macherey-Nagel, Duren, Germany) into HPLC vials. Vials with the samples were stored in the freezer at −20 °C until further analyses.

The fruit extract was prepared in 10 repetitions.

2.4. Preparation of Organic Acid and Sugar Extracts

For the extraction of sugars and organic acids the flesh of fruit was separated from stones. The flesh (3 g) of destoned fresh fruits was homogenized with 25 mL of double distilled water. The homogenized mixture was left for 30 min at room temperature with

frequent stirring at 250 rpm (Unimax 1010, Heidolph, Schwabach, Germany). Samples were then centrifuged for 9 min at 12.857 g (Eppendorf centrifuge with rotor F-34-6-38). Then the extract was filtered through a 0.2 µm cellulose filter (Macherey-Nagel) into HPLC vials and stored at −20 °C until further analysis.

The fruit extract was prepared in 10 repetitions.

2.5. HPLC-DAD MS^n Analysis of Phenolic Compounds and Cyanogenic Glycosides

Detection of phenolic compounds was performed in the Thermo Scientific Dionex HPLC system with a diode array detector (Thermo Scientific, San Jose, CA, USA) using the Chromeleon workstation software. The method of analysis was previously described [22]. Compounds were analyzed at three wavelengths of 280, 350 and 530 nm. The used column was Gemini C18 (150 × 4.6 mm 3 µm; Phenomenex, Torrance, CA, USA). The mobile phases consisted of phase A: 3% acetonitrile/0.1% formic acid/96.9% double-distilled water; phase B: 3% water/0.1% formic acid/96.9% acetonitrile. The injected extract volume was 20 µL, flow rate was 0.6 mL/min, column temperature 25 °C.

Phenolics were identified using a mass spectrometer (LTQ XL Linear Ion Trap Mass Spectrometer, Thermo Fisher Scientific, USA) with electrospray ionization (ESI) in the negative and positive ion mode (for anthocyanins). The scanning range was between m/z 110 and 1700. The capillary temperature was 320 °C, the sheath gas and auxiliary gas were 20 and 8 units, the source voltage was 4 kV for negative ionization and 0.1 kV for positive ionization. The normalized collision energy was set between 20% and 35%. The injection volume was 10 µL, flow rate was 0.6 mL/min. For compounds lacking standards, quantification was carried out with a chemically similar standard. Thus, feruloylquinic acids were quantified in equivalents of ferulic acid, *p*-coumaroylquinic acids in equivalents of *p*-coumaric acid, caffeoylquinic acids in equivalents of chlorogenic acid, caffeic acid hexoside in eqv. of caffeic acid, all procyanidins in eqv. of procyanidin B1, kaempferol glycosides in eqv. of kaempferol-3-glucoside, isorhamnetin glycosides in eqv. of isorhamnetin-3-glucoside and cyanidin derivatives in eqv. of cyanidin-3-galactoside. All available standards were dissolved in methanol to obtain stock solution of 1 mg/mL, which was stored at −80 °C until analyzed. Calibration curves were constructed using six standard solutions containing 0.05, 0.01, 0.025, 0.005, 0.001 and 0.0001 mg/mL of each standard. Concentrations of identified compounds in fruits were expressed in mg/kg of fresh weight.

2.6. HPLC-MS^n Analysis of Cyanogenic Glycosides

The presence of cyanogenic glycosides was confirmed on a TSQ Quantum Access Max quadrupole mass spectrometer (MS). The MS instrument was operated using an (ESI) source in the positive ion mode. All instrument parameters and analytical conditions were described in [23]. Prunasin and amygdalin were analyzed in the selected reaction monitoring (SRM) mode, which provides excellent sensitivity for the quantification of target compounds, and additionally by comparison with the SRM data of both standards. Contents were expressed in mg per g fruit.

2.7. Analysis of Organic Acids and Sugars by HPLC-DAD

Analysis of organic acids and sugars was conducted using HPLC (Thermo Scientific, San Jose, CA, USA). The methods were previously described [24]. For organic acids the Rezex ROA column was used (300 × 7.8 mm) (Phenomenex). For sugars the Rezex RCM-monosaccharide column was used (300 × 7.8 mm) (Phenomenex). Both columns were heated at 65 °C. The mobile phase for organic acids was 4 mM sulfuric and for sugars it was double-distilled water. Detection was performed by an ultraviolet detector (UV) set to 210 nm for organic acids and a refractive index (RI) detector for sugars. Quantification was performed with the help of the standard calibration curve with the known concentration. Calibration curves of sugars were composed using standard solutions containing 0.50, 1.00, 2.50 and 5.00 mg/mL of each standard. Where calibration curves of organic acids

were constructed using standard solutions containing 0.05, 0.10, 0.20, 0.50 mg/mL of each standard, the contents were expressed in g/kg FW fruits.

