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Physicochemical and antioxidative properties of Cornelian cherry beer

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ABSTRACT

The aim of this study was to determine the possibility of the use of Cornelian cherry (CC) juices in brewing technology. We analyzed basic physicochemical properties, concentration of polyphenols and iridoids, and antioxidative activity of brewed beer. The concentration of total polyphenols (F-C) in CC beer ranged from 398.1 to 688.7 mg GAE/L beer. The antioxidative activity measured with the DPPH˙ and FRAP assays was the highest in the beer with the addition of juice from red-fruit CC cultivar. Among the identified iridoids, loganic acid was the predominating compounds and its highest concentration, accounting for 184.6 mg LA/L beer, was found in the beer with juice made of coral-fruit CC cultivar. The identified polyphenols included anthocyanins and flavonol derivatives. The novelty of this study was to brewed beers containing compounds from the group of iridoids.

1. Introduction

Beer is one of the oldest and most often consumed alcoholic beverages across the world. It is produced via alcoholic fermentation by yeast which transform sugars contained in malt wort mainly to ethyl alcohol and carbon dioxide. Beer contains also hop and, optionally, some additives (Denby et al., 2018). It is rich in carbohydrates, amino acids, minerals, vitamins, and phenolic compounds, that derive mainly from malt and hop (Sohrabvandi, Mortazavian, & Rezaei, 2012). The global beer market faces a significant increase in artisan beer because beer-lovers search for products other than those produced on the mass scale (Aquilani, Laureti, Poponi, & Secondi, 2015).

Enrichment of beer with fruits may impart new flavors to it and may also increase concentrations of its bioactive compounds (Ducruet et al., 2017). A wide spectrum of compounds with antioxidative effects is offered by, e.g., fruits of Cornelian cherry (*Cornus mas* L.). Extracts and products manufactured from these fruits are characterized by high contents of phenolic and iridoid compounds which exhibit hypotensive, antibiotic or anti-inflammatory potential (Kucharska, 2012; Kucharska, Szumny, Sokół-Łętowska, Piórecki, & Klymenko, 2015; Kawa-Rygielska, Adamenko, Kucharska, & Piórecki, 2018). Their content in Cornelian cherry fruits and products is mainly cultivar-dependent (Kucharska, 2012; Kucharska et al., 2015). In addition, the phenolic compounds (anthocyanins in particular) are sensitive to many factors of the production process. Hence, the appropriate choice of a cultivar and the use of the optimal technological process are essential to preserve high levels of active compounds in the finished product. We found no works in the available literature on the characteristics and properties of Cornelian cherry beer. Therefore, the aim of this research was to determine the possibility of using Cornelian cherry fruits for brewing beer including iridoids and characterized by very high antioxidative activity.

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2. Materials and methods

2.1. Materials

2.1.1. Biological material

Saccharomyces cerevisiae Safbrew S-33 (S-33) yeast from the Fermentis company (Lesaffre, France) were used for wort fermentation. Yeast was rehydrated in pure water for 30 min at 25 °C, than inoculated into the wort in the amount of 0.58 g d.m./L, in accordance with the manufacturer's recommendations.

2.1.2. Raw material

Cornelian cherry fruits (*C. mas* L.) from 3 cultivars: 'Podolski' (red color), 'Yantarnyi' (yellow color) and 'Koralovyi' (coral color) were harvested in the Arboretum in Bolestraszyce, near Przemyśl, Poland in September 2016, and immediately frozen at −20 °C. Fruits were pressed through the Zodiak laboratory hydraulic press from SRSE company (Warsaw, Poland). The extract content in fruit juices were: 16%w/w of yellow juice, 15%w/w of coral juice and 18% of red juice. Pale Ale malt (Bath number: 292/2017) and hops 'Marynka' (Bath numbers: F029006) were purchased from the Viking Malt company (Strzegom, Poland) and Biowin company (Łódź, Poland), respectively. Malt was ground through a laboratory grinder from Brabender company (Duisburg, Germany), which is intended for crumbling malt with proper grind.

2.2. Brewing technology

For mashing 5 kg of malt and 17.5 kg of water were used, i.e. in a weight ratio of 1:3.5 (malt:water). The infusion mashing with stirring was conducted under laboratory conditions: water was heated to a temperature of 67 °C, malt was then added whilst maintaining the temperature 70 min; next, the temperature was increased to 76 °C with to 10 min of step time. The ready mash was filtrated by means of filter paper MN 614 ¼ from MACHEREY-NAGEL company (Düren, Germany) and the resultant wort was boiled with hop (1 g/L) for 60 min. The boiled wort was cooled to a temperature of 25 °C and filtered again to obtain the control wort (W0), in which the initial extract content was measured by the use of Densito 30PX densimeter (Mettler Toledo, Columbus, OH, USA) by cooling wort to the temperature of 20 °C and established at 12 °Bx (Analytica-EBC, 2010). Table 1 provides explanations of all acronyms used in the manuscript. The scheme of preparation of particular experimental variants is provided in Scheme 1 (supplementary material). Fermentation was carried out in 2 L glass fermentation flasks.

