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Characteristics of Cornelian cherry sour non-alcoholic beers brewed with the special yeast *Saccharomycodes ludwigii*

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ABSTRACT

Recipes for traditional and sour non-alcoholic beers were developed in this study employing a special yeast species *Saccharomycodes ludwigii.* They were characterized for their basic physicochemical properties, antioxidative activity as well as subjected to the quantitative and qualitative analysis of their biologically-active compounds, and to the sensory assessment. Sour non-alcoholic beers were brewed with the addition of juice from fruits of red-colored Cornelian cherry (*Cornus mas* L.) variety, which are characterized by naturally sour taste and aroma. Ethyl alcohol content in the beers manufactured ranged from 0.41%v/v in traditional non-alcoholic beers to 0.43%v/v in sour non-alcoholic beers. The final products had a low energy value, ranging from 116 to 148 kcal/500 mL of beer. The sour beers had several times higher antioxidative potential and significantly higher polyphenols concentration compared to the control ones. In addition, they were rich in anthocyanins and iridoids, and presented novel sensory attributes.

1. Introduction

Today, beer is the most often consumed alcoholic beverage worldwide. However, low-alcohol and non-alcoholic beers have recently raised increased interest and boosted consumption rates [https://www.euromonitor.com/beer/]. Beers are regarded non-alcoholic in different countries of the world depending on the permitted alcohol content set by legal regulations binding therein. In many countries, like e.g. in: Germany, Austria, Poland, Finland, Switzerland, Portugal, USA or China, ethanol content in the non-alcoholic beer should be < 0.5% v/v (Müller, Bellut, Tippmann, & Becker, 2017).

Non-alcoholic beers have no or a low content of alcohol, hence they can quench thirst well and represent a low-calorie alternative to sweetened non-alcoholic beverages (Silva et al., 2016). Their lower production costs and lower tax burden offer significant economic profits to breweries, but consumption of these beers has also certain benefits for consumers, including e.g.: lower calorie intake, no negative outcomes of alcohol consumption or provision of some valuable compounds like vitamins, minerals, polyphenols or soluble dietary fiber (Puerari et al., 2016).

Non-alcoholic beers can be manufactured with various methods, both the physical ones, including: rectification, membrane separation, evaporation (Jackowski & Trusek, 2018), reverse osmosis (Alcantara et al., 2016), and the biological ones, like: limited fermentation, changed mashing, special yeast, and continuous fermentation, which are based on limited alcohol production by the yeast in the fermentation process (Bellut & Arendt, 2019). Their production method has been proved to exert a direct effect on their sensory attributes, e.g. the thermal or membrane methods are implied to contribute to great losses of their aromatic compounds (Müller et al., 2017; Krebs, Müller, Becker, & Gastl, 2019). Non-alcoholic beers produced with the biological methods have also some disadvantages including the risk of contamination and essential pasteurization, and also some sensory defects like relatively high concentration of diacetyl, undesirable worty-taste, bread-like, cereal-like and sweet taste or no typical aromas in wheat non-alcoholic beers. (Bellut & Arendt, 2019). Despite the above disadvantages, in this study, non-alcoholic beers were brewed with the biological method in which the wort was fermented with a special *Saccharomycodes ludwigii* WSL 17 yeast species intended for brewing beers of this type.

We have found no works that would describe the brewing of non-alcoholic beers with the use of a fruit additive. There are only a few

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related works concerning classic non-alcoholic beers and some which address classic low-alcoholic beers (De Francesco et al., 2018; Bellut & Arendt, 2019). An innovation of our study was the development of a brewing technology for sour non-alcoholic beers (non-alcoholic Sour Ale). In this case, we used juice from fruits of red-colored variety of Cornelian cherry (*Cornus mas* L.), which made the use of lactic acid bacteria unnecessary.

We used Cornelian cherry fruits because, apart from interesting sensory values like sour taste or aroma, they have been proved to offer other valuable properties like a strong antioxidative potential, but also to contain iridoids which were detected in few fruit species only (Kucharska, Szumny, Sokół-Łętowska, Piórecki, & Klymenko, 2015). The usability of these fruits has been tested in the production of various food products, e.g. in alcoholic beverages, including beer, or meads which showed significantly higher contents of natural antioxidants compared to the traditional products without Cornelian cherry fruit addition (Kawa-Rygielska, Adamenko, Kucharska, & Piórecki, 2018; Adamenko, Kawa-Rygielska, Kucharska, & Piórecki, 2018; Kawa-Rygielska, Adamenko, Kucharska, Prorok, & Piórecki, 2019). Considering that beer contains a variety of biologically active compounds and that it is the most often consumed alcoholic drink worldwide, it can represent an additional source of natural antioxidants, including polyphenolics. These compounds are included in malt, hop, cereals and in some additives, especially fruits (Adadi, Kovaleva, Glukhareva, Shatunova, & Petrov, 2017). Polyphenols can directly contribute to the characteristics of beer, mainly body, haze, flavor, fullness, and astringency. Some of these compounds have very interesting health-promoting properties. Antioxidants can protect the beer from oxidative degradation through the whole manufacture process (Collin, Jerkovic, Bröhan, & Callemien, 2013). Because of a high antioxidant activity and high contents of polyphenols and iridoids, the manufactured non-alcoholic beers can also be produced as a functional drink.

