Journal of Food Composition and Analysis 40 (2015) 95–102

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Iridoids and anthocyanins in cornelian cherry (*Cornus mas* L.) cultivars

Alicja Z. Kucharska [a,*], Antoni Szumny [b], Anna Sokół-Łętowska [a], Narcyz Piórecki [c,d], Svitlana V. Klymenko [e]

[a] *Wrocław University of Environmental and Life Sciences, Department of Fruit Vegetable and Cereals Technology, Chełmońskiego 37/41, 51-630 Wrocław, Poland*
[b] *Wrocław University of Environmental and Life Sciences, Department of Chemistry, Norwida 25/27, 50-375 Wrocław, Poland*
[c] *Arboretum and Institute of Physiography in Bolestraszyce, 37-700 Przemyśl, Poland*
[d] *University of Rzeszów, Towarnickiego 3, 35-959 Rzeszów, Poland*
[e] *National Botanical Gardens of the Ukrainian National Academy of Sciences, Kiev, Ukraine*

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 6 December 2014
Accepted 8 December 2014
Available online 12 February 2015

Keywords:
Cornelian cherry
Cornus mas L.
Iridoids
Loganic acid
Cornuside
Anthocyanins
Pelargonidin 3-galactopiranoside
Cultivar difference
NMR
LC–MS
HPLC-DAD
Biodiversity and nutrition
Food composition
Food analysis

ABSTRACT

The qualitative and quantitative characteristics of iridoids and anthocyanins of cornelian cherry (*Cornus mas* L.) ripe fruits were investigated. The characteristics of these compounds were determined using HPLC-DAD, LC–ESI-MS, and NMR methods. Two iridoids (loganic acid and cornuside) and five anthocyanins (delphinidin, cyanidin, and pelargonidin glycosides) were identified in cornelian cherry fruits. The MS fragmentation pathways of the two iridoids were studied. A total of 26 different cultivars and 2 ecotypes, harvested from five locations in the years 2007–2011 were analyzed. The content of total iridoids in cornelian cherry fruits covered a wide range, i.e. from 86.91 to 493.69 mg/100 g fw. Loganic acid was the most dominant iridoid compound identified in cornelian cherry fruits and amounted to 88–96% of total iridoids. The total content of anthocyanins in red cultivars of cornelian cherry fruits was in a wide range from 5.59 to 134.57 and 341.18 mg/100 g fw. In most cornelian cherry fruits, pelargonidin 3-galactopyranoside was dominant.

1. Introduction

The genus *Cornus* consists of about fifty-five species that are distributed in various regions of the world. The subgenus *Cornus* consists of four species: *Cornus officinalis* Sieb. et Zucc., *Cornus mas* L., *Cornus chinensis* Wanger and *Cornus sessilis* Torr. Usually, plants belonging to it take the form of large shrubs or small trees. The most interesting, taking into account the usage in both the pharmacy and the food industry, is the *C. mas* L. species.

C. mas grows in natural conditions in Central and South-Eastern Europe and in Asia, while it is cultivated in many countries such as Ukraine, Georgia, Armenia, the Czech Republic, Slovakia, Turkey, Serbia, Austria, and Poland. Its mature fruits have an attractive red or, less frequently, yellow color and sour tart taste. They contain a single stone. Fruits can be eaten fresh or after being processed, in the form of jams, jellies, wines, liqueurs, fruit compotes, or pickles (Kucharska et al., 2007, 2010a,b, 2011a,b). Recent studies have shown that *C. mas* fruits contain biologically active compounds, such as vitamin C, organic acids, pectin, phenolic acids, flavonoids (anthocyanins, flavonols), triterpenoid (ursolic acid) and iridoids (loganic acid, cornuside) (Yamabe et al., 2007; Pawlowska et al., 2010; Rop et al., 2010; Kucharska et al., 2010a,b, 2011a,b; Kucharska, 2012; West et al., 2012; Deng et al., 2013). These compounds have high antioxidant and anti-inflammatory properties (Seeram et al., 2002; Vareed et al., 2006; West et al., 2012; Deng et al., 2013). Consumption of cornelian cherry fruits or their derivative products, rich in bioactive compounds, may have a positive influence on human health, particularly in the prevention of cancer, as well as inflammatory or cardiovascular diseases

* Corresponding author. Tel.: +48 713207712; fax: +48 713207707.
E-mail addresses: alicja.kucharska@up.wroc.pl, alicja.kucharska@wnoz.up.wroc.pl (A.Z. Kucharska).

http://dx.doi.org/10.1016/j.jfca.2014.12.016
0889-1575/

(Mikaili et al., 2013). The results of the research of Jayaprakasam et al. (2006) suggest that compounds from cornelian cherry fruits improve certain metabolic parameters associated with diets rich in saturated fats and obesity. Rop et al. (2010) indicated chosen cultivars of cornelian cherry as a new food source for human nutrition.

Iridoids belong to the monoterpenoid compound group. They are found mainly in the leaves and young stems, but very rarely in fruits. In literature, iridoids were reported in various parts of plants from the *Cornacae* family. The iridoids were fractionated and isolated from Corni Fructus extract (*C. officinalis* Sieb. et Zucc.) (Yamabe et al., 2007). Jensen et al. (1973), Tanaka et al. (2001) and Stermitz and Krull (1998) also tested iridoids in the roots of *Cornus capitata* Wall. ex Roxb. and in the leaves of *Cornus canadensis* L., *Cornus florida* L., *Cornus kousa* (Miq) and *Cornus nuttallii* Audubon. Kucharska et al. (2010a,b), Kucharska (2012), West et al. (2012), and Deng et al. (2013) identified iridoids in the fruits of *C. mas* L.

Anthocyanins are red colorants belonging to the flavonoids and widely appearing in fruits, vegetables and flowers. Fruits of cornelian cherry contain anthocyanins, such as cyanidin and pelargonidin derivatives. According to Du and Francis (1973a,b) there are five anthocyanins in the fruits of cornelian cherry. However, later work showed that anthocyanins of cornelian cherry were a mixture of three compounds These anthocyanins were identified by different methods: HPLC, LC–ES/MS, and NMR (Seeram et al., 2002), HPLC (Tural and Koca, 2008), and LC-PDA-MS (Pawlowska et al., 2010).

In the previous research (Kucharska, 2012), one of the authors of this article described the contents of active compounds in fruits of 10 Polish cultivars of cornelian cherry. There is, however, no literature, in which quantitative characteristics of various compounds from phenols and iridoids groups present in many other European cornelian cherry cultivars would be presented. Therefore, the main purpose of this work was to investigate the contents of those compounds in fruits of 26 cultivars and 2 ecotypes of cornelian cherry, harvested in Poland and Ukraine in the period 2007–2011. In this work, we also fractionated and isolated loganic acid, cornuside and anthocyanins from cornelian cherry (*C. mas* L.) fruits. These compounds were characterized using HPLC-DAD, UPLC–Q TOF ESI-MS, and NMR methods.

2. Materials and methods

2.1. Chemicals

Acetonitrile, formic acid, and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). Acetonitrile for LC–MS was purchased from POCh (Gliwice, Poland). Loganic acid, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-galactoside, pelargonidin 3-*O*-glucoside and pelargonidin 3-*O*-rutinoside were purchased from Extrasynthese (Lyon Nord, France).