2.3. Analytical methods

2.3.1. Carbohydrate profile and glycerol

Carbohydrate profile and glycerol were analyzed by High Performance Liquid Chromatography (HPLC) (Pietrzak, Kawa-Rygielska, Król, Lennartsson, & Taherzadeh, 2016). Samples of beer were degassed and centrifuged (2675 centrifugal force (RCF), 6000 rpm, 10 min) than both samples of beer and worts were diluted with bi-distilled water in the ratio of 1:2 (in volume). Further analysis was performed as described in the studies of Kawa-Rygielska et al. (2018). The samples were analyzed using a Prominence liquid chromatography system (Shimadzu Corp., Kyoto, Japan) with 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 analyzed compounds were determined based on a five-point calibration curve integrated in Chromax 10.0 software by Pol-Lab (Wilkowice, Poland). All measurements were performed in triplicate.

2.3.2. Basic physico-chemical parameters

Extract content, degree of fermentation, concentration of ethyl alcohol, and density of beer were measured with the near infrared (NIR) spectroscopy using an Anton Paar DMA 4500 M oscillating densitometer (Graz, Austria). Beer were degassed and centrifuged as in point 2.3.1 of section, and then filtered on laboratory filter papers and subjected to analyses. The pH value of beer was measured with a Mettler Toledo MP 240 pH-meter (Columbus, USA). All measurements were performed in triplicate.

2.3.3. Analysis of beer bitterness (IBU)

To determine bitterness level of the brewed beers, 10 mL of degassed beer were transferred to Falcon tubes (35 mL), and 0.5 c of a hydrochloric acid solution (6 N HCl) and then 20 mL isoacetate were added to the tube (Analytica-EBC, 2010). The tubes were shaken manually for 5 min. Next, 10 mL of the sample were transferred to Falcon centrifugation tubes (15 mL) and centrifuged (3000 rpm, 5 min). A sample for analysis was collected from the isoacetate layer and determined spectrophotometrically by measuring its absorbance at a

Table 1
Symbols and description of fruit juices, beer production methods, worts and beer obtained in the study.

Symbol	Description	
Y	cherry fruit juices	juice from a yellow fruits of Cornelian cherry
C		juice from a coral fruits of Cornelian cherry
R		juice from a red fruits of Cornelian cherry
M-1	beer production methods	method 1 of Cornelian cherry beer brewed with juice addition, which was added in the amount of 10% of wort volume before the beginning of the primary (effervescent) fermentation
M-2		method 2 of Cornelian cherry beer brewed with juice addition, which was added in the amount of 10% of wort volume before the beginning of the secondary (silent) fermentation
SF		primary fermentation
CF		secondary fermentation
W0	worts	wort without addition
WY		wort with the addition of juice from yellow fruits of Cornelian cherry
WC		wort with the addition of juice from coral fruits of Cornelian cherry
WR		wort with the addition of juice from red fruits of Cornelian cherry
B0	beer	beer without juice
BY1		beer with the addition of juice from yellow fruits of Cornelian cherry brewed with method 1
BC1		beer with the addition of juice from coral fruits of Cornelian cherry brewed with method 1
BR1		beer with the addition of juice from red fruits of Cornelian cherry brewed with method 1
BY2		beer with the addition of juice from yellow fruits of Cornelian cherry brewed with method 2
BC2		beer with the addition of juice from coral fruits of Cornelian cherry brewed with method 2
BR2		beer with the addition of juice from red fruits of Cornelian cherry brewed with method 2

Table 2
Basic physico-chemical parameters of the obtained worts and beer.

Variety of wort or beer	Ethyl alcohol		Extract		Degree of fermentation		Wort extract	density
	%v/v	%w/w	apparent (%w/w)	real (%w/w)	apparent (%)	real (%)	%w/w	g/mL
W0	na[1]	na	na	na	na	na	12.69 ± 0.07[f]	1.0338 ± 0.02[b]
WY	na	na	na	na	na	na	12.99 ± 0.05[cd]	1.0500 ± 0.00[a]
WC	na	na	na	na	na	na	12.96 ± 0.01[d]	1.0499 ± 0.00[a]
WR	na	na	na	na	na	na	13.13 ± 0.03[ab]	1.0507 ± 0.00[a]
B0	4.68 ± 0.04[c2]	3.65 ± 0.04[c]	3.70 ± 0.05[ab]	5.39 ± 0.04[a]	70.30 ± 0.46[d]	56.72 ± 0.00[d]	12.44 ± 0.04[h]	1.0126 ± 0.00[c]
BY1	5.05 ± 0.01[a]	3.94 ± 0.00[a]	3.36 ± 0.00[c]	5.18 ± 0.01[a]	73.71 ± 0.02[a]	59.49 ± 0.00[a]	12.78 ± 0.01[e]	1.0113 ± 0.00[c]
BC1	4.89 ± 0.02[b]	3.81 ± 0.01[b]	3.42 ± 0.08[c]	5.18 ± 0.08[a]	72.79 ± 0.54[b]	58.74 ± 0.14[b]	12.55 ± 0.05[g]	1.0115 ± 0.00[c]
BR1	5.09 ± 0.01[a]	3.97 ± 0.01[a]	3.41 ± 0.03[c]	5.24 ± 0.03[a]	73.57 ± 0.23[a]	59.38 ± 0.37[a]	12.90 ± 0.00[d]	1.0115 ± 0.00[c]
BY2	5.04 ± 0.01[b]	3.93 ± 0.01[a]	3.68 ± 0.00[b]	4.71 ± 0.00[a]	71.81 ± 0.04[c]	57.96 ± 0.01[c]	13.07 ± 0.00[bc]	1.0125 ± 0.00[c]
BC2	5.08 ± 0.04[a]	3.95 ± 0.03[a]	3.79 ± 0.03[a]	5.61 ± 0.00[a]	71.36 ± 0.17[c]	57.59 ± 0.37[c]	13.21 ± 0.06[a]	1.0129 ± 0.00[c]
BR2	5.05 ± 0.02[a]	3.93 ± 0.01[a]	3.74 ± 0.01[ab]	5.56 ± 0.00[a]	71.49 ± 0.13[c]	57.66 ± 0.14[c]	13.13 ± 0.01[ab]	1.0128 ± 0.00[c]

