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Fruit Low-Alcoholic Beverages with High Contents of Iridoids and Phenolics from Apple and Cornelian cherry (*Cornus mas* L.) Fermented with *Saccharomyces bayanus*

Kinga Adamenko[1,], Joanna Kawa-Rygielska[1], Alicja Z. Kucharska[2], Narcyz Piórecki[3,4]*

[1]Department of Fermentation and Cereals Technology, Faculty of Food Science, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland;
[2]Department of Fruit, Vegetable and Plant Nutraceutical Technology, Faculty of Food Science, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland;
[3]Arboretum and Institute of Physiography in Bolestraszyce, 37-700 Przemyśl, Poland;
[4]Faculty of Physical Education, University of Rzeszów, Towarnickiego 3, 35-959 Rzeszów, Poland

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In this study, we produced novel, natural and fermented apple-Cornelian cherry beverages rich in natural antioxidants. These products were examined for their physicochemical parameters, and antioxidative properties as well as subjected to the quantitative and qualitative identification of iridoids and phenolics. The highest concentration of total phenolics determined with the Folin-Ciocialteu method (964.28 mg GAE/L) and the strongest antioxidative properties measured with the DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP tests (7.90, 11.04, and 12.86 mmol TE/L) were determined in the beverages with the addition of juice from red-fruit Cornelian cherry. The most numerous group of compounds in the analyzed beverages were iridoids, with loganic acid (LA) found to predominate (424 mg/L). Results obtained demonstrate that the addition of juice from Cornelian cherry fruits during the production of fermented apple beverages causes a significant increase in their antioxidative properties, modifies their phenolics profile, and allows enriching them with iridoids.

INTRODUCTION

Latest research indicates the global alcohol consumption to decline in recent years. However, this decrease has been found to result from the fact that more aware consumers search for novel, special, and valuable products characterized by interesting and diversified flavors [Podstawski *et al*., 2017; Muggah & McSweeney, 2017]. For this reason, products that have exceptional sensory attributes and also valuable composition seem to respond to these needs. Fermented fruit beverages represent an interesting group of products considering their sensory diversity and their multiple health-promoting compounds which exhibit various biological activities like, *e.g*.: phenolics, vitamins, organic acids or carotenoids [Cusano *et al*., 2018; Escudero-López *et al*., 2018].

In turn, apples are commonly available fruits, whose global production accounts for *ca*. 77.3 million tons annually (data for 2017/2018), [USDA Foreign Agricultural Service, 2017]. Apart from the production of juices, one of the significant sectors of apple processing includes the manufacture of ciders, *i.e.* natural fermented alcoholic beverages produced as a result of apple must fermentation, containing from 1.2 to 8.5%v/v of ethanol and exhibiting antioxidative and antimicrobial activities [Verdu *et al*., 2013]. Apples, which are the basic ingredients in cider production, contain a wide spectrum of polyphenolic compounds and dietary fiber; in addition they exert a bacteriostatic effect and prevent digestive system disorders [Condezo-Hoyos *et al*., 2014]. Considering their exceptional values and their availability, in this study we produced novel alcoholic beverages with the addition of Cornelian cherry fruits characterized by a very high content of natural antioxidants compared to other fruits.

Cornelian cherry (*Cornus mas* L.) is a fruit growing wild in Europe and Asia. It may be consumed fresh or may be processed to produce liqueurs, juices, jams, concentrates or alcoholic beverages [Kucharska *et al*., 2007; Kucharska, 2012; Sokół-Łętowska *et al*., 2014; Bozdogan, 2017]. Numerous investigations have proved Cornelian cherry fruits and products made of them to be characterized by an exceptionally rich phenolics composition [De Biaggi *et al*., 2018], and by other health-promoting properties like *e.g.* hypocholesterolemic [Hosseinpour *et al*., 2017] or anti-inflammatory ones [Moldovan *et al*., 2016]. Regular consumption of small amounts of natural fermented beverages rich in antioxidants may supplement an everyday fruit-vegetable diet. This study was aimed at developing a production technology for apple-Cornelian cherry low-alcoholic beverage with strong antioxidative

* Corresponding Author: Tel.: +4871 323-9417;
E-mail: kinga.adamenko@upwr.edu.pl (K. Adamenko)

properties and high concentrations of iridoids and phenolics, and to identify the best Cornelian cherry variety and the best production method of these beverages.

MATERIALS AND METHODS

Standards and reagents

Dimethyl sulfoxide (DMSO), 2,4,6-tri(2-pyridyl)-s--triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman--2-carboxylic acid (Trolox), acetonitrile, 1,1-diphenyl-2-picryl--hydrazyl radical (DPPH$^{\bullet}$), $FeCl_3$, sulfuric acid, formic acid, and sodium hydroxide were purchased from Sigma-Aldrich (Taufkirchen, Germany). Acetic acid was obtained from Chempur (Piekary Śląskie, Poland). Acetonitrile was acquired from POCh (Gliwice, Poland), whereas acetic acid from Chempur (Piekary Śląskie, Poland). Loganic acid (LA), loganin (L), sweroside (S), ellagic acid (EA), 5-*O*-caffeoylquinic acid (5-CQA, chlorogenic acid), *p*-coumaric acid (*p*-CuA), quercetin 3-*O*-glucoside (Q glc), kaempferol 3-*O*-glucoside (Kf glc), cyanidin 3-*O*-glucoside (Cy glc), (+)-catechin (C), procyanidin dimer B1, procyanidin dimer B2, (–)-epicatechin, and phloretin 2'-*O*-glucoside (Ph 2'-glc) were purchased from Extrasynthese (Lyon Nord, France). All reagents were of analytical grade. The Folin-Ciocialteu phenol reagent was purchased from Chempur (Piekary Śląskie, Poland).

Biological material

Apple juice and apple-Cornelian cherry juices were fermented with wine yeast *Saccharomyces bayanus* – Safspirit fruit, purchased from the Fermentis company (Lesaffre, Marcq-en-Barœul, France). Dry yeast were rehydrated in bi-distilled water at a temperature of 25°C for 30 min. Fermentation worts were inoculated with the activated yeast cells in a dose of 0.5 g of dry matter per liter (g d.m./L).