2.2. Cornelian cherry cultivars

Cornelian cherry fruits from 19 cultivars ('Aurea', 'Bolestraszycki', 'Dublany', 'Elegantissima', 'Elegantnyi', 'Flava', 'Florianka', 'Yantarnyi', 'Juliusz', 'Koralovyi', 'Kostia', 'Kresowiak', 'Paczoski', 'Podolski', 'Raciborski', 'Radost', 'Schonbrunner', 'Słowianin', 'Szafer') were harvested in the Arboretum and Institute of Physiography in Bolestraszyce, near Przemyśl, Poland, 7 cultivars ('Ekzoticheskiy', 'Kostia', 'Lukianovskiy', 'Pervenets', 'Radost', 'Semen', 'Szafer') were harvested in the National Botanical Gardens of the Ukrainian National Academy of Sciences, Kiev, Ukraine, 3 cultivars ('Elegantissima', 'Jolico', 'Schonbrunner') were harvested in the Research Station for Cultivar Testing in Zybiszów, near Wrocław, Poland, 2 cultivars ('Flava', 'Golden Glory') were harvested in the Warsaw University Botanic Garden, and 2 ecotypes ('Czarny', 'Jurek') were harvested in the Wrocław University Botanical Garden, Wrocław, Poland. The plant materials were authenticated by Prof. Jakub Dolatowski (Arboretum and Institute of Physiography in Bolestraszyce, Poland), Prof. Tomasz Nowak (Wrocław University Botanical Garden, Poland), Elżbieta Melon, M.Sc. (Warsaw University Botanic Garden, Poland), Prof. Svitlana Klymenko (National Botanical Gardens of the Ukrainian National Academy of Sciences, Kiev, Ukraine), and the adequate voucher specimens ('Aurea' – BDPA 7329; 'Bolestraszycki' – BDPA 3951; 'Czarny' – BDPA OBWR 1; 'Dublany' – BDPA 3938; 'Elegantissima' – BDPA 7331; 'Elegantnyi' – BDPA 14132; 'Ekzoticheskiy' – BDPA OBK 1; 'Flava' – BDPA 8795; 'Florianka' – BDPA 1463; 'Golden Glory' – BDPA 14540; 'Yantarnyi' – BDPA 14131; 'Jolico' – BDPA 14586; 'Juliusz' – BDPA 6753; 'Jurek' – BDPA OBWR 2; 'Koralovyi' – BDPA 14136; 'Kostia' – BDPA 14135; 'Kresowiak' – BDPA 3946; 'Lukianovskiy' – BDPA 14123; 'Paczoski' – BDPA 3966; 'Pervenets' – BDPA OBK 2; 'Podolski' – BDPA 10462; 'Raciborski' – BDPA 3967; 'Radost' – BDPA 14130; 'Schonbrunner' – BDPA 14599; 'Semen' – BDPA 14595; 'Słowianin' – BDPA 3965; 'Szafer' – BDPA 10884; 'Svetliachok' – BDPA 14589) has been deposited at the Herbariums of Arboretum and Institute of Physiography in Bolestraszyce, Poland.

Fruits were harvested in September or October 2007–2011, and immediately frozen at −20 °C.

2.3. Extraction, purification, and fractionation of iridoids and anthocyanins for NMR and LC–MS analysis

Frozen ripe fruits of cornelian cherry (*C. mas* L.) (1 kg) were shredded and heated for 5 min at 95 °C using a Thermomix (Vorwerk, Wuppertal, Germany). The pulp was subsequently cooled down to 40 °C and depectinized at 50 °C for 2 h by adding 0.5 mL of Panzym Be XXL (Begerow GmbH & Co., Darmstadt, Germany) per 1 kg. After depectinization and the removal of stones, the pulp was pressed in a Zodiak laboratory hydraulic press (SRSE, Warsaw, Poland). The pressed juice was filtered and run through an Amberlite XAD-16 resin column (Rohm and Haas, Chauny Cedex, France). Impurities were washed off with distilled water, while pigments and iridoids were eluted with 80% ethanol. The eluate was concentrated under vacuum at 40 °C. The solvent was evaporated using a Rotavapor (Unipan, Warsaw, Poland). The concentrated extract of pigments and iridoids was purified using ethyl acetate to remove non-polar impurities and other flavonoids. The purification procedure was repeated three times. The sample of purified compounds was concentrated under vacuum at 40 °C and lyophilized (Alpha 1–4 LSC, Christ, Germany). As a result of drying, 5.4 g of freeze-dried lyophilisate was obtained. The lyophilisate was dissolved in a small vol. of 25% aqueous ethanol, containing 1% acetic acid. The dissolved sample was fractionated by polyamide (Macherey-Nagel-CC 6.6, Düren, Germany) column chromatography (150 mm × 30 mm) using 50% aqueous ethanol containing 0.5% acetic acid as eluent, to give three fractions. The fraction I (compound 1) was monitored at 254 nm, fractions II (compounds 4 and 6), and III (compounds 2, 3, 5, 7) – at 520 nm and 254 nm. The fractions II and III were further separated with RP-18 (Merck, Darmstadt, Germany) column chromatography (150 mm × 30 mm), using 50% aqueous ethanol containing 0.5% acetic acid as eluent to give two (II-1 and II-2) and three fractions (III-1, III-2 and III-3). The column (C 10/40, Pharmacia) was connected to the detector (Model 229 UV/VIS, Polymer Laboratories) and the recorder (C-R6A Chromatopac, Shimadzu). The sample was eluted with 50% methanol containing 0.5% acetic acid. The resulting alcohol–water fractions were concentrated and then freeze-dried (lyophilized). The fractions were analyzed by NMR.

2.4. Extraction of iridoids and anthocyanins for HPLC analysis

The frozen fruits of cornelian cherry (*C. mas* L.) without stones were homogenized. 10 mL of 80% aqueous methanol, acidified with 1% of HCl were added to 5 g of homogenate. Samples were sonicated (Sonic 6D, Polsonic, Warsaw, Poland) twice for 10 min. After this time, extracts were gradually filtered under vacuum using a Schott funnel. Precipitates were collected into 50 mL flasks. The extract was centrifuged at 19,000 × *g* for 15 min at 4 °C and filtered with a 0.45 μm filter before HPLC analysis. Two parallel extracts was done from each cultivar.

2.5. NMR analysis

The chemical structures of the purified compounds were determined and proved by means of nuclear magnetic resonance (NMR) in Laboratory of Structural Analyses, Wroclaw University of Technology, Wroclaw, Poland. NMR spectra were recorded on the NMR Bruker Avance™ 600 MHz spectrometer at 600.6 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR, respectively, in 297 K, using DMSO-D_6 (loganic acid and anthocyanins) or D_2O (cornuside) as the solvents. To determine the structures of the compounds, they were tested with techniques using 1D: ^{1}H, ^{13}C, and 2D: HSQC, HMBC and ROESY spectra. All spectra were acquired using standard Bruker software. The processed 1D and 2D data were processed using MestreNova 6.02 program.

2.6. Identification of iridoids and anthocyanins with LC–MS

Iridoid and anthocyanin identification was performed on an Acquity ultra-performance liquid chromatography (UPLC) system, coupled with a quadruple-time of flight (Q-TOF) MS instrument (UPLC/Synapt Q-TOF MS, Waters Corp., Milford, MA, USA), with an electrospray ionization (ESI) source. Separation was achieved on the Acquity™ BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm; Waters). Detection wavelengths were set to 245 and 520 nm. The mobile phase was a mixture of 4.5% formic acid (A) and acetonitrile (B). The gradient program was as follows: initial conditions – 99% (A), 12 min – 75% (A), 12.5 min – 100% (B), 13.5 min – 99% (A). The flow rate was 0.45 mL/min and the injection volume was 5 μL. The column was operated at 30 °C. UV–vis absorption spectra were recorded on-line during HPLC analysis, and the spectral measurements were made in the wavelength range of 200–600 nm, in steps of 2 nm. The major operating parameters for the Q-TOF MS were set as follows: capillary voltage 2.0 kV, cone voltage 45 V, cone gas flow of 11 L/h, collision energy 50 eV, source temperature 100 °C, desolvation temperature 250 °C, collision gas, argon; desolvation gas (nitrogen) flow rate, 600 L/h; data acquisition range, *m*/*z* 100–1000 Da; ionization mode, negative and positive. The data was collected with Mass-Lynx™ V 4.1 software.