2.8. Statistical Evaluation

Obtained data were analyzed with the StatGraphics Plus 4.0 software (Manugistics, Rockville, MD, USA). The results are presented in Tables 1–3 as mean values ± standard deviation.

Table 1. Identification of phenolic compounds and cyanogenic glycosides in *Prunus serotina* fruits in positive and negative ion modes of HPLC-MS and MS^2. * $[M + H]^+$ (m/z) anthocyanins and cyanogenic glycosides were obtained in the positive ion mode.

	Peak Number	λ (nm)	$[M + H]^+$ * or $[M-H]^-$ (m/z)	MS^2 (m/z)
Hydroxycinnamic acids				
Vanillic acid hexoside	1	259,290	329	167,152,123,108
p-coumaric acid hexoside	6	324,275	325	163,119
5-feruloylquinic acid 1	9	322	367	191,173
5-feruloylquinic acid 2	17	322	367	191,173
5-caffeoylquinic acid 1 (chlorogenic acid)	10	234,328	353	191,179,135
5-caffeoylquinic acid 2	13	234,328	353	191,179,135
5-*p*-coumaroylquinic acid 1	16	312	337	191,173,163
5-*p*-coumaroylquinic acid 2	19	311	337	191,163,173
4-*p*-coumaroylqunic acid	14	312	337	173,191,145
Caffeic acid hexoside	11	295,322	341	179,161
Dicaffeoylquinic acid	27	324	515	353,191,179
Flavanols				
Procyanidin dimer 1	5	235,280	577	425,407,289
Procyanidin dimer 2	11	236,279	577	425,407,289
Procyanidin dimer 3	12	235,280	577	425,407,289
Procyanidin dimer 4	20	234,280	577	425,407,289
Catechin	8	234,279	289	245
Epicatechin	13	234,279	289	245
Procyanidin trimer 1	8	234,278	865	577,451,425,407,289
Procyanidin trimer 2	11	234,278	865	577,451,425,407,289
Procyanidin trimer 3	15	234,280	865	577,425,407,289
Procyanidin trimer 4	18	234,280	865	577,425,407,289
Procyanidin tetramer	16	235,279	1153	1135,983,865,577,575,407
Flavanones				
Eriodictyol hexoside 1	24	283,328	449	287
Eriodictyol hexoside 2	28	283,328	449	287
Flavonols				
Quercetin-3-rutinoside	21	255,355	609	301
Quercetin-3-glucoside	23	255,355	463	301
Quercetin-3-xyloside	24	356,255	433	301
Quercetin-3-arabinopyranoside	26	355,255	433	301
Quercetin-3-arabinofuranoside	27	355,255	433	301
Kaempferol-3-rutinoside	25	264,345	593	285
Kaempferol pentoside	29	266,346	417	285
Kaempferol hexoside	26	266,346	447	285
Kaempferol rhamnoside hexoside	22	265,346	593	447,285
Isorhamnetin-3-rutinoside	25	255,354	623	315
Isorhamnetin hexoside	27	256,354	477	315
Isorhamnetin pentoside 1	28	256,358	447	315
Isorhamnetin pentoside 2	29	256,358	447	315
Anthocyanins*				
Cyanidin-3-glucoside	2	279,516	449	287

Table 1. *Cont.*

	Peak Number	λ (nm)	$[M + H]^+$ * or $[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)
Cyanidin-3-rutinoside	3	279,516	595	287
Cyanidin-3-arabinoside	7	280,517	419	287
Peonidin-3-glucoside	4	279,516	463	301
Cyanogenic glycosides*				
Amygdalin		214	480	347,328
Prunasin		278	318	128,185

Table 2. Contents of phenolic compounds analyzed in black cherry fruits (mean ± standard deviations (SD) in mg kg^{-1} fresh weight (FW)); *n* = 10.