Raw material

Raw materials used in the study included: all-year round available on the Polish market, freshly pressed, naturally cloudy and pasteurized apple juice made of three apple varieties: Gloster, Champion, and Jonaprince, produced by the SOKPOL company (Myszków, Poland); as well as three varieties of Cornelian cherry: 'Podolski' var. with red fruits, 'Yantarnyi' var. with yellow fruits, and 'Koralovyi' var. with coral fruits. All fruits were harvested in the Arboretum in Bolestraszyce, near Przemyśl, Poland. The plant materials were authenticated by Prof. Jakub Dolatowski (Arboretum and Institute of Physiography in Bolestraszyce, Przemyśl, Poland), and the adequate voucher specimens ('Yantarnyi' – BDPA 14131; 'Koralovyi' – BDPA 14136; 'Podolski' – BDPA 10462) have been deposited at the Herbariums of Arboretum in Bolestraszyce, Poland. Fruits were harvested in September 2016, and immediately frozen at −20°C. Before fermentation, the fruits were pressed through a Zodiak laboratory hydraulic press from SRSE company (Warsaw, Poland) to obtain Cornelian-cherry juices.

Preparation of samples and fermentation process

Two methods differing in the course of the technological process were used in the study to produce apple-Cornelian cherry alcoholic beverages. The first method (M-1) consisted in the preparation of worts containing 90% of apple juice (630 mL) and 10% of juice from Cornelian cherry varieties differing in fruit color (70 mL). The prepared apple-Cornelian cherry worts were inoculated with yeast and fermented in 1-liter glass laboratory flasks at a temperature of 22°C for 7 days. Afterwards, the samples were decanted from above the yeast precipitate and left for aging at a temperature of 4°C for two weeks. In the second method (M-2), worts containing 100% of apple juice (700 mL) were fermented under the same conditions as in method M-1. Next, the samples were decanted from above the yeast precipitate and 10% of the resultant sample (sample after fermentation but before aging) was replaced by juice made of fruits of a given variety of Cornelian cherry, and the sample was left for aging under conditions as described in method M-1. A control sample (A) was prepared as well, in which 100% of apple juice was used for both the fermentation and aging processes which were conducted under the same technological conditions as in method M-1. All research variants were fermented in triplicate. Description of symbols used throughout the manuscript are presented in Table 1.

TABLE 1. Symbols and description of worts, beverage production methods, as well as worts and beverages manufactured in the study.

Symbol	Description	
A	wort	wort containing 100% of apple juice
AY		wort from apple juice with the addition of juice from yellow fruits of Cornelian cherry
AC		wort from apple juice with the addition of juice from coral fruits of Cornelian cherry
AR		wort from apple juice with the addition of juice from red fruits of Cornelian cherry
M-1	production method	production method in which 10% of Cornelian cherry juice was added to the wort before the primary fermentation process
M-2		production method in which 10% of post-fermentation liquid was replaced by Cornelian cherry juice
A-1	products manufactured with method M-1	fermented beverage containing 100% of apple juice
AY-1		fermented apple-Cornelian cherry beverage with juice from yellow fruits of Cornelian cherry manufactured with method M-1
AC-1		fermented apple-Cornelian cherry beverage with juice from coral fruits of Cornelian cherry manufactured with method M-1
AR-1		fermented apple-Cornelian cherry beverage with juice from red fruits of Cornelian cherry manufactured with method M-1
AY-2	products manufactured with method M-2	fermented apple-Cornelian cherry beverage with juice from yellow fruits of Cornelian cherry manufactured with method M-2
AC-2		fermented apple-Cornelian cherry beverage with juice from coral fruits of Cornelian cherry manufactured with method M-2
AR-2		fermented apple-Cornelian cherry beverage with juice from red fruits of Cornelian cherry manufactured with method M-2

Fermentation dynamics and pH value

Fermentation dynamics was determined based on carbon dioxide loss during the fermentation process. The mass loss of fermented samples was monitored every 24 h by weighing the containers on a WTB 2000 laboratory scale (RADWAG, Radom, Poland). The pH value of all samples was measured using an MP 220 pH-meter (Mettler Toledo, Greifensee, Switzerland).

Carbohydrates, ethanol, and glycerol concentrations

The concentrations of sucrose, glucose, fructose, ethanol, and glycerol were determined with the method of high performance liquid chromatography (HPLC) [Kawa-Rygielska *et al*., 2018]. The samples with 100% of apple juice were diluted with redistilled water in a water to sample ratio of 1:2 (v/v), whereas samples with Cornelian cherry were diluted in a ratio of 1:5. The samples were analyzed using a Prominence liquid chromatography system (Shimadzu Corp., Kyoto, Japan) equipped in a Rezed ROA-Organic Acid H+ column (300 × 4.6 mm) from Phenomenex (Torrance, CA, USA). The following parameters of measurements were applied: injection volume 20 μL, elution temperature 60°C, flow rate 0.6 mL/min, mobile phase 0.005 M H_2SO_4, and thermostat refractometric detector at 50°C. Concentrations of glucose, ethanol, and glycerol were determined based on a five-point calibration curve integrated in Chromax 10.0 software by Pol-Lab (Wilkowice, Poland).

Determination of phenolics concebtration and antioxidative activity

Total phenolics concentration

The total phenolics content was determined using the spectrophotometric method, with the Folin-Ciocalteu reagent test (F-C) [Prior *et al*., 2005]. The analyzed sample was mixed with the F-C reagent, and after 20 min Na_2CO_3 20% and distilled water were added. After 60 min of incubation, absorbance was measured at 765 nm. The results are expressed in mg of gallic acid equivalents (GAE) per liter of beverage (mg GAE/L), as the average of three replicates.

Antioxidative activity based on the DPPH$^\bullet$ test

The antioxidative properties of the analyzed samples were measured with the DPPH$^\bullet$ test [Yen & Chen, 1995]. The sample and the distilled water were pipetted into the cuvette and then the DPPH$^\bullet$ reagent was added. The samples were incubated for 10 min and next the absorbance was measured at 517 nm. The results are presented as the content of Trolox equivalents (TE) per liter of the sample (mmol TE/L), as the average of three replicates.

Antiradical activity based on the ABTS$^{\bullet+}$ test

The antioxidative activity of the analyzed beverages was also determined based on the ABTS$^{\bullet+}$ assay [Re *et al*., 1999]. A sample with the ABTS$^{\bullet+}$ solution was mixed in a cuvette. After 6 min of incubation, the absorbance was measured at 734 nm. The results are expressed as the average of three replicates, as the content of Trolox equivalents (TE) in a liter of the sample (mmol TE/L).

Antioxidative activity based on the FRAP test

Antioxidative activity of the analyzed samples was also measured with the spectrophotometric method, based on the FRAP test [Benzie & Strain, 1996]. After mixing the sample with distilled water, the FRAP reagent was added. The prepared samples were incubated for 10 min and then the absorbance was measured at 593 nm. Results are expressed as the average of three measurements, as the content of Trolox equivalents (TE) per liter of the sample (mmol TE/L).