2.7. Quantification of iridoids and anthocyanins by HPLC

The HPLC analysis was performed using a Dionex (Sunnyvale, CA, USA) system equipped with a diode array detector model Ultimate 3000, a quaternary pump LPG-3400A, an autosampler EWPS-3000SI, a thermostated column compartment TCC-3000SD, and controlled by Chromeleon v.6.8 software. An Atlantic T3 (250 mm × 4.6 mm i.d., 5 μm) column (Waters, Ireland), and an Atlantis T3 (20 mm × 4.6 mm i.d, 5 μm) guard column (Waters, Ireland) were used. The mobile phase was composed of solvent A (4.5% formic acid, v/v) and solvent B (acetonitrile). The elution system was as follows: 0–1 min 5% B, 1–37 min 5–25% B, 37–42 min 25–100% B, 42–47 min 100% B, 47–50 min, 100–5% B, 50–55 min 5% B. The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 20 μL. The column was operated at 30 °C. Iridoids were detected at 245 nm and anthocyanins at 520 nm. Iridoids were quantified as loganic acid and anthocyanins as cyanidin 3-*O*-glucoside. Results were expressed as mg/100 g fresh weight (fw). In this research, we have analyzed two independent samples of each material ($n = 2$).

The calibration curves were obtained by the external standard method on six levels of concentration of standard mixtures, with three injections per level. Chromatogram peak areas for 245 nm (loganic acid) and 520 nm (cyanidin 3-*O*-glucoside) were plotted against the known concentrations (0.02–0.3 mg/mL for loganic acid (Extrasynthese, ≥99% purity) and 0.01–0.1 mg/mL for cyanidin 3-*O*-glucoside (Extrasynthese, ≥96% purity)) of the standard solutions to establish the calibration equations. Linear regression equations were calculated by the least squares method. As the regression coefficients R^2 were ≥0.995, the dependences were considered linear, and – thus – acceptable for quantifying the compounds.

2.8. Statistical analysis

Statistical analyses were performed using Microsoft Office Excel 2010 upgraded with XLSTAT (ver. 2014.4.07.). Principal component analysis (PCA) was performed using XLSTAT on mean values of 43 samples (cornelian cherry cultivars and ecotypes) and 9 variables (loganic acid, cornuside, total iridoids, delphinidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-robinobioside, pelargonidin 3-*O*-galactoside, pelargonidin 3-*O*-robinobioside, total anthocyanins).

3. Results and discussion

3.1. Fractionation, purification and identification of iridoids and anthocyanins

A sample of the mixture of pure compounds (iridoids and anthocyanins) was obtained from ripe *C. mas* fruits by first purifying on Amberlite resin, and then purifying to remove non-polar impurities and other flavonoids. The compounds in fractions were identified by their UPLC retention times, elution order and also their spectroscopic and spectrometric characteristics (Tables 1 and 2 and Supplementary material Tables S1 and S2, Fig. S1), by comparison with the literature values (Seeram et al., 2002; Kucharska, 2012; West et al., 2012; Deng et al., 2013; Du and Francis, 1973a,b). The UPLC chromatograms of these mixtures, obtained in the UV spectral region, revealed two compounds 1 and 7 (iridoids) while obtained in the visible spectral region – and five compounds 2–6 (anthocyanins) (Fig. 1, Table 1).

A mixture of iridoids and anthocyanins was fractioned on a polyamide chromatographic column. The fractions of anthocyanins were chromatographed on the RP C18 column. The structures of iridoids (Fig. 2) and anthocyanins were established based on ^{1}H

Table 1
Chromatographic (UPLC) and spectrometric data of iridoids and anthocyanins of cornelian cherry fruits.

Peak no.	Compound	$t_{R\ UPLC\text{-}DAD}$ (min)	λ_{max} (nm)	Abs_{440}/Abs_{max} (%)
1	LA[a]	3.71	245	–
2	Df 3-gal	3.85	522	44
3	Cy 3-gal	4.49	515	32
4	Cy 3-rob	4.89	516	32
5	Pg 3-gal	5.09	426; 500	56
6	Pg 3-rob	5.54	426; 501	45
7	Co	9.53	243; 271	–

[a] LA, loganic acid; Df 3-gal, delphinidin 3-*O*-galactoside; Cy 3-gal, cyanidin 3-*O*-galactoside; Cy 3-rob, cyanidin 3-*O*-robinobioside; Pg 3-gal, pelargonidin 3-*O*-galactoside; Pg 3-rob, pelargonidin 3-*O*-robinobioside; Co, cornuside.

Table 2
MS data for identification of iridoids and anthocyanins of cornelian cherry fruits.

Peak no.	Compound	$[M-H]^-/[M+H]^+$ (m/z)	Other negative ions (m/z)	Other positive ions (m/z)
1	LA[a]	375.1276/377.1440	1503.5405 $[4M-H]^-$, 1127.4083 $[3M-H]^-$, 751.2686 $[2M-H]^-$, 213.0769 $[M-H-Glc]^-$, 169.0855 $[M-H-Glc-CO_2]^-$, 151.0771 $[M-H-Glc-CO_2-H_2O]^-$	1505.5663 $[4M+H]^+$, 1129.4250 $[3M+H]^+$, 753.2835 $[2M+H]^+$, 359.1347 $[M+H-H_2O]^+$, 341.1238 $[M+H-2\times H_2O]^+$, 215.0913 $[M+H-Glc]^+$, 197.0831 $[M+H-Glc-H_2O]^+$, 179.0701 $[M+H-Glc-2\times H_2O]$, 151.0763 $[M+H-Glc-2\times H_2O-CO]^+$, 133.0659 $[M+H-Glc-3\times H_2O-CO]^+$
2	Df 3-gal	463.0491/465.1034	303.0496 $[M-H-Gal]^-$	301.9950 $[M+H-Gal]^+$
3	Cy 3-gal	447.0916/449.1063	285.0360 $[M-H-Gal]^-$	287.0571 $[M+H-Gal]^+$
4	Cy 3-rob	593.1457/595.1713	447.0916 $[M-H-Rham]^-$, 285.0360 $[M-H-Gal]^-$	449.1150 $[M+H-Rham]^+$, 287.0571 $[M+H-Gal]^+$
5	Pg 3-gal	431.0994/433.1125	269.0435 $[M-H-Glc]^-$	271.0601 $[M+H-Glc]^+$
6	Pg 3-rob	577.1545/579.1766	431.0994 $[M-H-Rham]^-$, 269.0435 $[M-H-Gal]^-$	433.1125 $[M+H-Rham]^+$, 271.0601 $[M+H-Gal]^+$
7	Co	541.1566/543.1731	1625.4677 $[3M-H]^-$, 1083.3164 $[2M-H]^-$, 379.1022 $[M-H-Glc]^-$, 331.0634 ($C_{17}H_{16}O_7$), 169.0136 ($C_7H_6O_5$), 125.0241 ($C_6H_6O_3$)	381.1189 $[M+H-Glc]^+$, 211.0961 ($C_{11}H_{16}O_4$), 197.0831 ($C_9H_{10}O_5$), 153.0193 ($C_7H_6O_4$)

[a] LA, loganic acid; Df 3-gal, delphinidin 3-*O*-galactoside; Cy 3-gal, cyanidin 3-*O*-galactoside; Cy 3-rob, cyanidin 3-*O*-robinobioside; Pg 3-gal, pelargonidin 3-*O*-galactoside; Pg 3-rob, pelargonidin 3-*O*-robinobioside; Co, cornuside.

and ^{13}C NMR data and confirmed by comparison with those reported in the literature (Tables S1 and S2). Compounds 1–7 are given as below:

Compounds 1, 4, 6, 7: ^1H and ^{13}C NMR spectra of pure loganic acid, cyanidin 3-*O*-(6″-ramnosyl-*β*-galactopyranoside), pelargonidin 3-*O*-(6″-ramnosyl-*β*-galactopyranoside), and cornuside were consistent with the literature data (Kucharska, 2012). Compounds 2, 3, 5: ^1H and ^{13}C NMR spectra of pure delphinidin 3-*O*-*β*-galactopyranoside, cyanidin 3-*O*-*β*-galactopyranoside and pelargonidin 3-*O*-*β*-galactopyranoside were consistent with the literature data (Seeram et al., 2002; Slimestad and Andersen, 1998).