[1]na – not applicable.
[2]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).

wavelength of 275 nm. Pure isoacetate was used as the standard. The formula that was used to calculate the IBU was: IBU = 50*A (A – absorbance at 275 nm). All measurements were performed in triplicate.

2.3.4. Total polyphenols content and antioxidative activity

2.3.4.1. Total polyphenols content. The total polyphenolic content of the beer was determined using the Folin-Ciocalteu (F-C) spectrophotometric method (Prior, Wu, & Schaich, 2005). Beer samples and F-C reagent were pipetted into cuvettes. After 3 min, 1 mL of a 20% aqueous solution of sodium carbonate (Na_2CO_3) and 2 mL of distilled water were added. The absorbance at 765 nm was measured after 1 h, and the results were expressed as mg of gallic acid equivalents (GAE) per liter of beer. Data were expressed as the mean value for three measurements.

2.3.4.2. Free-radical-scavenging ability by the use of a DPPH radical. The antiradical activity was determined using a $DPPH^{\cdot}$ assay (Yen & Chen, 1995). 0.1 mL samples of beer were mixed with 2 mL of 0.04 mmol/L $DPPH^{\cdot}$ in methanol and 0.4 mL of H_2O. After 10 min of incubation at room temperature, the absorbance was measured with a spectrophotometer at 517 nm using disposable polystyrene cuvettes. A calibration curve was prepared with Trolox solution (0.05 × 10–1 mmol/L). The data were expressed as Trolox equivalent (TE) of antioxidative capacity per liter of the beer (mmol TE/L). All measurements were performed in triplicate. Calibration curves, in the range 2–10 µmol TE/L, showing good linearity (r2 ≥ 0.998).

2.3.4.3. Free-radical-scavenging ability by the use of a ABTS radical cation. The antioxidative activity of beer was determined using the $ABTS^{\cdot+}$ assay (Re et al., 1999). 0.06 mL samples of beer were mixed with 3 mL of $ABTS^{\cdot+}$ solution with measured absorption of 0.700 at a wavelength of 734 nm. After 6 min the absorbance of samples was measured. Each sample was tested in triplicate. The data were expressed as mmol Trolox equivalent of antioxidative capacity per liter of the beer (mmol TE/L). Calibration curves, in the range 1.70–21.70 µmol TE/L, showed good linearity (r2 ≥ 0.999).

2.3.4.4. Ferric Reducing/Antioxidant Power (FRAP) assay. The FRAP is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe (III)-TPTZ] to the ferrous complex at low pH, followed by spectrophotometric analysis (Benzie & Strain, 1996). Briefly, the reagent was prepared by mixing 10 mmol 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)/L reagent with 20 mmol/L ferric chloride in acetate buffer (pH 3.6). Quantitative analyses were performed by the external standard method using ferrous sulfate (2 × 10–1 mmol/L) as the reference standard and correlating the absorbance (λ 593 nm) with the concentration. 0.1 mL samples of beer were mixed in polystyrene cuvettes with 0.9 mL of distilled water and 3 mL of ferric complex. The results were calculated and expressed as millimoles of Trolox per liter of the beer. The absorbance was read in disposable polystyrene cuvettes using a spectrophotometer. All measurements were performed in triplicate. Calibration curves, in the range 1.25–12.50 µmol TE/L, showed good linearity (r2 ≥ 0.998).

2.3.5. Quantification of iridoids and polyphenols by HPLC-PDA

The details of the analysis are described in the publication by Kucharska et al. (Kucharska, Sokół-Łętowska, Oszmiański, Piórecki, & Fecka, 2017). The HPLC-PDA analysis was performed using a Dionex (Germering, Germany) system equipped with the diode array detector model Ultimate 3000, quaternary pump LPG-3400A, autosampler EWPS-3000SI, thermostated column compartment TCC-3000SD, and controlled by Chromeleon v.6.8 software (Thermo Scientific Dionex, Sunnyvale, CA, USA). Iridoids were detected at 245 nm, flavonols at 360 nm, and anthocyanins at 520 nm. The results are expressed as mg per liter of the beer.