2. Materials and methods

2.1. Materials

2.1.1. Biological material

Yeast *Saccharomycodes ludwigii* WSL 17 (SL) were purchased from a yeast bank Hefebank Weihenstephan (München, Germany). The yeast medium (YM) was prepared for yeast propagation (pH = 7.3, 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L glucose), and sterilized at a temperature of 121 °C for 20 min in an ASL 80B autoclave (SMS, Góra Kalwaria, Poland). Yeast were transferred from an agar slant to 20 mL of the YM medium and propagated for 24 h. Afterwards, the culture was transferred to 100 mL of the medium, and after another 24 h – it was transferred from 100 mL to 500 mL of the medium and propagated for another 24 h. SL propagation was conducted in 50–750 mL conical laboratory flasks under aerobic conditions and continuous stirring in a 358A laboratory shaker (ElpinPlus, Lubawa, Poland), in a thermostat laboratory, at a temperature of 15 °C.

2.1.2. Raw material

The Pale Ale type malt was purchased from Viking Malt (Strzegom, Poland), whereas hop pellets of Australian variety Topaz (with the isoalpha-acid content of 14.5%) from Twój Browar distributor (Wrocław, Poland). Fruits of Cornelian cherry (*Cornus mas* L.) of red-colored 'Podolski' variety were obtained from Arboretum in Bolestraszyce (Poland). Fruits were picked up and immediately frozen at a temperature of −20 °C. After thawing, they were pressed through a Zodiak laboratory hydraulic press (SRSE, Warsaw, Poland) to produce fruit juice. The basic physicochemical parameters of red-colored Cornelian cherry juice were as follows: extract 14.1%, acidity 2.74% (as malic acid), pH = 2.84, dry mass 14.3, ash 0.62%, and vitamin C 26.77 mg/mL. The quantitative and qualitative composition of iridoids and polyphenols was described in the article Adamenko et al., 2018).

2.2. Brewing technology

Mashing was conducted under laboratory conditions. Water (50% of tap water and 50% of distilled water) was acidified with 80% edible lactic acid to the pH value of pH = 6.2 and enriched with mineral salts as follows: 1% $CaCO_3$, 1% $CaCl_2$, and 1% $CaSO_4$. Water (6.6 L) was heated to a temperature of 72 °C, and then 2.2 kg of malt were added. Mashing was performed with the single infusion method modified for the needs of brewing non-alcoholic beers with *Saccharomycodes ludwigii* yeast. Temperature of 72 °C was maintained for 50 min. The mash was filtered using 17 L of water. The resultant wort was boiled with hops (0.5 g/L) for 60 min. The hopped wort was filtered and cooled to a temperature of 17 °C, at which initial extract content was established at 7°Bx. Extract content was determined at a temperature of 20 °C using a Densito 30PX manual oscillating densitometer (Mettler Toledo, Columbus, USA). 20 L of the produced wort were inoculated with SL yeast in the amount of $1x10^6$ yeast/mL. Fermentation was carried out at a temperature of 17 °C for 3 days (time needed to achieve permitted ethanol content), then the beer was cooled to 8 °C and cold-hopped (1 g/L). After another 3 days, the beer was decanted from above the yeast precipitate and portioned to be used in two experimental variants: the first which included control beer (B0) and the second which included beer with 10% addition of juice from fruits of red-colored variety of Cornelian cherry (BJ). Glucose (0.5 g/L) was added to B0 to make its extract content equal with that of BJ. Next, the beers were bottled into 500-mL brown glass bottles and re-fermented at 28 °C in a laboratory thermostat for 24 h. The fermented beers were pasteurized at 65 °C for 20 min in an MLL147 water bath (AJL Electronic, Cracow, Poland). After cooling, they were left for maturation in a cold store, at a temperature of 15 °C for 4 weeks.

2.3. Analytical methods

2.3.1. Carbohydrate profile and contents of glycerol, lactic acid, and acetic acid

High-performance liquid chromatography (HPLC) was employed to analyze the carbohydrate profile (dextrins, maltotriose, maltose, glucose, fructose) of beers before pasteurization and of the final products (Kawa-Rygielska et al., 2018). Contents of glycerol was determined as well. Degassed and centrifuged (2675 centrifugal force (RCF), 6000 rpm, 10 min) samples were double diluted with redistilled water and filtered through nylon filters (mesh size of 0.22 μm) to chromatographic vials. Beer samples were analyzed using a Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped in a Rezed ROA-Organic Acid H^+ column (300 × 4.6 mm; Phenomenex, Torrance, USA). Measurement parameters were as follows: 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 carbohydrates, acetic acid, ethyl alcohol, and glycerol were determined based on a five-point calibration curve integrated in Chromax 10.0 software (Pol-Lab, Wilkowice, Poland). Analyses were carried out in three replications.