ESI-MS spectra of iridoids and anthocyanins in both negative and positive ionization modes were investigated (Table 2). The structures of iridoids and anthocyanins in cornelian cherry extracts and fractions were identified earlier (Kucharska et al., 2010a,b; Kucharska, 2012; West et al., 2012; Deng et al., 2013; Du and Francis, 1973a,b) but not by a quadrupole/time-of-flight mass spectrometer (Q/TOF-MS), which offers high mass accuracy. The structure of compound 1 was compared to the structure of the loganic acid standard and was confirmed by the results of NMR and LC–MS analysis. For compound 1, the ions in the MS spectra were $[M-H]^-$ (375.1276), $[2M-H]^-$ (751.2686) and $[3M-H]^-$ (1127.4015) in negative ESI mode and $[M+H]^+$ (377.1440), $[2M+H]^+$ (753.2835) and $[3M+H]^+$ (1129.4250) in positive ESI mode (Table 2). The major ions from the MS^2 fragmentations of compound 1were m/z 213.0769 $[M-H-Glc]^-$ and m/z 215.0913 $[M+H-Glc]^+$ for negative and positive ionization modes, respectively. The loss of a glucose unit (162 Da) generated the aglycone ion ($C_{10}H_{15}O_5$). In the MS^3 experiment, for negative ionization mode, the aglycone ion yielded fragment ions at m/z 179.0349, 169.0855, 151.0771, 135.0440 and 113.0260 which

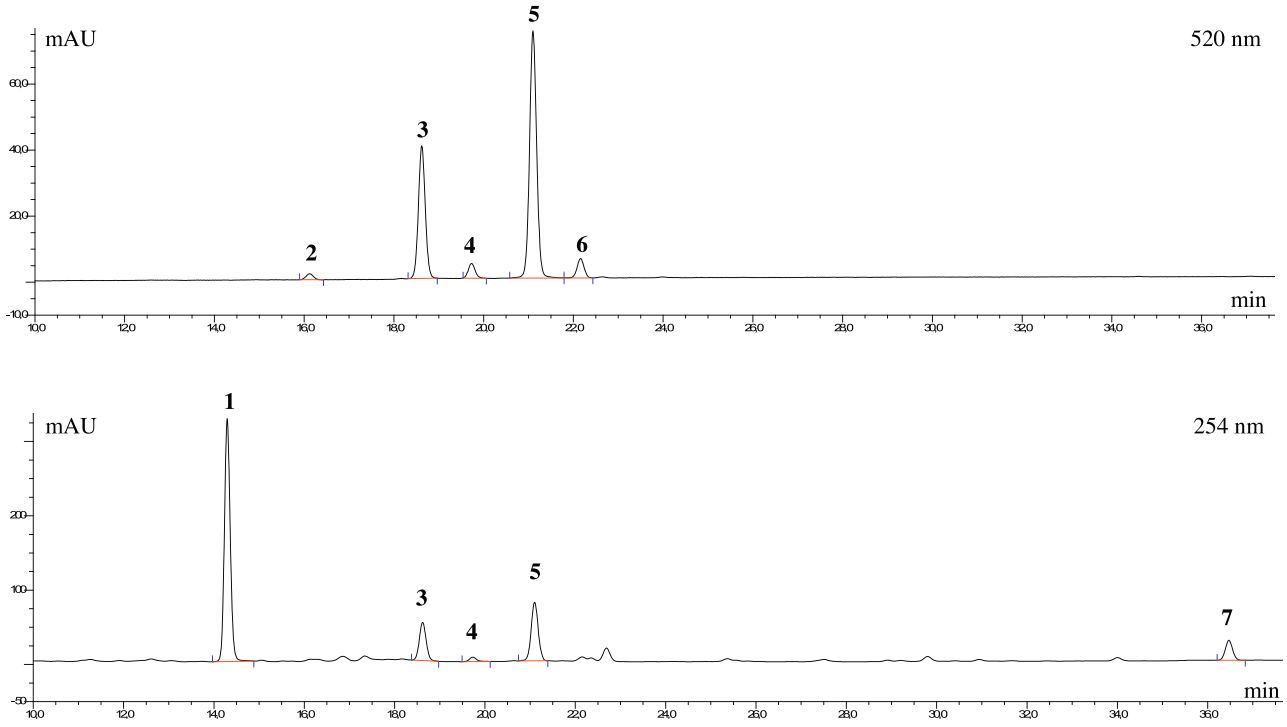

Fig. 1. HPLC profiles of anthocyanins (520 nm) and iridoids (254 nm) in cornelian cherry fruits extract. The peak number corresponds to the number in Tables 1–2.

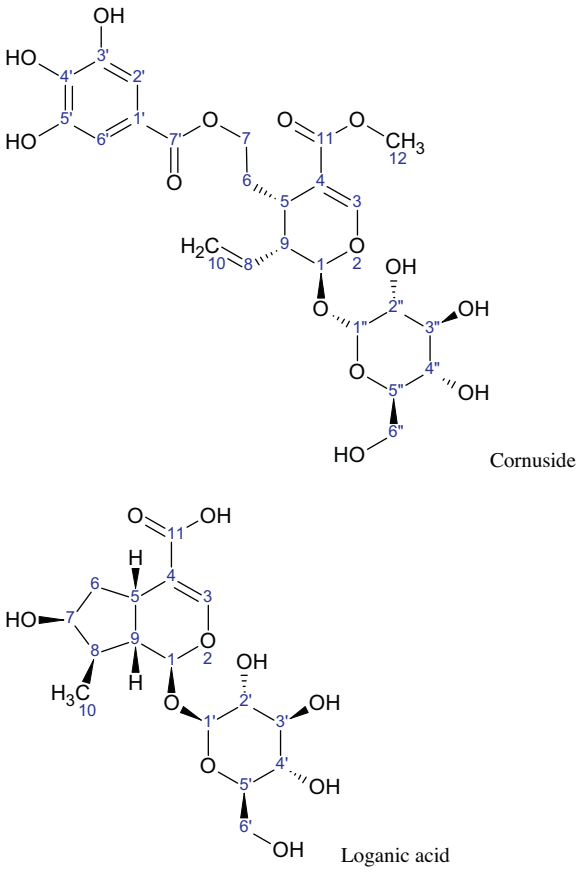

Fig. 2. The chemical structure of iridoids from cornelian cherry fruits.