Compound	Content (mg kg^{-1})	Content Previously Reported [16] (mg kg^{-1})
Vanillic acid hexoside	58.1 ± 3.80	
p-coumaric acid hexoside	10.3 ± 0.98	
5-feruloylquinic acid 1	2.14 ± 0.17	
5-feruloylquinic acid 2	15.4 ± 7.92	
5-caffeoylquinic acid 1 (chlorogenic acid)	71.7 ± 5.79	188
5-caffeoylquinic acid 2	12.2 ± 2.50	
5-*p*-coumaroylquinic acid 1	5.71 ± 2.21	
5-*p*-coumaroylquinic acid 2	26.4 ± 16.4	
4-*p*-coumaroylqunic acid	16.4 ± 1.80	
Caffeic acid hexoside	2.71 ± 0.61	
Dicaffeoylquinic acid	1.74 ± 0.94	
Total hydroxycinnamic acids	**222 ± 28.3**	
Catechin	303 ± 89.5	454
Epicatechin	2933 ± 300	972
Procyanidin dimer 3	325 ± 43.1	
Procyanidin dimer 4	649 ± 108	
Procyanidin trimer 1	63.0± 18.6	
Procyanidin trimer 2	120 ± 27.0	Total proanthocyanidins 655
Procyanidin trimer 3	1574 ± 637	
Procyanidin trimer 4	172 ± 88.6	
Procyanidin tetramer	906 ± 3500	
Total flavanols	**7017 ± 543**	
Eriodictyol hexoside 1	6.89 ± 2.43	
Eriodictyol hexoside 2	2.40 ± 1.29	
Total flavanones	**9.30 ± 3.12**	
Quercetin-3-rutinoside	35.5 ± 7.99	
Quercetin-3-glucoside	10.0 ± 1.53	Total quercetin derivatives 72
Quercetin-3-xyloside	9.77 ± 1.14	
Quercetin-3-arabinopyranoside	5.55 ± 1.02	
Quercetin-3-arabinofuranoside	45.1 ± 5.75	
Kaempferol-3-rutinoside	0.82 ± 0.09	Total kaempferol derivatives 3
Kaempferol pentoside	4.87 ± 0.99	
Kaempferol hexoside	1.79 ± 0.33	
Kaempferol rhamnoside hexoside	4.66 ± 0.71	
Isorhamnetin-3-rutinoside	0.75 ± 0.09	
Isorhamnetin hexoside	1.66 ± 0.21	
Isorhamnetin pentoside 1	0.8 2± 0.17	
Isorhamnetin pentoside 2	2.49 ± 1.14	
Total flavonols	**123 ± 11.4**	
Cyanidin-3-glucoside	2138 ± 484	
Cyanidin-3-rutinoside	1780 ± 242	Total Cyanidin glycosides 89
Cyanidin-3-arabinoside	62.4 ± 5.23	
Peonidin-3-glucoside	40.2 ± 3.79	
Total anthocyanins	**4020 ± 719**	
Total phenolics	**11,394 ± 1221**	**2433**

Table 3. Contents of organic acids and sugars in black cherry fruits (mean ± SD in g kg^{-1} of fresh flesh material FFW), n = 10.

Sugars	Content (g kg^{-1})	Organic Acids	Content (g kg^{-1})	Ratio of Sugars/Acids
Sucrose	5.97 ± 0.47	Citric acid	28.2 ± 4.79	
Glucose	111 ± 8.55	Malic acid	21.1 ± 4.33	
Fructose	95.9 ± 5.64	Shikimic acid	0.23 ± 0.03	
Sorbitol	21.9 ± 2.46	Fumaric acid	0.06 ± 0.01	
Total	235 ± 13.52	Total	49.6 ± 9.16	4.79 ± 0.55

3. Results and Discussion

3.1. HPLC Analyses of Phenolic Compounds

To our knowledge this paper is the first report on HPLC-MS analysis of phenolic compounds in *Prunus serotina* fruits. All identified compounds are listed in Table 1 and the chromatogram shown in Figure 1.