2.3.2. Basic physico-chemical parameters

The final degree of fermentation, extract content, energy value (calories), and density of beers, as well as concentration of ethyl alcohol were measured using an Anton Paar DMA 4500 M oscillating densitometer (Graz, Austria). Alcohol content was measured with the near infrared (NIR) spectroscopy, whereas density was measured using an oscillating U-tube. Other parameters were calculated on the basis of the density value. Beers were degassed and centrifuged as in point 2.3.1. of Material and Methods section, and then filtered on laboratory filter papers and subjected to analyses. Their pH value was measured with a Mettler Toledo MP 240 pH-meter (Columbus, USA). Calories were also measured with the NIR using an Anton Paar Alex 500 m (Graz, Austria). on the basis of density (ρ), real extract (Er), and alcohol content (A),

according to the below equation. Analyses were carried out in three replications.

$$Cal[kcal/100mL] = (7 \times A_{[\%w/w]} + 3.5 \times Er_{[\%w/w]} \times \rho_{sample})$$

2.3.3. Analysis of beer bitterness (IBU)

To determine bitterness level of the manufactured beers, 10 cm^3 of degassed beer were transferred to Falcon tubes (35 cm^3), and 0.5 cm^3 of a hydrochloric acid solution (6 N HCl) and then 20 cm^3 of isoacetate were added to the tubes. The tubes were shaken manually for 5 min. Next, 10 cm^3 of the sample were transferred to Falcon centrifugation tubes (15 cm^3) 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 wavelength of 275 nm. Pure isoacetate was used as the standard (Kawa-Rygielska et al., 2019). Analyses were carried out in three replications.

2.3.4. Antioxidative activity and polyphenolic profile

2.3.4.1. Total polyphenols content. Total polyphenols content was determined with the spectrophotometric method based on the reaction with the Folin-Ciocialteu (F-C) reagent (Prior, Wu, & Schaich, 2005). A diluted beer sample and the F-C reagent were mixed in a cuvette and incubated for 3 min. Next, a 20% Na_2CO_3 solution and redistilled water were added to the mixture. The samples were then incubated in the dark for 60 min, and afterwards their absorbance was measured at a wavelength of 765 nm. Results were expressed as gallic acid equivalents (GAE) per 1 L of beer. Analyses were carried out in three replications.

2.3.4.2. Antioxidative activity

2.3.4.2.1. Antioxidative activity assayed based on the test with DPPH$^{\bullet}$ reagent. A diluted beer sample was mixed in a cuvette with DPPH$^{\bullet}$ dissolved in ethanol and water. The mixture was incubated at a room temperature for 10 min and, afterwards, its absorbance was measured at a wavelength of 517 nm (Yen & Chen, 1995). Results were expressed as Trolox equivalents (TE) per 1 L of beer (mmol TE/L). Analyses were carried out in three replications.

2.3.4.2.2. Antiradical activity assayed based on the reaction with ABTS$^{\bullet+}$. A diluted beer sample was mixed in a cuvette with an ABTS$^{\bullet+}$ solution, the absorbance of which measured at a wavelength of 734 nm reached 0.700 (Re et al., 1999). Sample absorbance was measured after 6-minute incubation. Results were expressed as Trolox equivalents per 1 L of beer (mmTE/L). Analyses were carried out in three replications.

2.3.4.2.3. Antioxidative activity assayed based on the FRAP test. A diluted beer sample was mixed in a cuvette with a ferric complex and redistilled water. After 10-minute incubation, absorbance was measured at a wavelength of 593 nm (Benzie & Strain, 1996). Results were expressed as Trolox equivalents per 1 L of beer (mmol TE/L). Analyses were carried out in three replications.

2.3.4.3. Quantification of iridoids and polyphenols with HPLC-PDA. The details of the analysis are described in the publication by (Kawa-Rygielska et al., 2018). The HPLC-PDA analysis was performed using a Dionex (Germering, Germany) system equipped with an Ultimate 3000 diode array detector, an LPG-3400A quaternary pump, an EWPS-3000SI autosampler, a TCC-3000SD thermostated column compartment, and controlled by 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, 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, anthocyanins at 520 nm, and flavonols at 360 nm. Results were expressed as mg per 1 L of beer.

2.4. Sensory analysis

The beers manufactured were subjected to the statistical analysis using a descriptive card, prepared accordingly to the guidelines available at the website of the Polish Homebrewers Association [https://pspd.org.pl/sedziowie/materialy/]. They were also assessed in a five-point scale for their: clarity (significance points × 1), color (significance points × 1), saturation (significance points × 1), foaminess (significance points × 2), bitterness (significance points × 4), aroma (significance points × 4), and taste (significance points × 7). The maximal number of points the beer could get was 100. The beers were assessed by a group of 10 students aged 22–26 years, including 6 women and 4 men. The students were not familiarized with characteristics of the samples, which were served to them in coded plastic cups. The consumer assessment was performed at a sensory analysis laboratory with individual boxes for organoleptic assessment of food products. Temperature of the samples was 12 °C.