corresponded to losses of 34 (CO), 44 (CO_2), 62 (CO_2 and H_2O), 78 (CO and CO_2) and 100 ($4\times H_2O$ and CO_2), respectively. In the MS^3 experiment, for positive ionization mode, the aglycone ion yielded fragment ions at *m/z* 197.0810, 179.0711 and 151.0763, which corresponded to losses of 18 (H_2O), 36 ($2\times H_2O$) and 62 ($2\times H_2O$ and CO), respectively. For the second iridoid (compound 7), the ions in the MS spectra were $[M-H]^-$ (541.1566), $[2M-H]^-$ (1083.3164) and $[3M-H]^-$ (1625.4677) in negative ESI mode and $[M+H]^+$ (543.1731) and $[2M+H]^+$ (1085.3318) in positive ESI mode. The ions from the MS^2 fragmentations of compound 7 were *m/z* 379.1022 $[M-H-Glc]^-$ and *m/z* 381.1189 $[M+H-Glc]^+$ for negative and positive ionization modes, respectively. The MS^3 fragmentation pattern of compound 7 showed signals at *m/z* 331.0634 ($C_{17}H_{16}O_7$), 169.0137 ($C_7H_6O_5$) and 125.0241 ($C_6H_6O_3$) for negative ionization modes, and signals at *m/z* 211.0961 ($C_{17}H1_6O_7$), and 197.0831 ($C_9H_{10}O_5$), 153.0195 ($C_7H_6O_4$) for positive ionization modes. In earlier publications (Kucharska, 2012; West et al., 2012; Deng et al., 2013) MS and MS^2 ions were described, but without MS^3. MS fragmentation pathways of iridoids, especially of MS^3, run differently for positive and negative ionization modes, which may be helpful in identification of compounds belonging to this group. Other authors detected similar metabolites: loganic acid and cornuside in Fructus Corni (Cao et al., 2011) obtained from *C. officinalis*.

Compounds 2, 3, and 5 were recognized as delphinidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside, and pelargonidin 3-*O*-galactoside, respectively (Table 2). These results were in agreement with previous studies (Du and Francis, 1973a,b; Seeram et al., 2002; Kucharska, 2012).

Compound 4 (t_R 4.89 min) had a molecular ion at *m/z* 595.1713 $([M+H]^+)$ and a fragment ion at *m/z* 287.0536 $([M+H-disach]^+)$, which corresponded to the molecular ion of the cyanidin aglycone, after loss of a rutinose or robinobiose (308 Da) The retention time of compound 4 was not similar to the retention time of cyanidin 3-*O*-rutinoside standards, which suggests that this compound is probably cyanidin 3-*O*-robinobioside. This was confirmed by NMR analysis. Multiplicities and coupling constants for the proton resonances of compound 4 indicated that the primary sugar, *O*-linked at C-3 (corresponding to the anomeric proton δ_H 5.28) was β-galactopyranose (Table S1). Compound 4 lost rhamnose (*m/z* 146), and then galactose (*m/z* 162), and it was identified as cyanidin 3-*O*-rhamnopyranosyl-galactopyranosyl. Compound 6 (t_R 5.54 min) exhibited a molecular ion at *m/z* 579.1766 $([M+H]^+)$ and a fragment ion at *m/z* 271.0601 $([M+H-disach]^+)$, which corresponded to the molecular ion of the pelargonidin moiety, after loss of a rutinose or robinobiose (308 Da). NMR studies indicate that robinobiose (rhamnopyranosyl-galactopyranosyl) was attached to aglicon in compound 6, like in the case of compound 4. Our results of the identification of anthocyanins in cornelian cherry are consistent with the results obtained by Du and Francis (1973a,b), who, in the seventies, using simple methods of identification, determined three galactosides of delphinidin, cyanidin, and pelargonidin, and two rhamnosylgalactosides of cyanidin and pelargonidin. However, the qualitative assessment of composition of anthocyanins in cornelian cherry cultivars/ecotypes, described in this paper, is not the same as the qualitative description of composition of anthocyanins in fruits of cornelian cherry recently investigated by other authors (Seeram et al., 2002; Tural and Koca, 2008; Pawlowska et al., 2010; Lachman et al., 1995). Publications from the last 10 years report that, among cornelian cherry red colorants, there are three anthocyanins. According to Seeram et al. (2002), they are: delphinidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside and pelargonidin 3-*O*-galactoside, according to Tural and Koca (2008) – cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside and pelargonidin 3-*O*-glucoside, and according to Pawlowska et al. (2010), – cyanidin 3-*O*-galactoside, pelargonidin 3-*O*-glucoside and pelargonidin 3-*O*-rutinoside. Those authors identified anthocyanins in cornelian cherry using different modern methods: HPLC, LC–ES/MS and NMR (Seeram et al., 2002) HPLC (Tural and Koca, 2008), and LC–PDA–MS (Pawlowska et al., 2010).

3.2. Quantitative analysis of iridoids and anthocyanins in cornelian cherry fruits

Influence of the genotype (cultivar/ecotype) and environment (location and growing season) on the contents of the iridoids and anthocyanins was studied. 43 different samples of fruits harvested from five locations in the years 2007–2011 were analyzed. The quantitative results are shown in Table 3. The total iridoid content in cornelian cherry fruits covered a wide range from 86.91 to 493.69 mg/100 g of fresh weight (fw). The average content of total iridoids found in the cornelian cherry was 260.4 mg/100 g of fresh weight (fw), with significant differences depending upon the cultivar, place and time (year) of harvesting. The 'Ekzoticheskiy' cultivar contains the least iridoids while the 'Raciborski' and 'Koralovyi' cultivars contain the most of these compounds. The amount of iridoids in 'Aurea', 'Elegantnyi', 'Golden Glory', 'Koralovyi', and 'Kostia' cultivars covered a wide range 256.98–302.60 mg/100 g, 289.49–347.11 mg/100 g, 200.12–248.15 mg/100 g, 383.04–443.24 mg/100 g and 121.35–264.28 mg/100 g, respectively, which depended on the location and year of harvesting. The iridoids in the Polish cultivars were described in a previous work (Kucharska, 2012), while in the European cultivars they were determined for the first time by us. Loganic acid was the most predominant iridoid compound identified in cornelian cherry fruits (Kucharska, 2012; West et al., 2012; Deng et al., 2013). It

Table 3
The iridoids and anthocyanins content of cultivars/ecotypes of cornelian cherry fruits (mg/100 g fw).