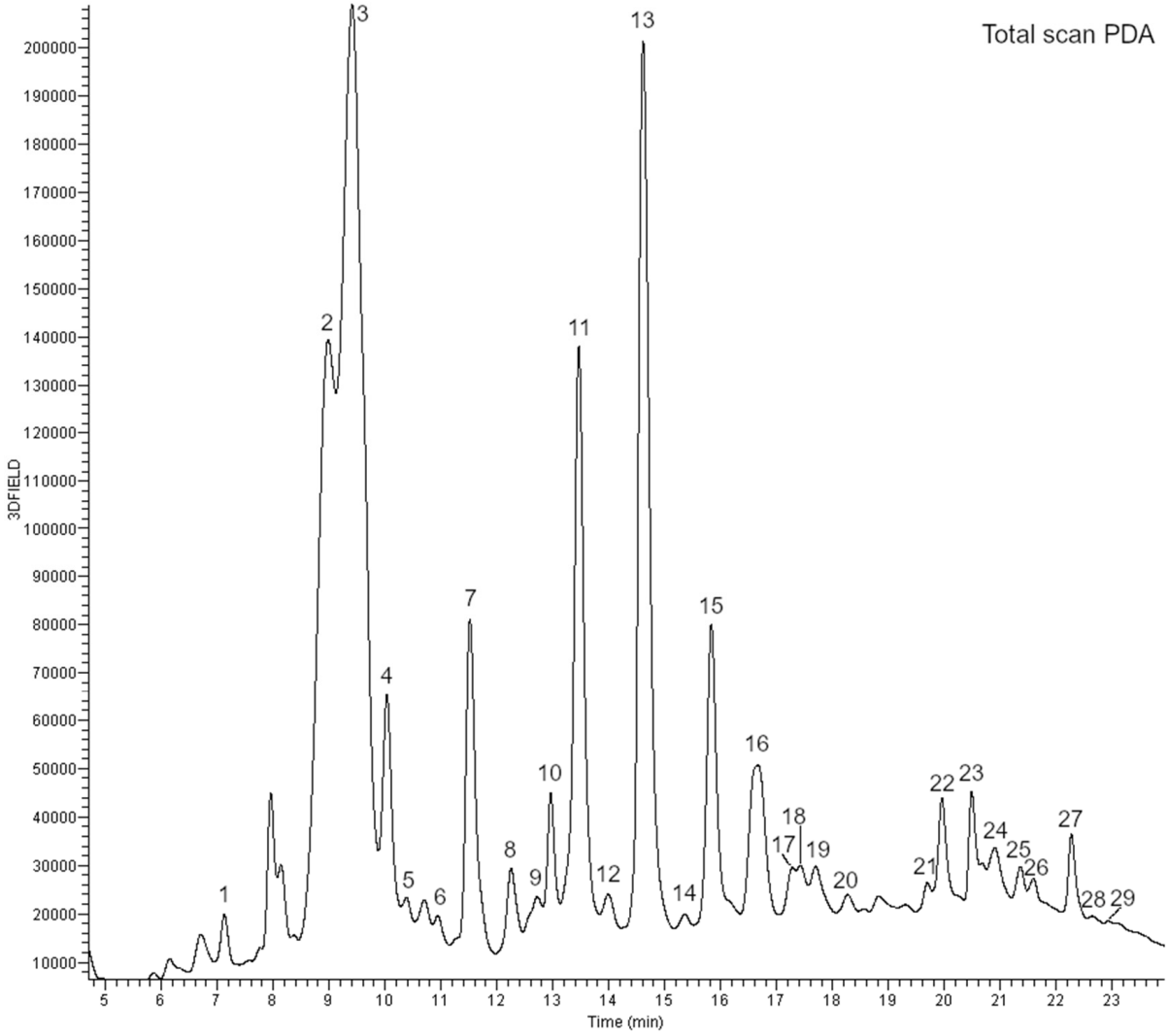

Figure 1. Chromatograms of *Prunus serotina* fruits. Peak numbers are described in Table 1.

Table 2 shows data on the phenolic composition of black cherry fruits. Identified compounds are divided into five groups: hydroxycinnamic acids, flavanols, flavanones, flavonols, and anthocyanins.

Flavanols are the most abundant group of phenolic compounds in the fruit of black cherry. Their level is 7017 mg per kg of fresh fruits, which indicates that *P. serotina* fruits are rich sources of these compounds in comparison with other *Prunus* species: *P. padus* at 124.14 mg/kg and *P. avium* at 546 mg/kg [25]. Four dimers and four trimers of procyanidin were identified as well as one tetramer. It is noticeable that fruits of black cherry are very abundant in catechins. The sum of catechin and epicatechin is 3236 mg/kg of fresh fruits, more than the previously reported 1426 mg/kg FW [16]. The analysis of catechins in fruits shows that in *Prunus avium* it is 62.7 mg/kg of catechins in fruits, while in *Prunus cerasus* it is only 9.8 mg/kg FW. In that study the most abundant in catechins was apricot (*Prunus armeniaca*) with 156.0 mg/kg FW [26].