2.5. Statistics

Selected data were processed using Statistica 13.5 software (StatSoft, Tulsa, OK, USA), based on ANOVA ($\alpha = 0.05$). Duncan test was used to analyze differences between mean values ($p < 0.05$). The tables show values of standard deviation.

3. Results and discussion

3.1. Carbohydrate profile and concentrations of alcohol and glycerol based on HPLC analysis

Table 1 presents concentrations of carbohydrates (dextrins, maltotriose, maltose, glucose, and fructose), ethyl alcohol, and glycerol in beers before and after the pasteurization process. Among the

Table 1
Concentrations of carbohydrates and glycerol in the analyzed beers [g/L].

Variety of Beer	Dextrin	Maltotriose	Maltose	Glucose	Fructose	Glycerol
	[g/L]					
Before Pasteurization						
B0[1]	16.77 ± 1.10[a2]	6.60 ± 0.59[a]	19.71 ± 1.82[a]	0.23 ± 0.02[b]	0.65 ± 0.06[b]	0.07 ± 0.10[b]
BJ	16.39 ± 0.69[a]	6.81 ± 0.26[a]	20.09 ± 0.76[a]	6.39 ± 0.24[a]	3.43 ± 0.11[a]	0.08 ± 0.02[b]
Final Beer						
B0	18.00 ± 0.26[a]	7.14 ± 0.08[a]	21.31 ± 0.23[a]	0.23 ± 0.07[b]	0.67 ± 0.03[b]	0.14 ± 0.00[a]
BJ	17.93 ± 0.18[a]	7.31 ± 0.08[a]	21.38 ± 0.22[a]	6.74 ± 0.07[a]	3.82 ± 0.04[a]	0.16 ± 0.00[a]

[1]B0 – control non-alcoholic beer; BJ – Cornelian cherry non-alcoholic beer;
[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).

carbohydrates identified in beers, the highest concentration was found for maltose, followed by dextrins and by maltotriose.

The control non-alcoholic beer (B0) and the Cornelian cherry non-alcoholic beer (BJ) did not differ in concentrations of the above-mentioned sugars before and after pasteurization. The production of non-alcoholic beers by means of limited fermentation consists in arresting the fermentation process when the highest permitted alcohol level is achieved, therefore the mashing process requires high amounts of higher dextrins and thus low amounts of fermentable sugars, which are typical of the non-alcoholic beers produced with this method (Krebs et al., 2019). The analyzed beers had high concentrations of maltose and maltotriose, because *Saccharomycodes ludwigii* yeast are incapable of their fermenting (Bellut et al., 2018). The beers were also analyzed for their contents of glucose and fructose. The control beers (without Cornelian cherry juice) contained their trace amounts, i.e. 0.23 g/L of glucose and 0.66 g/L of fructose. The pasteurization process had no effect on concentrations of these sugars in the final products. Compared to the control beers, the beers with the addition of Cornelian cherry juice (BJ) had a 20-fold higher concentration of glucose and over 3-fold higher concentration of fructose. The significantly higher concentrations of these sugars in the non-alcoholic beers with 10% addition of juice from red fruits of Cornelian cherry are due to the fact that both, glucose and fructose represent the major monosaccharides in fruits of the 'Podolski' variety of Cornelian cherry, i.e. glucose accounts for 59% and fructose for 35% of total monosaccharides (Kucharska, 2012). Analyses of saccharides composition in other non-alcoholic beverages also demonstrated that the pasteurization process had no effect on their content in the final products (Wibowo et al., 2019). The beers with the addition of Cornelian cherry juice (BJ) had a slightly higher (by 0.02% v/v on average) content of ethanol than the control beers (B0), which proves no effect of the pasteurization process on its final content. Senkarcinova, Dias, Nespor, and Branyik (2019) also manufactured non-alcoholic beers with the use of yeast species *Saccharomyces cerevisiae* var. *boulardii*, however via the fermentation process which lasted only 24 h. In turn, Bellut et al., 2018) used various yeast species not belonging to the *Saccharomyces cerevisiae* species to produce non-alcoholic beers (< 0.5%v/v) by fermenting wort having the initial extract content of 7°Bx, for 72 h or 96 h at latest. Other scientists who used *Saccharomycodes ludwigii* yeast for beer wort fermentation produced low-alcohol beers with ethanol content above 0.5%v/v (De Francesco et al., 2018). In the present study, the beers produced were also found to contain traces of glycerol, however its content was twofold higher in the pasteurized beers. Traditional beers have higher contents of glycerol regardless of raw material they had been made of (Mastanjević et al., 2018; Kawa-Rygielska et al., 2019). Glycerol, being one of the byproducts of alcoholic fermentation run by yeast, affects the character of beer, including the sensation of sweetness, and beer viscosity (Mastanjević et al., 2018).