Absorbance measurements in the above tests were made using a DU 650 spectrophotometer from the Beckman Coulter company (Atlanta, GA, USA).

Quantification of bioactive compounds with HPLC-PDA

Detailed description of the quantification procedure was described in works by Kucharska *et al*. [2017] and Kawa-Rygielska *et al*. [2018]. It was performed using a Dionex (Germering, Germany) system equipped with an Ultimate 3000 model diode array detector, LPG-3400A quaternary pump, EWPS-3000SI autosampler, TCC-3000SD thermostated column compartment, and controlled by the Chromeleon v.6.8 software (Thermo Scientific Dionex, Sunnyvale, CA, USA). A Cadenza Imtakt C5-C18 column (75×4.6 mm, 5 m) was used (Imtakt, Kyoto, Japan). The mobile phase was composed of solvent A (4.5% aq. formic acid, v/v) and solvent B (100% acetonitrile). The elution system was as follows: 0–1 min 5% B in C, 20 min 25% B in A, 21 min 100% B, 26 min 100% B, and 27 min 5% B in A. 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, phenolic acid at 320, ellagic acid at 254 nm, anthocyanins at 520 nm, flavanols at 280 nm, dihydrochalcones at 280 nm, and flavonols at 360 nm. Loganic acid and cornuside were expressed as loganic acid, loganin, sweroside; and secoxyloganin as loganin; caffeoylquinic acids as 5-*O*-caffeoylquinic acid' *p*-coumaroylquinic acid as *p*-coumaric acid; ellagic acid as ellagic acid; anthocyanin derivatives as cyanidin 3-*O*-glucoside; (–)-epicatechin and procyanidin dimers as (–)-epicatechin; (+)-catechin as (+)-catechin; phloretin derivatives as phloretin 2'-*O*-glucoside; quercetin derivatives as quercetin 3-*O*-glucoside; and kaempferol 3-*O*-galactoside as kaempferol 3-*O*-glucoside. Results provided in the manuscript represent a mean value of three replications and are expressed as mg per liter of the sample. Results obtained were controlled and analyzed by Chromeleon v.6.8 software (Thermo Scientific Dionex, Sunnyvale, CA, USA). Iridoids and phenolics were identified by their HPLC retention times, UV-Vis spectra, the HPLC profile, and by comparison with the known standards and literature data.

Statistical analysis

Analysis of selected data was conducted with Statistica 13.5 software (StatSoft, Tulsa, OK, USA) based on one-way analysis of variance (ANOVA test), at a significance level of $\alpha=0.05$. Differences between mean values were computed with the Duncan test (p-value <0.05). Standard deviations are provided in tables.

RESULTS AND DISCUSSION

Fermentation dynamics control

Figure 1 depicts the dynamics of the alcoholic fermentation of the analyzed worts. The fermentation dynamics of all worts were similar in the first 24 h. After 48 h, weaker dynamics was demonstrated for the sample with the addition of juice made of yellow fruits of Cornelian cherry (AY), compared to all other variants. On the third day, the greatest amount of CO_2 was emitted from the wort made of apple juice (A), and this CO_2 loss was by 20% greater than in the other samples. After 96 h, the highest fermentation dynamics was again determined in the wort produced from apple juice (A) and in the sample with the addition of yellow-fruit juice (AY). On the same day, the loss of CO_2 was by 10% lower in the variant with the addition of coral fruit juice (AC) and by 20% lower in the wort with the addition of red fruit juice (AR). In the last three days of the fermentation process, its dynamics decreased in the control sample (A) and in the variant with the addition of yellow-fruit juice. Since that moment till the end of the process, the average loss of carbon dioxide reached 12%, whereas in the other worts (AC and AR), CO_2 loss accounted for *ca.* 21%. The control of the dynamics of alcoholic fermentation in the study of fermentation processes is important because it allows for the proper course of the process [Kawa-Rygielska *et al*., 2018]. The use of various additives in specified amounts may modify parameters of the dynamics of alcoholic fermentation of fermented beverages [Roldán *et al*., 2011].

Carbohydrates, ethanol, glycerol concentrations, and pH value

Table 2 presents concentrations of sucrose, glucose, fructose, ethanol, and glycerol as well as pH values of the worts and fermented apple-Cornelian cherry beverages. The highest concentration of carbohydrates in the analyzed worts (80 g/L) was determined in the variants with the addition of juices from coral and red fruits of Cornelian cherry (AC and AR), whereas in the control sample its concentration was lower by 50 g/L. Sugars concentration in worts depends on the composition of a fruit juice, whereas sugars content in apples depends on both their variety and color of their fruits, with the red varieties having a higher sugar content [Kumar *et al*., 2018]. The worts with 10% addition of Cornelian cherry juice had a higher concentration of sugars because they represent 60% of dry matter in these fruits [Kucharska, 2012].

The fermented apple beverage (A-1) contained trace amounts of sugars, while no sucrose and glucose were detected in the fermented apple-Cornelian cherry beverages produced with the first method (M-1), irrespective of Cornelian cherry variety (AY-1, AC-1, AR-1). In the beverages produced with the second method (M-2), glucose and fructose concentration was higher (20.0 g/L), because prior to the aging process, 10% of fermented apple juice was replaced with fresh juice pressed from Cornelian cherry fruits. The addition of this fresh juice increased the concentration of sugars which were not consumed by wine yeast because the fermented beverages were decanted from above the yeast precipitate before aging, and the aging process was conducted at a temperature of 4°C at which these yeast are inactive. The sensation of sweetness is one of the most important traits of fermented beverages in the sensory assessment made by consumers who usually prefer slightly sweeter flavors [de Jesus Filho *et al*., 2018]. In the case of apple-Cornelian cherry alcoholic beverages produced in this study with the second method (M-2), the level of sweetness was not high despite the higher sugars