The second group are anthocyanins with two dominant compounds: cyanidin-3-glucoside (2.1 g/kg FW) and cyanidin-3-rutinoside 1.8 g/kg FW). Two other identified anthocyanins were cyanidin-3-arabinoside and peonidin-3-glucoside with amounts below 100 mg/kg FW. In comparison, in European bird cherry the anthocyanin level was determined in the works [24–27], and it ranges from 207 to 581 mg/100 g FW with two identified anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside in a study [27] and four: cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and cyanidin rhamnosyl hexoside in a publication [25]. The content of total identified anthocyanins in fruit of black cherry in this study was in the same range (402 mg/100 g FW) as in the European bird cherry. Two of the identified anthocyanins are common for *P. padus* and *P. serotina*: cyanidin-3-glucoside and cyanidin-3-rutinoside. The same two compounds were reported as major anthocyanins in black cherry in a paper [13]. Additionally, the work [28] shows three common anthocyanins for sweet and black cherry, i.e., cyanidin-3-glucoside, cyanidin-3-rutinoside and peonidin-3-glucoside. *Prunus serotina* fruits with the anthocyanin level of 402 mg/100 g of fresh fruits can be considered as a rich source of anthocyanins [29,30].

The third group by content in mg/kg are hydroxycinnamic acids. In total they amount to 222 mg/kg FW. The main component in this group is 5-caffeoylquinic acid 1 (chlorogenic acid) at 71 mg/kg FW, followed by vanillic acid hexoside at 58 mg/kg FW. The level of hydroxycinnamic acids in black cherry fruits was lower than in most popular *Prunus* species. In the work [25] the reported content of hydroxycinnamic acids varied, depending on the species, from 442 to 660 mg/kg FW, which is approximately 2-fold lower than in black cherry.

The least abundant groups were flavonols (123 mg/kg FW) and flavanones (9 mg/kg FW). Among flavonols, there were two main phenolics quercetin-3-arabinofuranoside (45 mg/kg FW) and quercetin-3-rutinoside. Flavonols were the least abundant group among flavonoids. The total flavonol content was 123 mg/kg FW, which was higher than the content of flavonols (10–60 mg/kg FW) reported in [31].

In the study [32] the total polyphenolic content in sour cherry varied depending on the cultivar from 15,395 to 29,825 mg/kg, i.e., it was higher than the content of total phenolics in our study (11,394 mg/kg), comparing with the work of Vasco [16] at 2433 mg/kg FW the amount of total phenolics determined in our work is more than four times higher.

3.2. Sugars and Organic Acids

Table 3 shows sugar and organic acid contents in fresh *P. serotina* fruits. The main sugar in black cherry fruits is glucose; this is also the main sugar in many *Prunus* species [12,25].

Glucose (111 g/kg) and fructose (95.9 g/kg) are also the main sugars in sweet cherry fruits, varying between cultivars, but in *P. avium* it is more than two times lower than in black cherry [33]. The third compound was a sugar alcohol, sorbitol (21.9 g/kg FW) and the last was a disaccharide, sucrose, accounting for 6.0 g/kg FW. The concentration of glucose and fructose in sweet cherry is approx. 45 g/kg for glucose and 40 g/kg for

fructose, respectively. Black cherry with total sugar contents of 235 g/kg of fresh flesh is similar to the level of sugars in *P. avium* fruit reported in a study [12], whereas it is 2-fold higher according to the work [33]. The main organic acid was citric acid (28.2 g/kg FFW), which ranked second as a major organic acid in *P. padus* in a study [34]. The second most abundant organic acid for *P. serotina* was malic acid at 21.1 g/kg FFW. Shikimic and fumaric acids were detected only in trace amounts. Malic acid is also a common organic acid in *P. padus*. In the sweet cherry the main organic acid is malic acid, followed by citric and shikimic acids. The total organic acid content is approx. 13 g/kg FW in sweet cherry and approx. 49 g/kg in black cherry [33]. The sugar/acid ratio of 4.79 suggests a sour taste of fruit [25] and a bitter taste due to the high content of flavanols. The sugar/acid ratio of the above-mentioned *Prunus avium* is 8.15, which indicates a sweet taste of its fruit.