Cultivar	Harvest		Iridoids			Anthocyanins					
	Place[b]	Time	LA[a]	Co	Total	Df 3-gal	Cy 3-gal	Cy 3-rob	Pg 3-gal	Pg 3-rob	Total
			mg LA/100 g			mg CyGlc/100 g					
'Aurea'	B	2009	245.05	11.93	256.98	0.13	1.98	0.13	38.51	1.59	42.32
	B	2010	285.86	16.74	302.60	0.08	3.01	0.39	47.12	3.79	54.39
'Bolestraszycki'	B	2011	277.97	12.44	290.41	1.08	45.66	9.47	44.20	4.26	104.66
'Czarny'[c]	Wr	2007	268.76	14.77	283.52	16.68	234.40	45.32	40.69	4.10	341.18
'Dublany'	B	2011	195.02	24.66	219.68	0.63	12.83	1.91	12.83	2.23	30.43
'Elegantissima'	B	2010	158.98	21.10	180.08	1.46	5.08	1.32	76.08	5.52	89.45
	Z	2010	247.02	26.21	273.22	1.98	16.35	3.08	104.82	5.40	131.63
'Elegantnyi'	B	2010	264.72	21.77	286.49	0.32	49.41	2.00	24.46	0.42	76.61
	B	2011	326.75	20.36	347.11	1.91	52.85	1.40	37.06	0.68	93.91
'Ekzoticheskiy'	K	2009	81.53	5.38	86.91	0.39	41.04	7.62	23.59	1.86	74.50
'Flava'	B	2010	298.44	20.62	319.05	0.00	0.00	0.00	0.00	0.00	0.00
	Wa	2011	238.73	21.09	259.82	0.00	0.00	0.00	0.00	0.00	0.00
'Florianka'	B	2011	171.28	11.35	182.63	1.49	11.52	2.63	54.90	6.72	77.27
'Golden Glory'	Wa	2007	233.75	14.41	248.15	0.64	11.8	0.00	47.39	1.62	61.50
	Wa	2008	186.95	13.17	200.12	1.49	5.13	0.29	42.23	1.22	50.36
'Yantarnyi'	B	2009	383.67	18.73	402.40	0.00	0.00	0.00	0.00	0.00	0.00
	B	2010	267.78	17.52	285.30	0.00	0.00	0.00	0.00	0.00	0.00
	B	2011	272.39	18.02	290.41	0.00	0.00	0.00	0.00	0.00	0.00
'Jolico'	Z	2010	290.72	23.12	313.84	1.03	33.66	4.97	42.73	5.46	87.84
	Z	2011	268.62	36.25	304.87	2.40	56.19	9.40	44.84	3.85	116.68
'Juliusz'	B	2011	154.82	10.74	165.57	0.94	8.31	0.20	36.49	0.73	46.66
'Jurek'[a]	Wr	2007	120.60	10.20	130.80	0.24	7.60	1.39	47.29	4.00	60.53
'Koralovyi'	B	2010	365.53	17.51	383.04	0.00	0.57	0.00	4.02	0.00	4.59
	B	2011	427.02	16.22	443.24	0.00	0.78	0.00	6.27	0.00	7.05
'Kostia'	K	2010	111.25	10.10	121.35	0.56	32.55	0.91	38.02	0.49	72.53
	B	2010	134.66	11.95	146.62	0.91	33.75	0.79	26.21	0.47	62.13
	B	2011	249.19	15.09	264.28	1.18	54.42	1.93	32.29	0.00	89.82
'Kresowiak'	B	2011	258.54	13.09	271.63	0.85	24.81	5.71	40.22	6.30	77.89
'Lukianovskiy'	K	2010	109.93	9.21	119.14	0.09	6.86	1.34	39.25	3.38	50.91
'Paczoski'	B	2011	256.25	10.80	267.06	1.30	33.55	2.17	48.16	1.60	86.78
'Pervenets'	K	2010	225.80	11.09	236.89	0.51	25.11	2.01	42.82	0.64	71.09
'Podolski'	B	2011	240.58	22.11	262.69	0.27	31.00	3.54	44.37	1.97	81.16
'Raciborski'	B	2011	461.08	32.61	493.69	2.85	53.78	6.31	50.64	4.04	117.61
'Radost'	K	2009	204.27	9.42	213.69	0.80	43.09	6.26	30.67	3.09	83.90
	B	2010	204.69	10.39	215.09	0.46	52.19	8.41	20.08	2.08	83.23
	B	2011	248.79	10.09	258.88	1.55	58.34	10.90	26.73	4.19	101.71
'Schonbrunner'	B	2009	187.90	12.57	200.47	1.86	10.36	2.05	65.13	5.36	84.76
	Z	2010	309.02	12.79	321.81	1.20	16.09	1.29	82.00	4.61	105.20
	Z	2011	308.25	15.40	323.64	1.02	22.47	1.56	59.60	1.85	86.49
'Semen'	K	2009	117.83	8.22	126.06	0.26	31.50	4.80	38.69	2.64	77.90
'Słowianin'	B	2011	194.90	12.10	207.01	1.19	36.37	4.10	47.69	3.72	93.08
'Szafer'	B	2011	233.93	13.90	247.83	1.54	59.17	13.72	55.62	4.50	134.57
'Svetliachok'	K	2009	286.69	11.53	298.22	0.39	38.50	6.51	30.34	2.95	78.69

Values in the table are means from two samples ($n = 2$).
[a] LA, loganic acid; Co, cornuside; Df 3-gal, delphinidin 3-*O*-galactoside; Cy 3-gal, cyanidin 3-*O*-galactoside; Cy 3-rob, cyanidin 3-*O*-robinobioside; Pg 3-gal, pelargonidin 3-*O*-galactoside; Pg 3-rob, pelargonidin 3-*O*-robinobioside; CyGlc, cyanidin 3-*O*-glucoside.
[b] B, Bolestraszyce; K, Kiev; Wa, Warszawa; Wr, Wrocław; Z, Zybiszów.
[c] Ecotype.

amounted from 88% to 96% of total iridoids. The average content of loganic acid was 244.80 mg/100 g fw. The 'Koralovyi', 'Yantarnyi', and 'Flava' cultivars with coral and yellow fruits were characterized by a high content of iridoids of an above average amount. Among the red cultivars, Polish cultivars of 'Raciborski' and 'Bolestraszycki', Ukrainian cultivars of 'Elegantnyi' and 'Svietliachok', Austrian cultivars of 'Schonbrunner' and 'Jolico' had more loganic acid then the average amount, while Polish ecotypes of 'Jurek' and 'Juliusz', Ukrainian cultivars of 'Ekzoticheskiy', 'Lukianovskiy', and 'Kostia' had the lowest content of this iridoid. Cornuside was the second iridoid compound identified in cornelian cherry fruits (Kucharska, 2012; West et al., 2012; Deng et al., 2013). It represented from 4% to 12% of total iridoids. The cornuside content ranged from 5.38 mg/100 g fw in 'Ekzoticheskiy' to 32 mg/100 g fw in 'Raciborski' and 36.2 mg/100 g fw in 'Jolico'. The average content of cornuside was 15.64 mg/100 g fw. Cultivars were less diverse in terms of content cornuside than in terms of loganic acid. 26 samples had lower cornuside contents than the average amount, while 17 samples, for example 'Jolico', 'Raciborski', 'Elegantissima', 'Podolski', 'Elegantnyi', 'Flava', 'Yantarnyi', and 'Koralovyi' had higher cornuside content than the average amount. The influence of cultivars, places and times (year) of harvesting on the concentration of phenolic compounds in various species of fruits was earlier investigated by other authors (Anttonen and Karjalainen, 2005; Khoo et al., 2011). However, up till now – according to the authors' knowledge – the influence of the mentioned factors on the concentration of iridoids in *C. mas* fruits has not been investigated.

In most samples five anthocyanins were determined, among which dominated galactosides of pelargonidin (up to 91% of total anthocyanins in the cultivar 'Aurea') and of cyanidin (up to 69% in the ecotyp 'Czarny') (Table 3). In the cultivar 'Koralovyi', we determined only two anthocyanins – galactosides of pelargonidin and of cyanidin. Among the tested samples, there were fruits, in which galactoside of pelargonidin predominated over galactoside of cyanidin, for example 'Aurea', 'Koralovyi', 'Aurea Elagantissima', 'Golden Glory', and 'Elagantissima' (from 80 to 91% of total anthocyanins), but there were also fruits in which prevailed