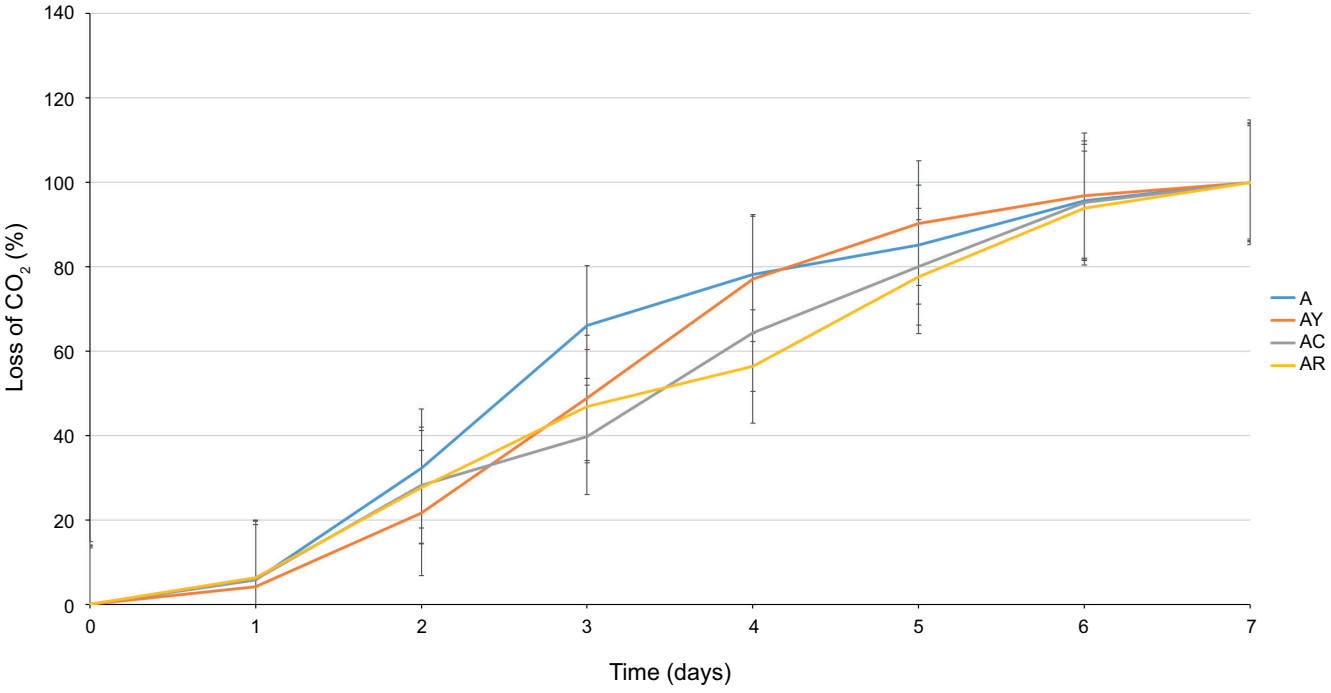

FIGURE 1. Fermentation dynamics expressed as the percentage loss of carbon dioxide.

A – apple wort, AY – apple wort with the addition of juice from yellow fruits of Cornelian cherry, AC – apple wort with the addition of juice from coral fruits of Cornelian cherry, AR – apple wort with the addition of juice from red fruits of Cornelian cherry.

TABLE 2. Concentrations of sucrose, glucose, fructose, ethanol, and glycerol (g/L) and the pH value of worts and beverages.

Variety of beverage	Stage of the process	Sucrose	Glucose	Fructose	Ethanol	Glycerol	pH
A	W[1]	1.68±0.02[c]	19.8±1.29[c]	12.7±0.02[b]	nd	nd	3.51±0.01[a3]
AY		6.93±0.07[a]	24.2±0.88[b]	49.1±0.46[a]	nd	nd	3.18±0.01[b]
AC		5.49±0.02[b]	32.3±0.49[a]	48.6±0.02[a]	nd	nd	3.25±0.01[b]
AR		6.45±0.15[a]	29.2±1.09[a]	48.3±0.01[a]	nd	nd	3.20±0.01[b]
A-1	M-1	nd[2]	0.35±0.02[e]	2.52±0.00[e]	49.1±4.88[a]	4.46±0.45[b]	3.19±0.00[b]
AY-1		nd	nd	1.44±0.01[f]	51.4±1.15[a]	5.48±0.85[a]	3.00±0.00[c]
AC-1		nd	nd	2.25±0.00[ef]	42.9±0.91[c]	4.73±0.00[b]	2.98±0.01[c]
AR-1		nd	nd	2.70±0.00[e]	47.4±0.52[b]	4.80±0.00[b]	3.02±0.04[c]
AY-2	M-2	nd	15.8±2.13[c]	10.8±0.16[cd]	42.9±1.41[c]	4.69±0.01[b]	2.93±0.01[c]
AC-2		nd	13.1±0.41[d]	11.6±0.12[c]	36.6±0.81[d]	3.34±0.08[c]	2.89±0.00[c]
AR-2		nd	13.4±0.78[d]	10.2±0.00[d]	39.0±7.79[c]	3.49±0.70[c]	2.90±0.01[c]

[1] W, wort before fermentation; M-1, beverages produced with the first method; M-2, beverages produced with the second method; [2] nd, not detected; [3] Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p-value < 0.05). Symbols: A, AY, AC, AR, A-1, AY-1, AC-1, AR-1, AY-2, AC-2, and AR-2 as explained in Table 1.

concentration than in the beverages made with the first method (M-1), because Cornelian cherry fruits are sour and bitter [Kucharska, 2012]. In addition, excessive consumption of products rich in simple sugars, including sweet beverages, may contribute to the development of many diseases [Crovetto *et al*., 2018]. For this reason, the products made with the first method (M-1) and being free of sucrose and glucose might contribute to a well-balanced diet.

Ethanol concentration of alcoholic beverages ranged from 36.60 g/L (4.6% v/v) in these produced with the addition of coral-fruit Cornelian cherry juice using the second method (AC-2) to 51.39 g/L (6.5% v/v) in these produced with the addition of yellow-fruit juice using the first method (AR-1). An equally high concentration of ethanol was determined in the control sample (A-1), which was correlated with results of their fermentation dynamics analysis. Results indicate that the first method (M-1) allows producing beverages with a higher ethanol concentration (by 7.7 g/L on average) compared to the second method (M-2). Girschik *et al*. [2017] produced ciders with a similar ethanol content – 6.1% v/v on average, while Gonzalez Flores *et al*. [2017] obtained ciders with a higher ethanol content (*ca*. 1% v/v) [Gonzalez Flores *et al*., 2017]. The level of alcohol in fermented fruit beverages depends on many factors which affect the fermentation process, including *e.g*.: variety and ripeness of fruits [Gonzalez Flores *et al*., 2017] or amount of fruits used in the production process [Caldeira *et al*., 2018]. It has been proved that the consumption of small amounts of low-alcohol orange beverages may have a beneficial effect on a human body [Hornero-Méndez *et al*., 2018; Escudero-López *et al*., 2018].