3.3. Cyanogenic Glycosides

Table 4 shows levels of cyanogenic glycosides in fruit of black cherry.

Table 4. Contents of cyanogenic glycosides (mean ± SD in mg kg^{-1} FW), n = 10.

Compound	Content (mg kg^{-1})
Amygdalin	19.24 ± 2.99
Prunasin	11.90 ± 3.06
Total	31.14 ± 5.65

Two cyanogenic glycosides (CGG) were identified in the extracts. The main one is amygdalin at 19.24 mg per kilogram of fresh fruits, while the recorded content of prunasin was 11.90 mg/kg FW.

Amygdalin, also called vitamin B-17, can be found in seeds and other parts of many *Prunus* species [35]. Among cyanogenic glycosides (CGG), prunasin is present in smaller amounts in the *P. serotina* fruit extract compared to amygdalin. However, prunasin bioavailability is approx. 50% of the oral dose, while amygdalin is almost impossible to absorb in an unchanged form [36]. The total content of CGG in fruits of black cherry is very low (31 mg/kg) compared to kernels of sweet cherry, which contain 1384 mg/kg [23]. However, if black cherry stones were crushed, the CGG content would be much higher, because they are stored there. Cyanogenic glycosides are known to be toxic by HCN hydrolysis, but their toxicity is lower than theoretical calculations suggest [37]. The level of 20 µg per kg body weight is described (by the European Food Safety Authority) as the maximum safe dose of cyanogenic glycosides [38]. According to the maximum dose of CGG, even 1 kg of processed fruit is not harmful for humans, bearing in mind that the fruit stone must not be crushed or damaged during processing; otherwise, the CGG content will be definitely higher.

4. Conclusions

The results of this study showed that fruits of this common invasive alien plant species of European forests can be considered rich in anthocyanins, which are known for their antioxidant activity. Although the total phenolic content is lower than in industrially used cherries, black cherry fruits are still a good source of dietary phenolics of natural origin. Wild species with an almond-like aroma are often considered harmful due to their content of cyanogenic glycosides. The study showed that the level of CGG in black cherry fruits is low, even 1 kg of fruits has lower amounts of prunasin and amygdalin than the level considered harmful to humans; nevertheless, it must be remembered that stones may not be crushed.

Author Contributions: Conceptualization, J.B.; data curation, J.B.; formal analysis, M.M.-P.; funding acquisition, M.H.; investigation, J.B., J.L. and M.M.-P.; methodology, M.M.-P.; project administration, R.V.; software, M.M.-P.; supervision, B.W., R.V. and M.M.-P.; validation, M.M.-P.; visualization, J.B.; writing—original draft, J.B.; writing—review and editing, B.W., R.V. and M.M.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Slovenian Research Agency (ARRS) Horticulture Program No. P4-0013-0481. Additionally, the results were obtained within the Erasmus+ scholarship programme of corresponding author.