2.4. Statistics

Selected data were processed using Statistica 13.5 software (StatSoft, Tulsa, OK, USA), using the ANOVA (α = 0.05). Duncan test analyzed the differences between mean (p < 0.05). The tables show the values of the mean and standard deviation deviation.

3. Results and discussion

3.1. Basic physicochemical parameters, bitterness level, pH value and concentrations of carbohydrates and glycerol

The basic physicochemical parameters of the prepared worts and brewed beer are presented in Table 2. The brewed beer were analyzed for the concentration of ethyl alcohol. The addition of juices allowed producing beer with a higher ethanol concentration compared to the control beer. A study conducted by Martínez, Vegara, Martí, Valero, & Saura (2017) on beer with persimmon fruit also demonstrated that fruit addition in the brewing process enabled brewing beer with a higher concentration of alcohol. In addition, ethanol concentration increased in the brewing process with an increasing addition of persimmon fruits. However, their addition at 50% of wort volume allowed producing beer with a lower alcohol concentration compared to the beer with 10% addition of Cornelian cherry fruit juice brewed in our study, despite the higher initial wort extract (Martínez, Vegara, Martí et al., 2017). Other investigations concerning beer with goji berries also showed that their

Table 3
Concentrations of carbohydrates and glycerol; pH values; and the level of bitterness of the obtained worts and beer.

Variety of wort or beer	dextrin	maltotriose	maltose	glucose	glycerol	pH	IBU
	g/L						
W0	27.79 ± 2.10[a2]	13.99 ± 0.22[a]	57.84 ± 1.08[a]	7.95 ± 1.18[b]	nd[1]	5.81 ± 0.01[a]	24.59 ± 0.97[a]
WY	25.72 ± 4.19[a]	12.93 ± 1.37[abc]	53.06 ± 4.99[a]	13.81 ± 1.11[a]	nd	3.69 ± 0.02[d]	19.51 ± 0.45[b]
WC	25.44 ± 2.84[a]	12.92 ± 1.02[abc]	53.23 ± 4.03[a]	13.29 ± 0.89[a]	nd	3.77 ± 0.04[c]	20.69 ± 1.32[b]
WR	27.08 ± 2.51[a]	13.29 ± 1.03[ab]	54.58 ± 1.98[a]	13.77 ± 0.34[a]	nd	3.55 ± 0.00[f]	19.15 ± 1.03[b]
B0	27.04 ± 0.49[a]	11.89 ± 0.29[abcd]	1.33 ± 0.03[b]	nd	1.58 ± 0.02[a]	4.59 ± 0.04[b]	16.98 ± 1.47[c]
BY1	25.09 ± 2.06[a]	9.01 ± 1.87[d]	1.37 ± 0.27[b]	0.16 ± 0.00[c]	1.48 ± 0.39[a]	3.64 ± 0.02[e]	14.03 ± 1.28[d]
BC1	30.44 ± 2.64[a]	10.76 ± 0.26[bcd]	1.68 ± 0.22[b]	0.47 ± 0.00[c]	1.76 ± 0.26[a]	3.71 ± 0.02[d]	13.98 ± 0.07[d]
BR1	31.20 ± 1.21[a]	10.74 ± 0.44[bcd]	1.98 ± 0.07[b]	0.56 ± 0.01[c]	1.78 ± 0.07[a]	3.56 ± 0.01[f]	14.03 ± 0.65[d]
BY2	24.15 ± 2.03[a]	10.51 ± 2.62[cd]	1.40 ± 0.06[b]	0.53 ± 0.00[c]	1.56 ± 0.15[a]	3.46 ± 0.00[gh]	14.90 ± 0.71[cd]
BC2	26.98 ± 0.02[a]	11.56 ± 1.36[abcd]	1.58 ± 0.86[b]	0.71 ± 0.00[c]	1.70 ± 0.01[a]	3.49 ± 0.01[g]	15.44 ± 0.07[cd]
BR2	30.49 ± 1.04[a]	11.23 ± 0.08[abcd]	1.87 ± 0.05[b]	0.59 ± 0.05[c]	1.67 ± 0.05[a]	3.43 ± 0.05[h]	15.01 ± 0.06[cd]

[1]nd – not detected.
[2]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).

addition enabled producing beer with a higher alcohol concentration. In that study, ethanol concentration in beer was also affected by production technologies which differed in the stage of fruit addition (Ducruet et al., 2017). After primary fermentation, the real extract content in young beers was: 5.48%w/w in the B0, 5.35%w/w in the BY1, 5.36%w/w in the BC1 and 5.47%w/w in the BR1. After wort fermentation (primary and secondary), extract content decreased by 9.5%w/w on average. The lowest degree of fermentation was determined in the case of beer B0, a slightly higher one – in the case of beers BY2, BC2, BR2, whereas the highest degree of fermentation was measured in the beers BY1, BC1, BR1. The real degree of fermentation (RDF) is an important factor in the brewing technology because it determines the efficiency of a beer brewery and measures the extent to which sugars were fermented in the wort to alcohol. Hence, its control is of outmost importance (Patindol, Mendez-Montealvo, & Wang, 2012; Cutaia, Reid, & Speers, 2009).