Glycerol levels in the produced apple-Cornelian cherry alcoholic beverages ranged from 3.34 to 5.48 g/L. The highest glycerol concentration was determined in the beverage with the addition of yellow Cornelian cherry juice and the lowest one – in products manufactured with the second method with the addition of coral-fruit (AC-2) and red-fruit (AR-2) juices. These levels of glycerol were comparable to the results reported for ciders by Suarez Valles *et al*. [2008], but slightly lower than these found in apple wines analyzed by Satora *et al*. [2016], *i.e.* 10.5–12.8 g/L. Glycerol is one of the basic by-products of yeast fermentation and has a significant effect on the sensory perception of the manufactured alcoholic beverages by affecting the fullness of their flavor, their sweetness sensations, and their viscosity [Gawel *et al*., 2007]. The produced worts and finished products were additionally analyzed for their acidity level based on their pH values. Among the analyzed worts, the highest pH value was measured in the variant without Cornelian cherry juice addition (A), whereas pH of the other worts was slightly lower. The mean pH value of all alcoholic apple-Cornelian cherry beverages was about 2.95, whereas the pH value of the product containing 100% of apple juice (A-1) was 0.2 higher. The lower pH values of the worts and finished beverages with the addition of juice from Cornelian cherry fruits are due to the naturally high acidity of these fruits not exceeding pH 3 [Kucharska *et al*., 2011]. The pH values of the apple-Cornelian cherry alcoholic beverages produced in our study are slightly lower compared to the values reported for the other fermented apple beverages, *i.e.* 3.5–3.7 [Girschik *et al*., 2017], 4.2–4.6 [Venkatachalam *et al*., 2018], and 3.1–3.4 [Gonzalez Flores *et al*., 2017].

Total concentration of phenolics and antioxidative properties

Results obtained in this study (Table 3) demonstrate that the addition of Cornelian cherry juice to worts caused a significant increase in their total phenolics content compared to the control wort containing 100% of apple juice (A). The highest total concentration of phenolics was determined in the worts with the addition of red-fruit juice (AR) and cor-

TABLE 3. Concentration of total polyphenols and antioxidative activity of worts and beverages.

Variety of beverage	Stage of the process	Total polyphenols content (mg GAE/L)	DPPH• (mmol TE/L)	ABTS•+ (mmol TE/L)	FRAP (mmol TE/L)
A	W[1]	683±0.70[e2]	3.99±0.04[d]	5.75±0.19[d]	7.69±0.35[gh]
AY		921±6.62b[c]	6.93±0.14[b]	11.0±0.97[ab]	8.40±0.47[e]
AC		1176±1.98[a]	7.98±0.32[a]	11.5±0.69[ab]	13.0±0.05[b]
AR		1163±1.62[a]	8.42±0.19[a]	12.2±0.63[a]	13.5±0.28[a]
A-1	M-1	482±2.25[f]	2.78±0.06[e]	4.93±0.24[d]	7.20±0.14[h]
AY-1		718±5.99[e]	5.46±0.22[c]	8.81±0.63[c]	10.0±0.30[e]
AC-1		825±5.97[d]	7.08±0.46[b]	10.1±0.44[abc]	11.9±0.17[cd]
AR-1		915±1.59[bc]	7.78±0.03[a]	10.5±0.86[abc]	12.5±0.13[bc]
AY-2	M-2	855±2.26[cd]	5.90±0.26[c]	9.75±2.95[bc]	11.5±0.22[d]
AC-2		901±4.06[bc]	7.13±0.60[b]	10.3±0.20[abc]	12.2±0.10[c]
AR-2		964±2.69[b]	7.90±0.07[a]	11.0±0.97[ab]	12.9±0.23[b]

[1] W, wort; M-1, beverages produced with the first method; M-2, beverages produced with the second method; [2] Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters: a, b, c etc. are statistically different ($p < 0.05$). Symbols: A, AY, AC, AR, A-1, AY-1, AC-1, AR-1, AY-2, AC-2, and AR-2 as explained in Table 1.

al-fruit juice (AC) and it was by *ca.* 490 mg GAE/L higher than in the control wort (A). The total phenolics content in the wort with the addition of juice made of yellow-fruit variety of Cornelian cherry (AY) was higher by 240 mg GAE/L than in the control wort. The antioxidative activity of the worts measured with the DPPH•, ABTS•+, and FRAP tests was the highest in the worts with the addition of red-fruit juice (AR) and coral-fruit juice (AC) and slightly lower in the wort with the addition of yellow-fruit juice (AY). The addition of Cornelian cherry fruit juice to worts significantly enhanced their antioxidative properties compared to the control wort (A), whose antioxidative capability was by even 6.4 mmol TE/L lower than that of the wort with red-fruit juice addition (AR) – according to results of the ABTS•+ test.

The analysis of the total phenolics content in the fermented fruit beverages demonstrated that the addition of juice from Cornelian cherry fruits caused *ca*. twofold increase in the concentration of these compounds compared to the 100% apple beverage (A). In addition, the second method of production, in which Cornelian cherry juice was added to wort before the beginning of the aging process (M-2), enabled manufacturing beverages with higher concentrations of phenolics compared to the first method (M-1) in which juice was added to wort before the fermentation process. The highest concentration of phenolics was determined in the samples with the addition of red-fruit juice (AR-1, AR-2), however, it was by *ca*. 50 mg GAE/L higher in the beverages produced with the second method (AR-2). The antioxidative activity determined in the finished products with the DPPH• test was the highest in the beverages with the addition of red-fruit juice (AR-1, AR-2), and slightly lower in the samples with coral-fruit juice (AC-1, AC-2) and yellow-fruit juice (AY-1, AY-2). The statistical analysis of results achieved with the DPPH• method showed no significant differences in the antioxidative capabilities of products manufactured with methods M-1 and M-2. According to the DPPH• assay, the antioxidative activity of the control sample (A-1) was nearly two- and three times lower than in the samples with 10% addition of Cornelian cherry juice. Similar results were obtained in the ABTS•+ assay which also demonstrated that the antioxidative capability of the control sample (A-1) was almost twofold lower than in the variants with Cornelian cherry juice addition. The fermented apple-Cornelian cherry beverages produced in our study had similar antioxidative properties. The strongest antioxidative properties assayed with the ABTS•+ test (like in the case of DPPH• test and determination of total phenolics content) were exhibited by the sample with the addition of juice from red fruits of Cornelian cherry (AR-1, AR-2). The antioxidative capabilities were stronger in the beverages produced with the second method. The above-discussed data confirm results obtained in the Fe^{3+} reducing capability test (FRAP), in which the control sample had a weaker antioxidative capability (by 4.6 mmol TE/L beverage on average) than the beverages produced with the addition Cornelian cherry juice. Irrespective of the production method, the highest antioxidative capability measured with the FRAP test was demonstrated for the beverages with the addition of juice from red and coral fruits of Cornelian cherry (AR-1, AR-2, AC-1, AC-2). Despite a lack of significant differences in the antioxidative activity between beverages with coral-fruit and red-fruit juice, measured with the FRAP assay, results obtained allow concluding that – like in the case of the other analytical methods – slightly stronger antioxidative capabilities were determined in the beverages produced with the second method (AC-2, AR-2) compared to these produced with the first method (AC-1, AR-1). This is confirmed by results achieved for the variant with yellow-fruit juice addition, in which the beverage manufactured with the second method (AY-2) exhibited significantly stronger antioxidative activity (by *ca.* 1.5 mmol TE/L) compared to the beverage manufactured with the first