Data Availability Statement: Data are available at corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Csiszár, Á.; Korda, M.; Schmidt, D.; Šporčić, D.; Süle, P.; Teleki, B.; Tiborcz, V.; Zagyvai, G.; Bartha, D. Allelopathic potential of some invasive plant species occurring in Hungary. *Allelopath. J.* **2013**, *31*, 309–318.
2. Deckers, B.; Verheyen, K.; Hermy, M.; Muys, B. Effects of landscape structure on the invasive spread of black cherry *Prunus serotina* in an agricultural landscape in Flanders, Belgium. *Ecography* **2005**, *28*, 99–109. [CrossRef]
3. Desiea, E.; Vancampenhoutb, K.; van den Berg, L.; Nyssena, B.; Weijterse, M.; den Ouden, J.; Muysa, B. Litter share and clay content determine soil restoration effects of rich litter tree species in forests on acidified sandy soils. *For. Ecol. Manag.* **2020**, *474*, 118377. [CrossRef]
4. Godefroid, S.; Phartyal, S.S.; Weyembergh, G.; Koedam, N. Ecological factors controlling the abundance of non-native invasive black cherry (*Prunus serotina*) in deciduous forest understory in Belgium. *Ecol. Manag.* **2005**, *210*, 91–105. [CrossRef]
5. Gentili, R.; Ferrè, C.; Cardarelli, E.; Montagnani, C.; Bogliani, G.; Citterio, S.; Comolli, R. Comparing negative impacts of *Prunus serotina, Quercus rubra* and *Robinia pseudoacacia* on native forest ecosystems. *Forests* **2019**, *10*, 842. [CrossRef]
6. Nestby, R.D.J. The status of *Prunus padus* L. (bird cherry) in forest communities throughout Europe and Asia. *Forests* **2020**, *11*, 497. [CrossRef]
7. Poyet, M.; Eslin, P.; Héraude, M.; Le Roux, V.; Prévost, G.; Gibert, P.; Chabrerie, O. Invasive host for invasive pest: When the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. *Agric. For. Entomol.* **2014**, *16*, 251–259. [CrossRef]
8. Douglas, G.W.; Meidinger, D.V.; Pojar, J. (Eds.) *Illustrated Flora of British Columbia. Volume 4: Dicotyledons (Orobanchaceae through Rubiaceae)*; B.C. Ministry of Environment, Lands & Parks and B.C. Ministry of Forests: Victoria, BC, Canada, 1999; pp. 328–329.
9. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* **2017**, *61*, 1361779. [CrossRef] [PubMed]
10. Lila, M.A. Anthocyanins and human health: An in vitro investigative approach. *J. Biomed. Biotechnol.* **2004**, *2004*, 306. [CrossRef] [PubMed]
11. Martín, J.; Kuskoski, E.M.; Navas, M.J.; Asuero, A.G. Antioxidant capacity of anthocyanin pigments. *Flavonoids-Biosynth. Hum. Health* **2017**, *3*, 205–255.
12. Usenik, V.; Fabčič, J.; Štampar, F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem.* **2008**, *107*, 185–192. [CrossRef]
13. Ordaz-Galindo, A.; Wesche-Ebeling, P.; Wrolstad, R.E.; Rodriguez-Saona, L.; Argaiz-Jamet, A. Purification and identification of Capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chem.* **1999**, *65*, 201–206. [CrossRef]
14. Hurtado, N.H.; Pérez, M. Identificación, estabilidad y actividad antioxidante de las antocianinas aisladas de la cáscara del fruto de capulí (*Prunus serotina* spp capuli (Cav) Mc. Vaug Cav). *Inf. Tecnol.* **2014**, *25*, 131–140. [CrossRef]
15. Jiménez, M.; Castillo, I.; Azuara, E.; Beristain, C.I. Antioxidant and antimicrobial activity of capulin (*Prunus serotina* subsp capuli) extracts. *Rev. Mex. Ing. Quím.* **2011**, *10*, 29–37.
16. Vasco, C.; Riihinen, K.; Ruales, J.; Kamal-Eldin, A. Phenolic Compounds in *Rosaceae* Fruits from Ecuador. *J. Agric. Food Chem.* **2009**, *57*, 1204–1212. [CrossRef] [PubMed]
17. Grajewski, S.; Miler, A.T.; Licznierski, M. Wykorzystanie opisu taksacyjnego do oceny występowania czeremchy amerykańskiej *Prunus serotina* Ehrh. *Rocz. Ochr. Śro.* **2013**, *15*, 1287–1301.
18. Grajewski, S.; Jankowski, K.; Licznierski, M. Czeremcha amerykańska *Prunus serotina* Ehrh. w polskich lasach na przykładzie drzewostanów nadleśnictwa doświadczalnego Zielonka oraz nadleśnictwa państwowego Szubin. *Zarządzanie Ochr. Przyr. W Lasach* **2010**, *4*, 39–55.
19. Halarewicz, A. Przyczyny i skutki inwazji czeremchy amerykańskiej *Prunus serotina* w ekosystemach leśnych. *Leśne Pr. Badaw.* **2011**, *72*, 267–272.
20. Rutkowski, P.; Maciejewska-Rutkowska, I.; Labedzka, M. Wlasciwy dobor skladu gatunkowego drzewostanow jako jeden ze sposobow walki z czeremcha amerykanska [*Prunus serotina* Ehrh.]. *Acta Sci. Pol. Silvarum Colendarum Ratio Et Ind. Lignaria* **2002**, *1*, 59–73.
21. Olszewska, M. Optimization and validation of an HPLC-UV method for analysis of corosolic, oleanolic, and ursolic acids in plant material: Application to *Prunus serotina* Ehrh. *Acta Chromatogr.* **2008**, *20*, 643–659. [CrossRef]
22. Mikulic-Petkovsek, M.; Ivancic, A.; Schmitzer, V.; Veberic, R.; Stampar, F. Comparison of major taste compounds and antioxidative properties of fruits and flowers of different *Sambucus* species and interspecific hybrids. *Food Chem.* **2016**, *200*, 134–140. [CrossRef]
23. Senica, M.; Stampar, F.; Veberic, R.; Mikulic-Petkovsek, M. Transition of phenolics and cyanogenic glycosides from apricot and cherry fruit kernels into liqueur. *Food Chem.* **2016**, *203*, 483–490. [CrossRef]