Concentrations of carbohydrates and glycerol, pH values and the level of bitterness are presented in Table 3. Among carbohydrates determined in worts, the highest concentration (over 53 g/L) was found for maltose which is released in the process of malt mashing and is the major fermenting sugar in brewing beer. Control wort (W0) contained by ca. 4 g/L more maltose compared to the worts containing juices made of Cornelian cherry fruits, because in these variants 10% of the basic wort were replaced by juices from yellow (WY), coral (WC) or red (WR) fruits, which caused this sugar concentration to decrease. Mean concentration of dextrins in worts accounting for 26.5 g/L. Also maltotriose was detected and its concentration was similar in all worts and reached 13 g/L. Another detected carbohydrate was glucose (ca. 13 g/L). Its concentration in the W0 was lower by 5.5 g/L than in the worts WY, WC, WR, because in Cornelian cherry fruits glucose constitutes 61% of all sugars (Kucharska, 2012). The pH value of the W0 was at pH = 5.81, however, it decreased significantly in the worts with the addition of Cornelian cherry juices due to their naturally low pH (pH = 2.9 on average) (Kucharska, Sokół-Łętowska, & Piórecki, 2011). The highest level of bitterness was found in the W0. In the case of worts with juices it was lower by 4 units, which might have resulted from the fact that part of the hopped wort was substituted with juice from Cornelian cherry fruits which has no bitterness properties. The highest percentage of maltose (99%) was fermented in the beer BR1; the consumption of this sugar in the other experimental variants reached 97%. Glucose was completely consumed by yeast in the variant B0 and in 99% in the BY1 variant; in the other variants the degree of its fermentation accounted for 96%. The highest fermentation degree of maltotriose (85%) was also measured in the BR1 sample. The lowest amount of this sugar (only 10%) was fermented by yeast in the beer BC2. In the other variants, the degree of its fermentation ranged from 15 to 30%. In general, lower level of sugar in beer brewed with the M-2 results from the fact, that in this method fruit juices were added to the wort after primary fermentation, in contrast to the M-1 in which juices were added before this process, during which the yeast converted the sugars from juices into ethyl alcohol. Starch hydrolysis during the mashing of malt results in the production of fermenting sugars (e.g. maltose, glucose or maltotriose), but also non-fermentable carbohydrates (like dextrins) which, depending on their concentration, may affect the organoleptic traits of beer. It is, therefore, necessary to control concentrations of these compounds to assure a desired quality of the finished product (Brandam, Meyer, Proth, Strehaiano, & Pingaud, 2003; Boulton & Quain, 2001). The carbohydrate profile analysis during the fermentation process of beer worts enables determining the course of the technological process, and investigating effects of technological parameters and substrates used on the degree of extract utilization (de Almeida, de Andrade Silva, Lima, Suarez, & da Cunha Andrade, 2018; Mastanjević et al., 2018; He et al., 2018).

The brewed beer were also analyzed for the concentration of glycerol, which turned out to be similar in all variants and reached 1.65 g/L on average. Glycerol is an important indicator of the quality of fermented beverages because it influences their sensory traits (Gawel, Sluyter, & Waters, 2007). The pH value of fermented worts with the addition of Cornelian cherry juices decreased slightly, whereas in the B0 sample it decreased by 1.2. The acidity of fruit beer is affected by both the type and quantity of fruits added to wort as well as by the fermentation process which decreases beer pH (Martínez, Vegara, Herranz-López et al., 2017; Adadi et al., 2017). The highest level of bitterness was achieved in beer BR2 and BY2, i.e. 19.16 and 18.21 IBU, respectively, whereas the lowest one in beers BY1, BC1, BR1, i.e. 14 IBU. Study results demonstrated that the bitterness decreased after fermentation, both in the control samples and in most of the samples with Cornelian cherry fruit juice addition. The bitterness of beer is attributable to hop, which additionally displays some antibacterial effect owing to which it contributes to the extension of beer stability, and affects its sensory traits like e.g. taste or aroma (Dresel, Vogt, Dunkel, & Hofmann, 2016). The most recent investigations on the sensory evaluation of different groups of beer conducted by Viejo, Fuentes, Howell, Torrico, and Dunshea (2018) have demonstrated that consumer preferences are focused on products with a low level of bitterness (Viejo et al., 2018). Hence, the Cornelian cherry beer brewed in our study could be widely acceptable in this respect.