3.2. Basic physicochemical parameters of beers

Table 2 presents values of basic physicochemical parameters of beers, including: extract content, alcohol content, fermentation degree, calories, density, bitterness, as well as pH and IBU values. Before fermentation, wort extract content reached 7%w/w in the beers without Cornelian cherry juice, whereas in the non-alcoholic beers with added juice it was higher by 1.3%w/w – as indicated by densitometry based on near infrared spectroscopy. The real extract content of the final control non-alcoholic beers (B0) reached 5.9%w/w, whereas that determined in the final non-alcoholic Cornelian cherry beers was higher by 1.7%w/w due to fruit juice addition. The statistical analysis demonstrated significant differences in extract contents between beers before and after pasteurization, i.e. value of this parameter was higher before pasteurization in both B0 and BJ beers. Wort extract content of beer depends on the contents of malt and other non-malted raw materials used in the brewing process (De Francesco et al., 2018; Ceccaroni, Marconi, Sileoni, Wray, & Perretti, 2019). Regardless of the addition of whole fruit, fruit juices or other processed fruit products, the fruit beers have a higher extract content than the control beers brewed without these additives (Ducruet et al., 2017; Kawa-Rygielska et al., 2019).

The degree of fermentation was lower in the non-alcoholic fruit beers than in the non-alcoholic control beers. This was due to the addition of fruit juice after the fermentation process, which increased extract content and, by this means, also the content of non-fermented sugars, including mainly glucose and fructose. The beers produced contained < 0.5%v/v of ethyl alcohol, therefore they may be classified as non-alcoholic beers based on provisions set in the Journal of Laws [Dz.U. 2018 item 1114]. Alcohol content of the fruit beers was higher by 0.02%v/v compared to the control B0 beers. The pasteurization process had no effect on changes in ethyl alcohol content of the beers. Alcohol content determined with the use of near infrared (NIR) spectroscopy corresponded to the results achieved with the HPLC method. Some literature works have elaborated on the feasibility of producing non-alcoholic beers using other technologies, like: pervaporation (Jackowski & Trusek, 2018) and reversed osmosis (Alcantara et al., 2016). It was proved, however, that the production technology of low-alcohol and non-alcoholic beers had a direct impact on their sensory traits, and that fermentation by special yeast allowed preserving the highest amount of aromatic compounds compared to other methods, including mainly these involving heat treatment (Liguori et al., 2015; Krebs et al., 2019). But, biological methods for non-alcoholic beers production carry some flavor disadvantages as well, including for example a too high level of sweetness, higher diacetyl content (more perceptible dieacetyl), undesirable wort-like flavor, weak aroma, no characteristic aromatic compounds in wheat beers (non-alcoholic) (Bellut & Arendt, 2019). The energy value of BJ was higher by 32 kcal/500 mL beer on average compared to B0; in addition it decreased by 0.8% on average in both types of beer (B0 and BJ) after pasteurization. We have found no works in the available literature that would describe analyses of the energy value of non-alcoholic beers. Bamforth (2005) determined energy value of various commercial beers and demonstrated it to depend on beer type, i.e. on raw materials and brewing technology used to produce them. The energy value of beers analyzed by this author ranged from 160 kcal/500 mL in lager type beers to even 420 kcal/500 mL in Barley Wine beer, in turn the energy value of low-alcohol beers and of these with a lower extract content was the lowest and reached ca. 140 kcal/500 mL beer. It has been proved that regular consumption of beers with a low alcohol content and thus with a low energy value has no significant effect on the increase of daily energy intake and no negative impact on a human body (Romeo, Gonzalez-Gross, Wärnberg, Diaz, & Marcos, 2007). Considering the international bitterness units – IBU, the bitterness of control beers (B0) was higher by 5 units than that of fruit beers (BJ). Results of IBU determination point to a low degree of bitterness of the manufactured non-alcoholic beers. Recent investigations into the preferences for various types of beers have demonstrated that consumers more often choose less bitter products (Viejo, Fuentes, Howell, Torrico, & Dunshea, 2019). The bitterness of beers derives from hops which impact also other sensory attributes of beers, like e.g.: typical taste or aroma (Dresel, Vogt, Dunkel, & Hofmann, 2016). In their study with light Cornelian cherry beers, Kawa-Rygielska et al. (2019) also demonstrated a slightly lower degree of bitterness in the fruit beers compared to the control ones, which was similar to that obtained in our study for non-alcoholic Cornelian cherry beers. There are no significant differences in IBU levels in both Cornelian cherry beers, despite using more hop by Kawa-Rygielska et al. (2019), which is due to the fact that the level of hop bitterness depends on contents of α-acids in a hope variety used for brewing. The non-alcoholic Cornelian cherry beers had a significantly higher acidity, namely: the pH value of BJ was lower by 1.7 on average than the pH value of B0. After the pasteurization process, the pH values of both B0 and BJ beers increased by 0.2 on average. The acidity of beers is

Table 2
Basic physicochemical parameters of non-alcoholic beers.