method (AY-1). The significant increase in the concentration of phenolics and antioxidative activity demonstrated in the beverages produced with the addition of juice from Cornelian cherry fruits results from the confirmed high content of natural antioxidants in these fruits [Kucharska, 2012; De Biaggi *et al*., 2018]. Studies have proved that various food products made of Cornelian cherry fruits or with their addition have a higher nutritional value [Bozdogan *et al*., 2017; Kawa-Rygielska *et al*., 2018]. This also applies to alcoholic beverages made of Cornelian cherry fruits, including fruit distillates or liqueurs [Kucharska, 2012; Sokół-Łętowska *et al*., 2014; Kawa-Rygielska *et al*., 2019]. Similar results were reported for fruit meads made with the addition of juices from different varieties of Cornelian cherry where the highest concentration of total phenolics and the strongest antioxidative capabilities measured with the same tests were determined for the mead produced with red-fruit juice [Adamenko *et al*., 2018]. Fruit wines analyzed by Ortiz *et al*. [2013] were characterized by a slightly higher concentration of total phenolics, reaching on average 999 mg GAE/L in apple-blackberry wines and 608 mg GAE/L in apple wines, but by a lower antioxidative activity measured with the DPPH$^{\bullet}$ test, accounting for 6.2 and 2.8 mmol TE/L, respectively. Substantially weaker antioxidative capability was demonstrated by Alberti *et al*. [2016] for apple ciders, *i.e.* barely 0.4–0.8 mmol TE/L cider according to the FRAP test. Polish apple ciders assayed with the ABTS$^{\bullet+}$ method by Kowalczyk *et al*. [2015] also exhibited a lower antioxidative activity, which reached 3.0 mmol TE/L on average.

Identification of iridoids and phenolics

Table 4 lists compounds identified in the produced worts and fermented apple-Cornelian cherry beverages which included four iridoids: loganic acid (LA), sweroside (S), loganin (L), and cornusides (Co and Co1); seven phenolic acids: 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA), 4-*O*-caffeoylquinic acid (4-CQA), caffeoylquinic acid derivatives (CQA1 and CQA2), *p*-coumaroylquinic acid (*p*-CoQA), and ellagic acid (EA); five anthocyanins: delphinidin 3-*O*-galactoside (Df 3-gal), cyanidin 3-*O*-galactoside (Cy 3-gal), cyanidin 3-*O*-robinobioside (Cy 3-rob), pelargonidin 3-*O*-galactoside (Pg 3-gal), and pelargonidin 3-*O*-robinobioside (Pg 3-rob); four flavanols: procyanidin dimer B1 (Dimer B1), (+)-catechin (Cat), procyanidin dimer B2 (Dimer B2), and (–)-epicatechin (epiC); four dihydrochalcones: 3-hydroxyphloretin 2'-*O*-xyloglucoside (3-OH-Ph 2'-xylglc), 3-hydroxyphloretin 2'-*O*-glucoside (3-OH-Ph 2'-glc), phloretin 2'-*O*-xyloglucoside (Ph 2'-xylglc), and phloretin 2'-*O*-glucoside (Ph 2'-glc) as well as four flavonols: quercetin 3-*O*-galactoside (Q 3-gal), quercetin 3-*O*-glucuronide (Q 3-glr) + quercetin 3-*O*-glucoside (Q 3-glc), kaempferol 3-*O*-galactoside (Kf 3-gal), and quercetin 3-*O*-xyloside (Q 3-xyl). 3-CQA, *p*-CoQA, dihydrochalcones, and quercetin 3-*O*-xyloside were detected only in the worts and beverages containing 100% of apple juice, which is due to the fact that these compounds are typical components of apples but do not occur in Cornelian cherry fruits [Alberti *et al*., 2016; Laaksonen *et al*., 2017]. Whereas compounds typical of the Cornelian cherry fruits, such as iridoids, ellagic acid, anthocyanins, quercetin 3-*O*-glucuronide, and kaempferol 3-*O*-galactoside, were identified only in worts and beverages containing juice pressed from these fruits (Table 3). In the apple-Cornelian cherry beverages, of all the identified compounds, the most abundant were iridoids, among which LA was the predominating compound (87–91%). The highest concentrations of these compounds were determined in worts and in beverages produced with the addition of coral-fruit juice (AC, AC-1, AC-2). 5-CQA was found to predominate among phenolic acids. Its highest concentration (93.5 mg/L on average) was assayed in the samples containing 100% of apple juice (A, A-1). In contrast, no EA was identified in these variants, but it was detected in all samples containing juice from Cornelian cherry fruits. Anthocyanins were not detected in worts and in the beverages made of apple juice (A, A-1) nor in the beverages containing juice from yellow fruits of Cornelian cherry (AY, AY-1, AY-2). The samples with coral-fruit juice (AC, AC-1, AC-2) contained only Cy 3-gal and Pg 3-gal, whose concentrations were several times lower than in worts and in the beverages with the addition of red-fruit juice (AR, AR-1, AR-2). In the latter samples, Cy 3-gal and Pg 3-gal were identified as the predominating anthocyanins and their contents constituted 73–76% of all detected anthocyanins. Among flavanols, Dimer B2, and epiCat were identified in all produced worts and fermented beverages, whereas Dimer B1 and Cat were detected only in the variants containing 100% of apple juice (A, A1). All worts and beverages contained a complete pool of identified dihydrochalcones. Flavonoles represented the smallest group of the identified phenolics; Kf 3-gal was identified only in the samples containing juice from red fruits of Cornelian cherry (AR, AR-1, AR-2), whereas concentration of Q 3-xyl was the same in all samples. Furthermore, Q 3-gal and Q 3-glr + Q 3-glc were detected in all analyzed variants. Their lowest concentrations were determined in the products without Cornelian cherry juice (A, A-1), whereas the highest concentrations were determined in the samples containing juice from coral and red fruits of Cornelian cherry. Our study demonstrated that the production method of fermented apple-Cornelian cherry beverages had no significant effect on concentrations of iridoids, phenolic acids, dihydrochalcones, and flavonols in the finished products. An opposite observation was made for anthocyanins and flavanols, whose higher concentrations were determined in the products manufactured with the second method in which Cornelian cherry juice was added after the effervescent fermentation (M-2). This points to greater degradation of anthocyanins and flavanols – being less stable compounds – during ethanolic fermentation compared to the other compounds. The composition of phenolics in the control variants without Cornelian cherry juice addition (A, A-1) is similar to the polyphenolic profiles reported by other authors [Venkatachalam *et al*., 2018;, Alberti *et al*., 2016; Laaksonen *et al*., 2017], who analyzed this group of compounds in apple ciders and also identified phenolics, flavonols, flavanols, and dihydrochalcones, however failed to identify any representatives of iridoids, which were detected in the fermented apple-Cornelian cherry beverages produced in our study. In turn, Marks *et al*. [2007] who investigated phenolics composition of 23 English ciders, demonstrated the presence of compounds from four groups of phenolics,