3.2. Concentration of total polyphenols and antioxidative activity

Fig. 1 presents results of determinations of the total polyphenol concentration in worts (W0, WY, WC, WR), in beer without juice

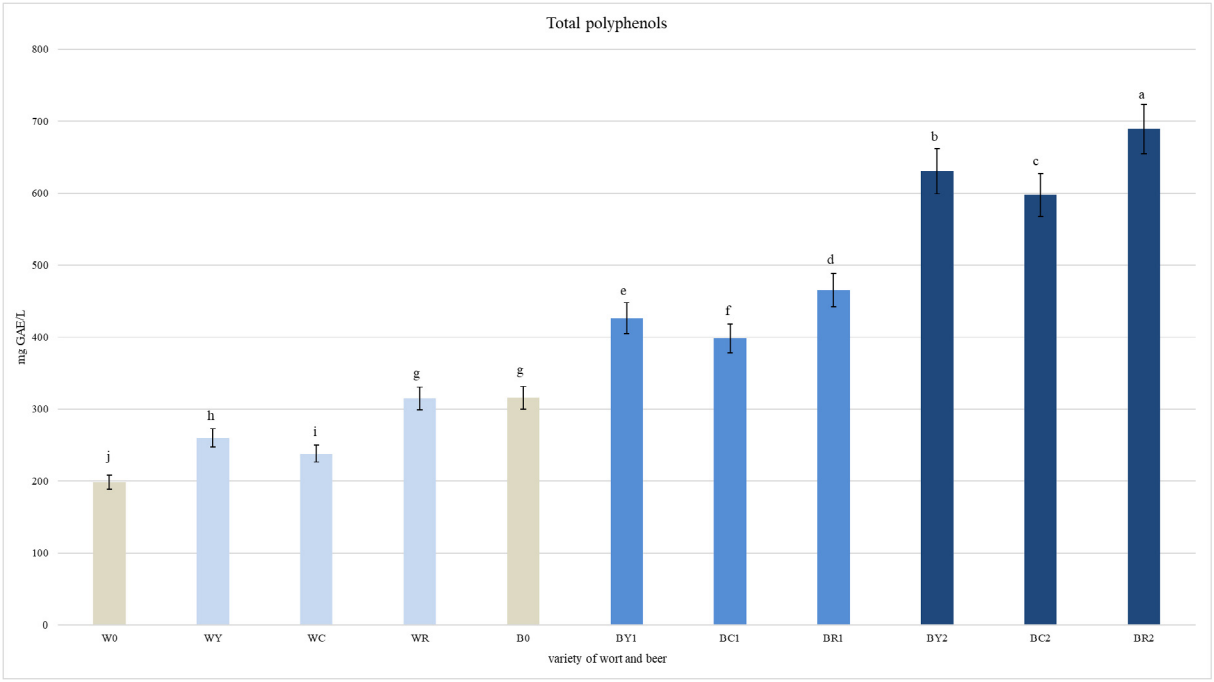

Fig. 1. Total polyphenol concentration in worts (W0, WY, WC, WR), control beer (B0) and beer produced with method M-1 (BY1, BC1, BR1), and with method M-2 (BY2, BC2, BR2) with the addition of juices from three cultivars of Cornelian cherry differing in fruit color. Values are expressed as the mean (n = 3). Mean values with different letters (a, b, c, etc.) are statistically different ($p < 0.05$).

addition (B0), and in beers brewed with method M-1 (BY1, BC1, BR1) and method M-2 (BY2, BC2, BR2). Worts with the addition of Cornelian cherry juice had a higher total concentration of polyphenols than the W0. The highest total polyphenol concentration was determined in the worth with juice made of red fruits. Study results demonstrate that fermentation of beer worts without additives (W0) and these with the addition of juices from Cornelian cherry fruits (WY, WC, WR) causes an increase in the total polyphenol concentration in the finished product.

The final concentration of total polyphenols in the brewed beer was determined by both method of their production and type of added juice. In beer without juice (B0), the total polyphenol concentration increased by 58% after fermentation, but still this increase was lower by half than in the beer produced with the M-2 method (BY2, BC2, BR2). The M-1 method also allowed producing fruit beers (BY1, BC1, BR1) with a higher by 90 mg GAE/L on average concentration of total polyphenols compared to the beer obtained from the B0. Our study demonstrated that the M-2 method allowed producing beer with total polyphenol content higher by 70% than in the beer produced with the M-1 method with the addition of juice from yellow and red fruits of Cornelian cherry (BY2, BR2). Polyphenolic compounds of beer are mainly derived from malt (75%) and hop (25%) and influence some traits of beer like its color, taste, bitterness (Collin, Jerkovic, Bröhan, & Callemien, 2013). In addition, they are claimed to be one of the major sources of beer antioxidants (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006). A research carried out by Martínez, Vegara, Martí et al. (2017) demonstrated that the addition of persimmon fruits during brewing reduced the concentration of total polyphenols in the finished product, and that this reduction was greater with a higher addition of fruits. This may be due to the fact that persimmon fruits have no phenolic compounds (Martínez, Vegara, Martí et al., 2017). In turn, a study addressing beer with the addition of goji berries demonstrated that they were characterized by a higher concentration of phenolic compounds than the control beer (Ducruet et al., 2017). In addition, findings reported by these authors are consistent with results of our study which showed that the method of beer production in which fruit juices were added before the stage of secondary fermentation enabled producing beer with a higher concentration of these compounds than the method in which fruit juices were added to wort before the beginning of the primary fermentation.