Variety of Beer	Wort Extract [%w/w]	Apparent Extract [%w/w]	Real Extract [%w/w]	Alcohol [%v/v]	Apparent Degree of Fermentation [%]	Real Degree of Fermentation [%]	Calories [kcal/500 mL]	Density [g/cm3]	IBU	pH
Before Pasteurization										
B0	6.50 ± 0.08^{b}	5.78 ± 0.01^{b}	5.93 ± 0.00^{c}	0.41 ± 0.01^{b}	11.86 ± 0.11^{a}	9.52 ± 0.08^{a}	117.08 ± 0.11^{c}	1.0209 ± 0.00^{c}	18 ± 0.23^{a}	4.33 ± 0.01^{b}
BJ	8.30 ± 0.01^{a}	7.52 ± 0.01^{a}	7.66 ± 0.01^{a}	0.43 ± 0.00^{a}	9.70 ± 0.00^{b}	7.77 ± 0.02^{b}	149.50 ± 0.21^{a}	1,0279 ± 0.00^{a}	13 ± 0.16^{b}	2.63 ± 0.01^{d}
Final Beer										
B0	6.51 ± 0.01^{b}	5.75 ± 0.00^{b}	5.90 ± 0.00^{d}	0.40 ± 0.01^{b}	11.61 ± 0.12^{a}	9.32 ± 0.09^{a}	116.43 ± 0.11^{d}	1.0208 ± 0.00^{c}	18 ± 0.19^{a}	4.59 ± 0.01^{a}
BJ	8.22 ± 0.01^{a}	7.46 ± 0.02^{a}	7.61 ± 0.01^{b}	0.43 ± 0.01^{a}	9.30 ± 0.34^{b}	7.46 ± 0.27^{b}	147.98 ± 0.18^{b}	1.0277 ± 0.00^{b}	13 ± 0.21^{b}	2.75 ± 0.01^{c}

[1]B0 – control non-alcoholic beer; BJ – Cornelian cherry non-alcoholic beer;
[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).

affected by both the fermentation process and additives used, including the fruit ones, which contribute to pH value decrease in the final products (Adadi et al., 2017).

3.3. Analysis of biologically-active compounds

3.3.1. Total polyphenols and antioxidative activity

Fig. 1 presents the total content of polyphenols and the antioxidative activity of the non-alcoholic beers assayed with DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP tests. Among the analyzed beers, the highest total polyphenols content was determined in the final beer with the addition of Cornelian cherry juice (BJ); it was 2.5-fold higher than in the control beer (B0). A similar tendency was noted before the pasteurization process, when BJ beer had a 2-fold higher content of total polyphenols than the B0 beer. The pasteurization process contributed to an increase in total polyphenols content in both types of beers, but in the case of BJ the increase was by as much as 132 mg GAE/L beer.

Similar results were achieved for the antioxidative activity of the manufactured non-alcoholic beers. All tests performed (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP) demonstrated the highest antioxidative activity of the non-alcoholic Cornelian cherry beer after the pasteurization process. In the case of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ tests, the activity was 2-fold higher and in the case of the FRAP test – over 5-fold higher compared to the pasteurized B0 beer. As in the case of the total polyphenols content, the antioxidative activity tests demonstrated that the process of pasteurization enhanced the activity in both B0 and BJ beers. The greatest increase in the antioxidative activity of B0 and BJ due to pasteurization was measured with the FRAP test and reached 59% and 48%, respectively. Czabaj, Kawa-Rygielska, Kucharska, and Kliks (2017) also demonstrated that the heat treatment of meads increased their total polyphenols content and their antioxidative activity. Some other authors (Azeez, Adebisi, Oyedeji, Adetoro, & Tijani, 2017; Huang et al., 2019; Yang et al., 2019) have confirmed various thermal processes to increase the total polyphenols content and antioxidative activity of foods as a result of the higher antioxidative activity of partly oxidized polyphenols caused by the heat treatment (Azeez, Adebisi, Oyedeji, Adetoro, & Tijani, 2017). The heat treatment of food products, either with or without amine compounds, induces degradation of reducing sugars, thus leading to the formation of dicarbonyl compounds which exhibit antioxidative properties (Kanzler, Haase, Schestkowa, & Kroh, 2016). Such properties have also been demonstrated for products of anthocyanins degradation (Patras, Brunton, O'Donnell, & Tiwari, 2010). The main sources of polyphenols in beer include the malt and hops which impart characteristic sensory attributes to these products, including: taste, aroma, bitterness, and color (Collin et al., 2013). Many published research works have proved the fruit additives used in the brewing to significantly increase the content of polyphenolic compounds in beers and, by this means, to enhance their antioxidative properties (Adadi et al., 2017; Ducruet et al., 2017). Cornelian cherry fruits are characterized by an exceptionally high content of biologically active compounds, including mainly natural antioxidants (Kucharska, 2012; Kucharska et al., 2015). In previous investigations of our research group, we have analyzed their usability for the production of various fermented products, including: Cornelian cherry vinegars, Cornelian cherry meads, Cornelian cherry light beers, and low-alcohol Cornelian cherry-apple beverages. We have proved that these products exhibited a significantly higher antioxidative potential than the traditional products as well as had a significantly higher content of polyphenolic compounds than the fermented products manufactured based on other fruits where the best effect was achieved upon the use of red-colored Cornelian cherry fruit (Kawa-Rygielska et al., 2018; Adamenko et al., 2018; Kawa-Rygielska et al., 2019). Results of the present study confirm that the addition of juice from red-colored Cornelian cherry fruit during brewing allowed manufacturing non-alcoholic beers with a significantly higher antioxidative potential and a higher content of polyphenolic compounds compared to the non-alcoholic beers analyzed by other scientists (Moura-Nunes et al., 2016; García-Guzmán et al., 2019). In turn, most of the Stout type beers analyzed by Alcantara et al., 2016) had a lower antioxidative potential than the non-alcoholic Cornelian cherry beers brewed in our study, however they had a higher total polyphenols content. This may be due to the stronger antioxidative properties of Cornelian cherry, and to the wider pool of polyphenolic compounds typical of dark malts used to produce Stout type beers.