TABLE 4. Concentrations of iridoids and phenolics (mg/L) in worts and beverages.

No	Compound	Wort before fermentation				Beverages produced with the first method				Beverages produced with the second method		
		A	AY	AC	AR	A-1	AY-1	AC-1	AR-1	AY-2	AC-2	AR-2
						Iridoids						
1	LA[1]	nd[3]	464±4.53[b4]	521±4.53[a]	327±4.33[d]	nd	437±6.31[c]	513±4.38[a]	322±4.35[d]	429±7.07[c]	516±7.35[a]	325±3.95[d]
2	S + L	nd	22.4±3.45[a]	23.2±2.96[a]	16.5±3.11[b]	nd	19.7±0.86[ab]	23.0±4.24[a]	16.2±1.63[b]	19.7±1.41[ab]	23.6±1.56[a]	16.4±2.90[b]
3	SecoL	nd	3.24±0.28[ab]	4.03±0.17[a]	3.56±0.42[ab]	nd	2.85±0.57[b]	3.45±0.28[ab]	2.93±0.18[b]	3.15±0.43[b]	3.65±0.14[b]	3.36±0.33[ab]
4	Co	nd	35.6±3.21[a]	35.2±1.67[a]	27.7±1.83[bc]	nd	22.5±2.10[d]	24.4±1.84[cd]	19.9±1.98[d]	31.0±1.41[ab]	34.9±2.34[a]	27.7±2.11[bc]
5	Co1	nd	tr[2]	2.19±0.08[a]	0.40±0.04[c]	nd	tr	1.44±0.06[b]	0.38±0.09[c]	tr	1.49±0.11[b]	0.36±0.03[c]
						Phenolic acids						
1	3-CQA	0.57±0.07[def]	0.55±0.05[def]	0.63±0.03[cde]	0.81±0.04[a]	0.54±0.04[def]	0.49±0.06[f]	0.65±0.01[cd]	0.80±0.04[a]	0.52±0.03[ef]	0.68±0.06[bc]	0.78±0.06[ab]
2	5-CQA	96.8±4.53[a]	81.1±4.30[b]	77.9±2.98[bc]	81.9±2.40[b]	90.3±2.86[a]	70.0±3.13[d]	74.9±3.53[bcd]	78.9±2.02[bc]	72.0±3.21[cd]	76.2±2.88[bcd]	79.8±3.12[bc]
3	4-CQA	6.95±0.67[bc]	8.38±0.92[a]	9.04±0.27[a]	6.66±0.28[c]	6.63±0.71[c]	6.33±0.45[c]	8.14±0.35[ab]	5.77±0.60[c]	6.33±0.69[c]	8.73±0.52[a]	6.32±0.28[c]
4	CQA 1	1.78±0.33[a]	1.55±0.11[ab]	1.41±0.25[ab]	1.35±0.18[ab]	1.49±0.36[ab]	1.26±0.06[b]	1.36±0.13[ab]	1.27±0.12[b]	1.23±0.08[b]	1.19±0.23[b]	1.13±0.13[b]
5	CQA 2	0.51±0.04[c]	0.51±0.03[c]	0.47±0.03[c]	0.47±0.03[c]	0.88±0.04[a]	0.53±0.08[c]	0.54±0.03[c]	0.55±0.01[c]	0.65±0.06[b]	0.65±0.03[b]	0.69±0.04[b]
6	p-CuQA	6.94±0.59[bcd]	7.43±0.16[abc]	7.05±0.38[bcd]	8.15±0.57[a]	6.22±0.45[de]	5.89±0.45[e]	6.50±0.34[cde]	7.77±0.37[ab]	6.05±0.23[de]	6.83±0.58[bcde]	7.79±0.31[ab]
7	EA	nd	0.10±0.02[e]	0.18±0.02[cd]	0.20±0.04[bc]	nd	0.13±0.03[de]	0.25±0.03[b]	0.22±0.02[bc]	0.10±0.01[e]	0.37±0.03[a]	0.18±0.01[cd]
						Anthocyanins						
1	Df 3-gal	nd	nd	nd	0.69±0.01[a]	nd	nd	nd	0.26±0.03[c]	nd	nd	0.58±0.02[b]
2	Cy 3-gal	nd	nd	0.47±0.03[b]	10.2±0.47[b]	nd	nd	0.20±0.01[b]	4.63±0.33[b]	nd	0.39±0.03[b]	8.86±0.28[a]
3	Cy 3-rob	nd	nd	nd	4.81±0.20[a]	nd	nd	nd	2.94±0.18[b]	nd	nd	4.41±0.12[a]
4	Pg 3-gal	nd	nd	3.37±0.33[d]	20.0±0.47[a]	nd	nd	1.78±0.17[e]	10.9±0.36[c]	nd	2.85±0.19[d]	17.8±0.13[b]
5	Pg 3-rob	nd	nd	nd	3.77±0.21[a]	nd	nd	nd	2.59±0.23[b]	nd	nd	3.49±0.22[a]
						Flavanols						
1	Dimer B1	4.46±0.37[a]	nd	nd	nd	4.32±0.42[a]	nd	nd	nd	nd	nd	nd
2	Cat	1.89±0.31[a]	nd	nd	nd	1.90±0.12[a]	nd	nd	nd	nd	nd	nd
3	Dimer B2	18.9±0.86[b]	19.3±0.57[b]	19.6±0.55[b]	22.3±0.94[a]	14.2±0.25[d]	14.2±0.51[d]	16.3±0.40[c]	19.0±0.42[b]	16.3±0.31[c]	18.9±1.00[b]	20.3±0.76[b]
4	epiCat	20.2±0.85[a]	14.8±0.98[de]	18.2±0.93[ab]	18.9±1.38[ab]	18.6±0.62[ab]	16.7±1.02[bcd]	15.7±0.87[cd]	13.3±0.61[e]	18.8±0.90[ab]	16.8±0.93[bcd]	17.8±0.99[bc]