galactoside of cyanidin, for example 'Radost' (51–63%), 'Elegantnyi' (56–64%), and 'Czarny' (69%). In the fruits of the cultivars 'Bolestraszycki', 'Dublany', 'Szafer' and 'Raciborski' there were similar shares of galactosides of pelargonidin and cyanidin (about 42%). Due to the fact that in some cultivars dominates pelargonidin, and in other – cyanidin, in this study we decided to use the cyanidin 3-*O*-glucoside standard for calculations of concentrations of individual anthocyanins. This anthocyanin is commonly found in most fruits and many authors use it for conversion, even if it is present in trace quantities (Cerezo et al., 2010; Ballistreri et al., 2013), therefore, it will be relatively easy to compare results obtained in the present study with those of other authors, who studied various species of fruits. The total anthocyanin content converted into cyanidin 3-*O*-glucoside in red cultivars of cornelian cherry fruits was in a wide range from 5.59 mg/100 g in 'Koralovyi' to 134.57 mg/100 g in 'Szafer' and 341.18 mg/100 g in 'Czarny' and depended mainly on the cultivar/ecotype, but also on the location and the year of harvest. This is consistent with the results of many authors investigating other species of fruits (Khoo et al., 2011; Ballistreri et al., 2013). Wide ranges of anthocyanin concentrations, determined by the spectrophotometry method, calculated as cyanidin 3-*O*-glucoside, were also obtained by other authors who studied different genotypes of cornelian cherry (Tural and Koca, 2008; Hassanpour et al., 2011; Popović et al., 2012). High contents of anthocyanins in cornelian cherry fruits harvested in natural conditions, measured with the pH differential absorbance method, is confirmed by the research of Pantelidis et al. (2007). The total anthocyanin content in cornelian cherry fruits is comparable to the total anthocyanin content in sour cherry, strawberry and raspberry (Khoo et al., 2011; Buendía et al., 2010; Bobinaite et al., 2012). The highest content of cyanidin 3-*O*-galactoside was observed in fruits of ecotype 'Czarny' (234 mg/100 g) and cultivars 'Radost', 'Elegantnyi', 'Raciborski', 'Kostia', 'Jolico' and 'Szafer' (50–60 mg/100 g), and of pelargonidin 3-*O*-galactoside – in cultivars 'Raciborski', 'Florianka', 'Szafer', 'Schonbrunner' and 'Elegantissima' (50–105 mg/100 g). The amounts of robinobiosides of cyanidin and of pelargonidin were far smaller than of galactosides, and the maximum of their shares in the total amount of anthocyanins accounted for 13% and 9%, respectively. In the cultivar 'Koralovyi' there were no such anthocyanins. The lowest concentration of cyanidin 3-*O*-robinobioside was in the fruit cultivars 'Golden Glory' (up to 0.3 mg/100 g) and 'Aurea' (0.4 mg/100 g), and the highest in fruit ecotype 'Czarny' (45.3 mg/100 g). The amount of pelargonidin 3-*O*-robinobioside was the lowest in the fruit cultivars 'Elagantnyi', 'Kostia', 'Juliusz' and 'Pervenets' (less than 1.0 mg/100 g), and the highest in the fruit cultivars 'Dublany', 'Florianka', 'Jolico', 'Kresowiak' and 'Schonbrunner' (above 5.0 mg/100 g). The content of the fifth anthocyanin delphinidin 3-*O*-galactoside in most varieties was low, and did not exceed 5% of the total amount of anthocyanins. The minimum concentration was approximately 0.1 mg/100 g ('Aurea', 'Lukianovskiy'), while the maximum – 16.7 mg/100 g ('Czarny'). Quantitative, as well as qualitative differences in the composition of the anthocyanins and iridoids in cornelian cherry showed that the cultivar, plant growth region, and the harvest period have an impact on active compounds in fruits. This is consistent with the results of other authors (Anttonen and Karjalainen, 2005; Khoo et al., 2011; Ballistreri et al., 2013).

3.3. Principal component analysis

PCA was performed based on the mean values of iridoids and anthocyanins obtained from 43 samples (Table 3). The first two factors (Fs) gave eigenvalues greater than 1.5 (3.977 and 2.520, respectively) and accounted for 72.18% of the total variability among the samples for all the investigated features (Table S3). The contribution of each parameter to the two factors and the distribution of cultivars on the plane are shown in Fig. 3. The first axe (F1) represented 44.19% of total variance and it was positively related to anthocyanins content, while the second factor (F2) accounted for 28.00% of the total variation and it was positively correlated with iridoids content. The biplot showed that some cultivars as 'Czarny', 'Raciborski', and cultivars with yellow fruits 'Flava', 'Yantarnyi', and 'Koralovyi' were clearly separated. The cultivars 'Czarny' and 'Raciborski' have large positive scores on the F1 and F2, due to high content of anthocyanins and iridoids respectively. Cultivars with yellow fruits, in which anthocyanins were no present, had negative scores on the F1. Other cultivars were similar to each other.

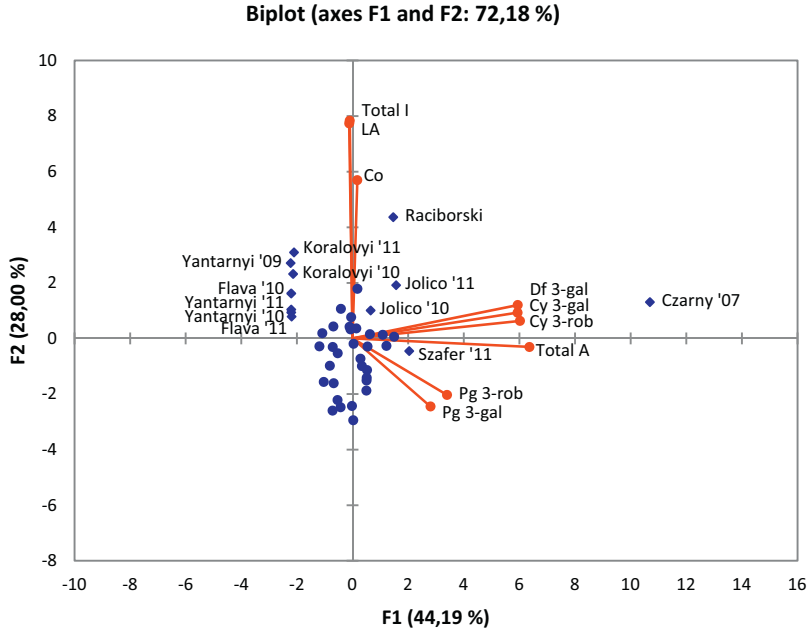

Fig. 3. PCA analysis biplot (axes F1–F2): contribution of studied variables (LA, loganic acid; Co, cornuside; Total I, total iridoids; Df 3-gal, delphinidin 3-O-galactoside; Cy 3-gal, cyanidin 3-O-galactoside; Cy 3-rob, cyanidin 3-O-robinobioside; Pg 3-gal, pelargonidin 3-O-galactoside; Pg 3-rob, pelargonidin 3-O-robinobioside; Total A, total anthocyanins) and plot of 43 cornelian cherry samples.

4. Conclusions

The qualitative and quantitative characteristics of iridoids and anthocyanins of cornelian cherry fruit were investigated using HPLC, UPLC–TOF-MS/MS and NMR methods. The studies have shown that the cornelian cherry is a fruit rich in biologically active compounds such as iridoids and anthocyanins. Active loganic acid is the predominant iridoid in *C. mas*, which rarely occurs in fruits of other botanical families. Analysis of the product ion spectra of iridoids was very useful for the identification of the functional groups in the structures of iridoids. Qualitative composition of anthocyanins of *C. mas* is specific for this species, as some of those colorants, either do not appear in the popular and less popular fruits, or are very rare and in trace amounts. The large quantity of pelargonidin 3-*O*-galactoside in comparison to other anthocyanins in cornelian cherry, reaching up to 91%, is very interesting in terms of the possible acquisition of this anthocyanin as a standard that is not found in available commercial catalogs. In addition, the presence of such a rare anthocyanin enables verification of the authenticity of cornelian cherry products. Differences of various kinds: genetic (cultivar), geographic (place of harvest) and environmental (year of harvest), affect the quantitative composition of iridoids and anthocyanins in cornelian cherry fruits; however, they do not affect the qualitative composition.

Acknowledgements

The authors are grateful to Prof. Tomasz Nowak (Wrocław University Botanical Garden), Elżbieta Melon, M.Sc. (Warsaw University Botanic Garden), and Joanna Laskowska, M.Sc. (Research Station for Cultivar Testing in Zybiszów) for sharing fruits of several varieties of cornelian cherry.