3.4. Quantitative and qualitative identification of polyphenols and iridoids

Two compounds from the group of iridoids: loganic acid (LA) and cornuside (Co); 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); as well as trace amounts of two flavonols: quercetin glucuronide (Q-glcr) and kaempferol galactoside (Kf-gal), were identified in the non-alcoholic Cornelian cherry beers (Table 3).

LA predominated among the iridoids and accounted for 92% of their total content. A predominating anthocyanin turned out to be Pg 3-gal, which accounted for 51% of the total content of these compounds; it was followed by Cy 3-gal (24%). Anthocyanins represented the smallest group of identified compounds, because the content of iridoids in the analyzed beers was over 14 times higher. Anthocyanins are relatively instable and susceptible to degradation under the influence of temperature, pH, oxygen or light, due to which their content could decrease in the final products (Castaneda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Kırca, Özkan, & Cemeroğlu, 2007). Previous studies have demonstrated the red fruits of Cornelian cherry of 'Podolski' variety to contain iridoids, anthocyanins, and flavonols (Kucharska, 2012; Kucharska et al., 2015). Our earlier investigations into the quantitative and qualitative identification of biologically active compounds in light Cornelian cherry

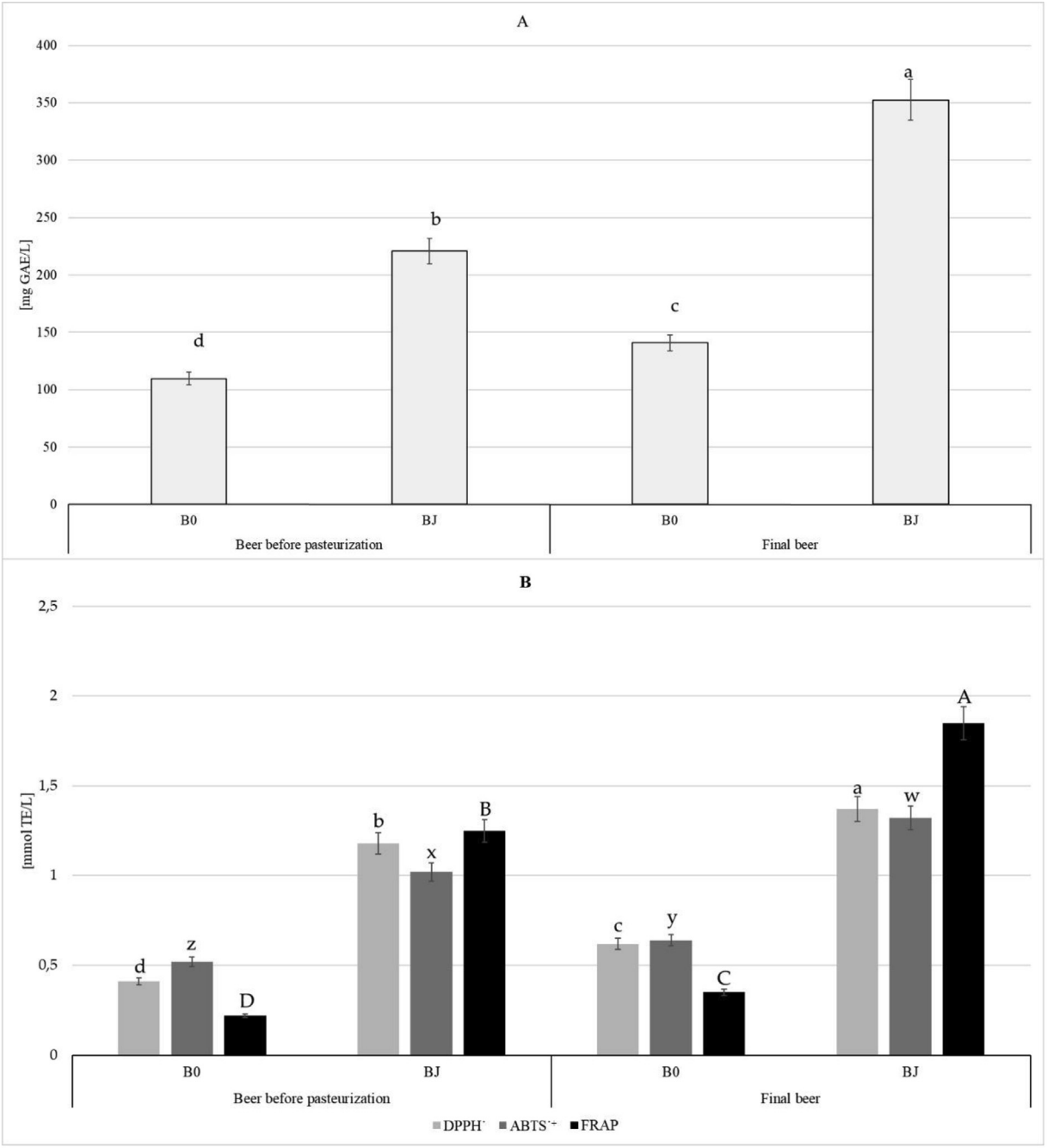

Fig. 1. Total polyphenols content (TPC) - (A) and antioxidative activity – (B) measured with DPPH˙, ABTS˙+, and FRAP tests in non-alcoholic beers. Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters: A, B, C, D (FRAP); a, b, c, d (TPC, DPPH˙); w, x, y, z (ABTS˙+) are statistically different ($p < 0.05$).

beers with alcohol content of 5%v/v have shown that the products made with the addition of juice of red-colored Cornelian cherry fruits also contained the aforementioned compounds, whose higher contents were obtained when fruit juice was added to young beer after effervescent fermentation. Therefore in the present study on non-alcoholic