Results of our study showed that the addition of juice from all studied cultivars of Cornelian cherry improved the antioxidative properties of the worts (Table 4). The highest antioxidative capability was determined in the case of wort WR, slightly weaker one in the WY, and the weakest one in the WC; nevertheless their antioxidative capabilities were stronger than that of the W0. Our study proves also that fermentation caused an increase in antioxidative potentials of the brewed beer, and that these potentials were additionally determined by beer production technology and type of added juice. The M-2 method allows brewing beer with stronger antioxidative capabilities compared to the M-1 method.

The strongest antioxidative capability was found in the case of beer with the addition of juice from red-fruit cultivar of Cornelian cherry

Table 4
Antioxidative activity ($DPPH^{\cdot}$, $ABTS^{\cdot+}$, FRAP) of worts (W0, WY, WC, WR), control beer (B0) and beer produced with method M-1 (BY1, BC1, BR1), and with method M-2 (BY2, BC2, BR2) with the addition of juices from three cultivars of Cornelian cherry differing in fruit color.

Variant of wort or beer	$DPPH^{\cdot}$ [mmol TE/L]	ABTS [mmol TE/L]	FRAP [mmol TE/L]
W0	2.02 ± 0.02^{i1}	2.60 ± 0.11^{z}	0.29 ± 0.03^{J}
WY	3.96 ± 0.07^{g}	4.54 ± 0.01^{x}	0.57 ± 0.03^{HI}
WC	2.89 ± 0.04^{h}	3.14 ± 0.02^{y}	0.43 ± 0.03^{IJ}
WR	4.48 ± 0.29^{f}	4.88 ± 0.11^{vwx}	0.70 ± 0.03^{GH}
B0	4.81 ± 0.23^{e}	4.63 ± 0.01^{wx}	0.86 ± 0.03^{G}
BY1	5.68 ± 0.02^{c}	5.08 ± 0.01^{vw}	1.58 ± 0.29^{E}
BC1	5.17 ± 0.01^{d}	4.79 ± 0.03^{vwx}	1.13 ± 0.03^{F}
BR1	6.18 ± 0.09^{b}	5.23 ± 0.08^{v}	1.78 ± 0.03^{D}
BY2	6.13 ± 0.17^{b}	5.67 ± 0.04^{u}	2.27 ± 0.02^{B}
BC2	5.59 ± 0.1^{c}	4.77 ± 0.06^{vwx}	1.95 ± 0.07^{C}
BR2	6.41 ± 0.1^{a}	6.51 ± 0.81^{t}	2.6 ± 0.02^{A}

[1]Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters: a, b, c etc. ($DPPH^{\cdot}$); A,B,C etc. ($ABTS^{\cdot+}$); t, u, v (FRAP) are statistically different ($p < 0.05$).

produced with the M-2 method (BR2). Slightly weaker capabilities were determined in the beer produced with yellow fruit juice and M-2 method (BY2) and in the beer produced with red fruit juice and M-1 method (BR1). The addition of juice from coral fruits, compared to juices from yellow and red fruits, had the weakest impact on the increase in the antioxidative activity of beer, however the antioxidative capability of beer with its addition was higher than that of the B0. Fruit juices and other beverages are characterized by high concentrations of natural antioxidants, including polyphenols (Ramadan-Hassanien, 2008). Beer is a very good source of antioxidants, and the composition of its antioxidants depends not only on the raw materials but also technology used to produce it (Jurková et al., 2012). Earlier investigations of other authors also demonstrate that the addition of fruits enables brewing beer with stronger antioxidative properties than the technology without their addition (Adadi et al., 2017; Ducruet et al., 2017). However, Ducruet et al. (2017) in their study on beer with goji berries as well as Martínez, Vegara, Martí et al. (2017) in their research on beers with persimmon fruits showed that the beer they produced were characterized by a lower antioxidant activity than the beer with Cornelian cherry fruit juices brewed in our experiment. Some works are available on the antioxidative activity of light and dark beer (Socha, Pająk, Fortuna, & Buksa, 2017; Niño-Medina, Diego Romo-Longoria, Valentina Ramirez-Gonzalez, Oziel Martinez-Reyna, & Urias-Orona, 2017; Flores-Calderón, Luna, Escalona-Buendía, and Verde-Calvo (2017)), however activities demonstrated therein are weaker than those reported in our study. A comparison of our study results with literature findings allows concluding that fruits of Cornelian cherry are appropriate additives in fruit beer production technology as they enable brewing beer with strong antioxidative capabilities.