This work was supported by the Polish Ministry of Science and Higher Education under Grant N N312 2864 33.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2014.12.016.

References

Anttonen, M.J., Karjalainen, R.O., 2005. Environmental and genetic variation of phenolic compounds in red raspberry. Journal of Food Composition and Analysis 18, 759–769.

Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S., Rapisarda, P., 2013. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. Food Chemistry 140, 630–638.

Bobinaite, R., Viskelis, P., Venskutonis, P.R., 2012. Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars. Food Chemistry 132, 1495–1501.

Buendía, B., Gil, M.I., Tudela, J.A., Gady, A.L., Medina, J.J., Soria, C., López, J.M., Tomás-Barberán, F.A., 2010. HPLC–MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. Journal of Agricultural and Food Chemistry 58, 3916–3926.

Cao, G., Zhang, Y., Feng, J., Cai, H., Zhang, C., Ding, M., Cong, X., 2011. Rapid and sensitive assay for determining the main components in processed Fructus Corni by UPLC–Q-TOF-MS. Chromatographia 73 (1–2) 135–141.

Cerezo, A.B., Cuevas, E., Winterhalter, P., Garcia-Parrilla, M.C., Troncoso, A.M., 2010. Isolation, identification, and antioxidant activity of anthocyanin compounds in Camarosa strawberry. Food Chemistry 123, 574–582.

Deng, S., West, B.J., Jensen, C.J., 2013. UPLC–TOF-MS characterization and identification of bioactive iridoids in *Cornus mas* fruit. Journal of Analytical Methods in Chemistry, http://dx.doi.org/10.1155/2013/710972, ID 710972.

Du, C.T., Francis, F.J., 1973a. Anthocyanins from *Cornus mas*. Phytochemistry 12, 2487–2489.

Du, C.T., Francis, F.J., 1973b. New anthocyanin from *Cornus mas*. HortScience 8, 29–30.

Hassanpour, H., Hamidoghli, Y., Hajilo, J., Adlipour, M., 2011. Antioxidant capacity and phytochemical properties of cornelian cherry (*Cornus mas* L.) genotypes in Iran. Scientia Horticulturae 129, 459–463.

Jayaprakasam, B., Olson, L.K., Schutzki, R.E., Tai, M.-H., Nair, M.G., 2006. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in cornelian cherry (*Cornus mas*). Journal of Agricultural and Food Chemistry 54, 243–248.

Jensen, S.R., Klaer, A., Nielsen, B.J., 1973. Glucosides in *Cornus capitata* and *C. kousa*. Phytochemistry 12, 2301.

Khoo, G.M., Clausen, M.R., Pedersen, B.H., Larsen, E., 2011. Bioactivity and total phenolic content of 34 sour cherry cultivars. Journal of Food Composition and Analysis 24, 772–776.

Kucharska, A.Z., Sokół-Łętowska, A., Piórecki, N., 2011a. Morphological, physical & chemical, and antioxidant profiles of polish varieties of cornelian cherry fruit (*Cornus mas* L.). Żywność, Nauka, Technologia, Jakość 3 (76) 78–89.

Kucharska, A., Szumny, A., Sokół-Łętowska, A., Oszmiański, J., 2010. Sposób otrzymywania kwasu loganowego (Method of Obtaining Loganic Acid). Bulletin of the Patent Office, 2, 4, P 388632. http://ipu.uprp.pl/portal/web/guest/wydawnictwawupbup.

Kucharska, A.Z., 2012. Active Compounds of Cornelian Cherry Fruit (*Cornus mas* L.) Wydawnictwo Uniwersytetu Przyrodniczego Wrocław.

Kucharska, A.Z., Kowalczyk, K., Nawirska-Olszańska, A., Sokół-Łętowska, A., 2010b. Effect of chokeberry, strawberry, and raspberry added to cornelian cherry purée on its physical and chemical composition. Żywność, Nauka, Technologia, Jakość 4 (71) 95–106.

Kucharska, A.Z., Piórecki, N., Sokół-Łętowska, A., Żarowska, B., 2011b. Characteristics of chemical composition and antioxidant properties of cornelian cherry fruit fermented in brine. Zeszyty Problemowe Postepow Nauk Rolniczych 566, 125–133.

Kucharska, A.Z., Sokół-Łętowska, A., Hudko, J., Nawirska, A., 2007. Influence of the preparation procedure on the antioxidant activity and color of liqueurs from cornelian cherry (*Cornus mas* L.). Polish Journal of Food and Nutrition Sciences 57 (4(B)) 343–347.

Lachman, J., Pivec, V., Šubrtová, D., 1995. Anthocyanins in the fruits of wild dogwood (*Cornus mas* L.) of central Bohemian origin. Scientia Agriculturae Bohemica 26 (4) 259–266.

Mikaili, P., Koohirostamkolaei, M., Babaeimarzangou, S.S., Aghajanshakeri, S., Moloudizargari, M., Gamchi, N.S., Toloomoghaddam, S., 2013. Therapeutic uses and pharmacological effects of *Cornus mas*: a review. Journal of Pharmaceutical and Biomedical Sciences 35, 1732–1738.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis, G.R., 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, red currants, gooseberries and cornelian cherries. Food Chemistry 102, 777–783.

Pawlowska, A.M., Camangi, F., Braca, A., 2010. Quali-quantitative analysis of flavonoids of *Cornus mas* L. (*Cornaceae*) fruits. Food Chemistry 119, 1257–1261.

Popović, B.M., Štajner, D., Slavko, K., Bijelić, S., 2012. Antioxidant capacity of cornelian cherry (*Cornus mas* L.) – comparison between permanganate reducing antioxidant capacity and other antioxidant methods. Food Chemistry 134, 734–741.

Rop, O., Mlcek, J., Kramarova, D., Jurikova, T., 2010. Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition. African Journal of Biotechnology 9 (8) 1205–1210.

Seeram, N.P., Schutzki, R., Chandra, A., Nair, M.G., 2002. Characterization, quantification, and bioactivities of anthocyanins in *Cornus* species. Journal of Agricultural and Food Chemistry 50 (9) 2519–2523.

Slimestad, R., Andersen, Ø.M., 1998. Cyanidin 3-(2-glucosylgalactoside) and other anthocyanins from fruits of *Cornus suecica*. Phytochemistry 49, 2163–2166.

Stermitz, F.R., Krull, R.E., 1998. Iridoid glycosides of *Cornus canadensis*: a comparison with some other *Cornus* species. Biochemical Systematics and Ecology 26, 845–849.

Tanaka, N., Tanaka, T., Fujioka, T., Furii, H., Mihashi, K., Shimomura, K., Ishimaru, K., 2001. An ellagic compound and iridoids from *Cornus capitata* root cultures. Phytochemistry 57, 1287–1291.

Tural, S., Koca, I., 2008. Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grow in Turkey. Scientia Horticulturae 116, 362–366.

Vareed, S.K., Reddy, M.K., Schutzki, R.E., Nair, M.G., 2006. Anthocyanins in *Cornus alternifolia*, *Cornus kousa* and *Cornus florida* fruits with health benefits. Life Sciences 78, 777–784.

West, B.J., Deng, S., Jensen, C.J., Palu, A.K., Berrio, L.F., 2012. Antioxidant, toxicity, and iridoid tests of processed Cornelian cherry fruits. International Journal of Food Science and Technology 47 (7) 1392–1397.

Yamabe, N., Kang, K.S., Matsuo, Y., Tanaka, T., Yokozawa, T., 2007. Identification of antidiabetic effect of iridoid glycosides and low molecular weight polyphenol fractions of Corni Fructus, a constituent of hachimi-jio-gan, in streptozotocin-induced diabetic rats. Biological and Pharmaceutical Bulletin 30 (7) 1289–1296.