24. Mikulic-Petkovsek, M.; Koron, D.; Rusjan, D. The impact of fruit processing on phenolic content in products made from juneberry (*Amelanchier lamarckii*) fruits. *J. Food Sci.* **2020**, *85*, 386–393. [CrossRef]
25. Mikulic-Petkovsek, M.; Stampar, F.; Veberic, R.; Sircelj, H. Wild *Prunus* fruit species as a rich source of bioactive compounds. *J. Food Sci.* **2016**, *81*, C1928–C1937. [CrossRef] [PubMed]
26. Tsanova-Savova, S.; Ribarova, F.; Gerova, M. (+)-Catechin and (−)-epicatechin in Bulgarian fruits. *J. Food Compos. Anal.* **2005**, *18*, 691–698. [CrossRef]
27. Kucharska, A.Z.; Oszmiański, J. Anthocyanins in fruits of *Prunus padus* (bird cherry). *J. Sci. Food Agric.* **2002**, *82*, 1483–1486. [CrossRef]
28. Mozetič, B.; Trebše, P.; Hribar, J. Determination and quantitation of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Gorica region (Slovenia). *Food Technol. Biotechnol.* **2002**, *40*, 207–212.
29. Pompeu, D.R.; Silva, E.M.; Rogez, H. Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using Response Surface Methodology. *Bioresour. Technol.* **2009**, *100*, 6076–6082. [CrossRef]
30. Rogez, H.; Pompeu, D.R.; Akwie, S.N.T.; Larondelle, Y. Sigmoidal kinetics of anthocyanin accumulation during fruit ripening: A comparison between açai fruits (*Euterpe oleracea*) and other anthocyanin-rich fruits. *J. Food Compos. Anal.* **2011**, *24*, 796–800. [CrossRef]
31. Henning, W.; Herrmann, K. Flavonol glykoside der Aprikosen (*Prunus armeniaca* L.) und der Pfirsiche (*Prunus persica* Batsch). *Z. Fuer Lebensm.-Unters. Und Forsch.* **1980**, *171*, 183–188. [CrossRef]
32. Wojdyło, A.; Nowicka, P.; Laskowski, P.; Oszmiański, J. Evaluation of sour cherry (*Prunus cerasus* L.) fruits for their polyphenol content, antioxidant properties, and nutritional components. *J. Agric. Food Chem.* **2014**, *62*, 12332–12345. [CrossRef]
33. Kelebek, H.; Selli, S. Evaluation of chemical constituents and antioxidant activity of sweet cherry (*Prunus avium* L.) cultivars. *Int. J. Food Sci. Technol.* **2011**, *46*, 2530–2537. [CrossRef]
34. Donno, D.; Mellano, M.G.; De Biaggi, M.; Riondato, I.; Rakotoniaina, E.N.; Beccaro, G.L. New findings in *Prunus padus* L. fruits as a source of natural compounds: Characterization of metabolite profiles and preliminary evaluation of antioxidant activity. *Molecules* **2018**, *23*, 725. [CrossRef] [PubMed]
35. Enculescu, M. Vitamin B17/laetrile/amygdalin (a review). *Bull. Uasvm Anim. Sci. Biotechnol.* **2009**, *66*, 20–25.
36. Rauws, A.G.; Olling, M.; Timmerman, A. The Pharmacokinetics of Prunasin, a Metabolite of Amygdalin. *J. Toxicol. Clin. Toxicol.* **1982**, *19*, 851–856. [CrossRef] [PubMed]
37. Cressey, P.; Reeve, J. Metabolism of cyanogenic glycosides: A review. *Food Chem. Toxicol.* **2019**, *12*, 225–232. [CrossRef]
38. EFSA (European Food Safety Authority). Acute health risks related to the presence of cyanogenic glycosides in apricot kernels and products derived from apricot kernels. *EFSA J.* **2016**, *14*, 4424.