Dihydrochalcones											
1	3-OH-Ph 2'-xylglc	12.9±1.30^{a}	11.2±1.03^{bc}	10.7±0.46^{bc}	10.9±0.40^{bc}	11.4±0.88^{ab}	9.8±0.74^{bc}	9.8±0.82^{bc}	10.1±0.44^{bc}	9.55±0.73^{c}	10.3±0.33^{bc}	9.96±0.42^{bc}
2	3-OH-Ph 2'-glc	6.82±0.53^{a}	5.27±0.37^{b}	4.99±0.49^{b}	5.51±0.64^{a}	6.81±0.25^{b}	5.18±0.33^{b}	5.01±0.46^{b}	5.69±0.40^{b}	5.16±0.41^{b}	5.28±0.41^{b}	5.73±0.34^{b}
3	Ph 2'-xylglc	9.85±0.85^{a}	8.05±0.79^{bc}	7.86±0.81^{c}	8.29±0.42^{abc}	9.00±0.63^{ab}	6.75±0.62^{bc}	7.10±0.77^{c}	7.46±0.52^{bc}	6.80±0.47^{c}	7.60±0.64^{bc}	7.71±0.82^{bc}
4	Ph 2'-glc	11.6±0.78^{a}	9.97±0.35^{b}	9.78±0.23^{b}	9.80±0.30^{b}	11.6±0.77^{a}	9.04±0.27^{b}	9.55±0.19^{b}	9.68±0.24^{b}	9.38±0.20^{b}	9.64±0.25^{b}	9.79±0.22^{b}
Flavonols												
1	Q 3-gal	0.80±0.07^{d}	1.06±0.12^{bcd}	1.46±0.19^{a}	1.32±0.18^{ab}	0.81±0.02^{d}	0.91±0.09^{d}	1.33±0.16^{ab}	1.26±0.11^{abc}	1.01±0.06^{cd}	1.37±0.07^{a}	1.37±0.10^{a}
2	Q 3-glcr + Q 3-glc	0.30±0.04^{d}	5.30±0.41^{b}	6.93±0.25^{a}	4.38±0.39^{c}	0.28±0.06^{d}	4.50±0.40^{c}	6.48±0.42^{a}	4.02±0.28^{c}	4.72±0.24^{bc}	6.84±0.29^{a}	4.34±0.28^{c}
3	Kf 3-gal	nd	nd	nd	3.32±0.06^{a}	nd	nd	nd	2.91±0.12^{a}	nd	nd	3.21±0.24^{a}
4	Q 3-xyl	1.38±0.11^{a}	1.22±0.25^{a}	1.26±0.16^{a}	1.19±0.13^{a}	1.32±0.08^{a}	1.05±0.14^{a}	1.16±0.08^{a}	1.12±0.06^{a}	1.11±0.12^{a}	1.24±0.18^{a}	1.18±0.08^{a}

[1]LA, loganic acid; S, sweroside; L, loganin; Co, cornuside; 3-CQA, 3-*O*-caffeoylquinic acid (neochlorogenic acid); 5-CQA, 5-*O*-caffeoylquinic acid (chlorogenic acid); 4-CQA, 4-*O*-caffeoylquinic acid (cryptochlorogenic acid); CQA, caffeoylquinic acid; *p*-CoQA, *p*-coumaroylquinic acid; EA, ellagic 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; Dimer B1, procyanidin dimer B1; C, (+)-catechin; Dimer B2, procyanidin dimer B2; epiC, (−)-epicatechin; 3-OH-Ph 2'-xylglc, 3-hydroxyphloretin 2'-*O*-xyloglucoside; 3-OH-Ph 2'-glc, 3-hydroxyphloretin 2'-*O*-glucoside; Ph 2'-xylglc, phloretin 2'-*O*-xyloglucoside; Ph 2'-glc, phloretin 2'-*O*-glucoside; Q 3-gal, quercetin 3-*O*-galactoside; Q 3-glcr, quercetin 3-*O*-glucuronide; Q 3-glc, quercetin 3-*O*-glucoside; Kf 3-gal, kaempferol 3-*O*- galactoside; Q 3-xyl, quercetin 3-*O*-xyloside; [2]tr, traces: detected but not quantified; [3]nd, not detected; [4]Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters: a, b, c etc. are statistically different ($p < 0.05$); Symbols: A-1, AY-1, AC-1, AR-1, AY-2, AC-2, and AR-2 as explained in Table 1.

i.e.: flavan-3-ols, hydroxycinnamates, flavonols, and dihydrochalcones. Analysis of the phenolics composition of the ciders indicated that it depended on the variety of apples and on the apple processing method. The English ciders were also characterized by a richer composition of phenolics compared to the earlier investigated apple juices [Marks *et al*., 2007].

CONCLUSIONS

Results achieved in this study prove that the produced apple-Cornelian cherry alcoholic beverages had strong antioxidative properties and high concentrations of natural antioxidants. The 10% addition of juice from red-fruit Cornelian cherry enabled producing beverages with the highest concentration of these compounds and, consequently, with the highest antioxidative capabilities. Apart from such phenolics as phenolic acids, anthocyanins or flavonols, they contained many compounds from the group of iridoids, in particular in the products with red and coral-fruit Cornelian cherry. Production method affected both concentrations of the mentioned compounds and antioxidative properties of the beverages. Juice addition after the primary fermentation caused better effects than its addition before this process. The novel natural fermented beverages produced in this study may be found interesting products on the market of alcoholic beverages and may also complete an everyday diet in phenolics. In the future, it is necessary to perform consumer sensory analysis for a deeper understanding of the scientific significance of the new products, as well as the possibility of introducing these beverages to the market.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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