3.3. Quantitative identification of iridoids and phenolic compounds

In worts and beer with the addition of Cornelian cherry fruit juices, we identified compounds from the group of monoterpenes (iridoids) and polyphenols (anthocyanins, flavonols) (Table 5). The quantitatively determined iridoids included loganic acid (LA) and cornuside (Co). A predominating iridoid turned out to be LA, which represented from 93% to 99% of the total iridoids content. The highest concentrations of LA and Co were determined in the WY and WC. Iridoids occur in a small group of fruits, including Cornelian cherry, blue honey suckle or cranberry (Kucharska, 2012; Kucharska et al., 2017; Jenen, Krogfelt, Cornett, Hansen, & Christensen, 2002), hence this groups of compounds has not been identified in the fruit beer investigated so far. Iridoids affect biological properties of food products (Dinda et al., 2016) as well as their taste. Some of them like secoiridoids (e.g. oleuropein) are responsible for the sensation of bitterness, however concentrations of iridoids in fruits of different Cornelian cherry cultivars and in products made of these fruits are low, because their predominating iridoid is a non-bitter loganic acid (Kucharska, 2012; Kucharska et al., 2015). Another group of compounds which influence the quality and biological properties of plant-based food products are polyphenols. In the group of anthocyanins, we identified: delphinidin galactoside (Df-gal), cyanidin galactoside (Cy-gal), cyanidin robinobioside (Cy-rob), pelargonidin galactoside (Pg-gal), and pelargonidin robinobioside (Pg-rob). The predominating anthocyanins turned out to be Cy-gal and Pg-gal. All discussed anthocyanins were detected in the samples with the addition of juice from red fruits of Cornelian cherry (WR, BR1, BR2). These were additionally the only variants in which we detected the presence of Df-gal and Pg-rob. Anthocyanins are relatively unstable and are susceptible to degradation upon the action of few factors, like: storage temperature, pH, oxygen or light access, hence their concentration decreased after the fermentation process (Castaneda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Kırca, Özkan, & Cemeroğlu, 2007; Martínez, Vegara, Herranz-López et al., 2017).

Of all detected flavonols, a derivative of kaempferol (Kf-gal) was identified only in the samples containing juice from red fruits of Cornelian cherry (WR, BR1, BR2), whereas a quercetin derivative was present in all experimental variants and control samples. Its highest concentration was determined also in the variants with the addition of juice from red fruits of Cornelian cherry. Moderate consumption of beer coupled with a healthy diet rich in fruits and whole-meal products brings some health benefits owing to the synergistic effects of polyphenols with other compounds present in various food products (Arranz et al., 2012).

4. Conclusions

Our research confirms that the addition of juices from Cornelian cherry allowed to obtain beer containing iridoids. The exception was beer with the addition of coral juice, which was brewed by the method in which the juice was added after primary fermentation. The final content of compounds with antioxidant properties is influenced by both the fruit variety and beer production technology. The highest antioxidant properties were obtained in beer with the addition of juice of red variety of Cornelian cherry fruit, furthermore, the method in which juice was added after primary fermentation was more profitable than the method of brewing beer with the addition of juice before this process. In addition, the technology of production of Cornelian cherry beer can be a good alternative to the classic and the most commonly used

Table 5
Iridoids and phenolic compounds content (mg/L) in worts (W0, WY, WC, WR), control beer (B0) and beer produced with method M-1 (BY1, BC1, BR1), and with method M-2 (BY2, BC2, BR2) with the addition of juices from three cultivars of Cornelian cherry differing in fruit color.

Variety of wort or beer	LA[1]	Co	Df-gal	Cy-gal	Cy-rob	Pg-gal	Pg-rob	Q-glcr	Kf-gal
	mg/L								
W0	nd[2]	nd	nd	nd	nd	nd	nd	0.6	nd
WY	191.7	11.8	nd	nd	nd	nd	nd	2.5	nd
WC	218.0	10.1	nd	0.1	0.1	0.6	nd	2.2	nd
WR	151.6	11.2	0.2	3.3	1.4	6.7	1.3	2.7	1.1
B0	nd	nd	nd	nd	nd	nd	nd	0.6	nd
BY1	139.0	3.4	nd	nd	nd	nd	nd	1.8	nd
BC1	184.6	2.9	nd	nd	nd	0.2	nd	2.2	nd
BR1	131.3	5.5	0.1	1.4	1.0	3.4	0.9	2.3	1.1
BY2	171.5	7.0	nd	0.2	nd	0.1	nd	1.9	nd
BC2	nd	nd	nd	nd	nd	nd	nd	0.4	nd
BR2	166.8	7.2	0.1	1.7	1.2	4.0	1.0	2.8	1.3

[1]LA, loganic acid; Co, cornuside; Df-gal, delphinidin galactoside; Cy-gal, cyjanidin galactoside; Cy-rob, cyjanidin robinoside; Pg-gal, pelargonidine galactoside; Pg-rob, pelargonidine robinoside; Q-glcr, quercetin glucuronide; Kf-gal, kaempferol galactoside.
[2]nd – not detected.

method of sour beer production with the use of lactic acid bacteria.

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Author contributions

J.K.-R., K.A., A.K., P.P.; the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted. N.P.: preparation specimens of fruits to deposited at the Herbariums of Arboretum in Bolestraszyce, critical revision of manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.12.093.

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