Cornelian cheery beers, the juice made of red-colored Cornelian cherry variety was added already after the completed fermentation process (Kawa-Rygielska et al., 2019). The BJ beers produced contained more anthocyanins and cornuside as well as less loganic acid and flavonols compared to the light Cornelian cherry beers manufactured by Kawa-Rygielska et al. (2019). Some works can be found in the available literature that report on anthocyanins content of various fermented products, however iridoids have so far been identified only in products made on the basis of Cornelian cherry fruit (Kawa-Rygielska et al., 2018; Adamenko et al., 2018; Kawa-Rygielska et al., 2019).

3.5. Sensory analysis

In the descriptive sensory analysis, the consumers evaluated control non-alcoholic beers (B0) and non-alcoholic Cornelian cherry beers (BJ) for their appearance, aroma, taste, and overall acceptability. In their opinion, the control beer (B0) had a straw-like color and was cloudy. Its aroma carried malt and bread-like notes as well as a delicate aroma of fruits (including mainly grapefruits and wild berries). Bitterness was evaluated as of low intensity with a short finish, and as derived from hops. The taste of beer was described as delicate and malt-like. In the

Table 3
Iridoids and phenolic compounds content (mg/L).

Variety of Beer	LA[2]	Co	Df 3-gal	Cy 3-gal	Cy 3-rob	Pg 3-gal	Pg 3-rob	Q 3-glcr	Kf 3-gal
	[mg/L]								
Before pasteurization									
B0[1]	nd[3]	nd	nd	nd	nd	nd	nd	nd	nd
BJ	113.80 ± 0.45[a4]	9.50 ± 0.06[a]	0.30 ± 0.00[a]	3.40 ± 0.02[a]	1.50 ± 0.01[a]	6.50 ± 0.05[a]	1.30 ± 0.01[a]	tr[4]	tr
Final beer									
B0	nd	nd	nd	nd	nd	nd	nd	nd	nd
BJ	149.60 ± 1.71[a]	12.30 ± 0.16[a]	0.20 ± 0.00[a]	2.70 ± 0.04[a]	1.30 ± 0.02[a]	5.70 ± 0.08[a]	1.30 ± 0.02[a]	tr	tr

[1]B0 – control non-alcoholic beer; BJ – Cornelian cherry non-alcoholic beer;
[2]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, Q-glcr, quercetin glucuronide; Kf-gal, kaempferol galactoside;
[3]nd, not detected;
[4]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);
[5]tr, traces.

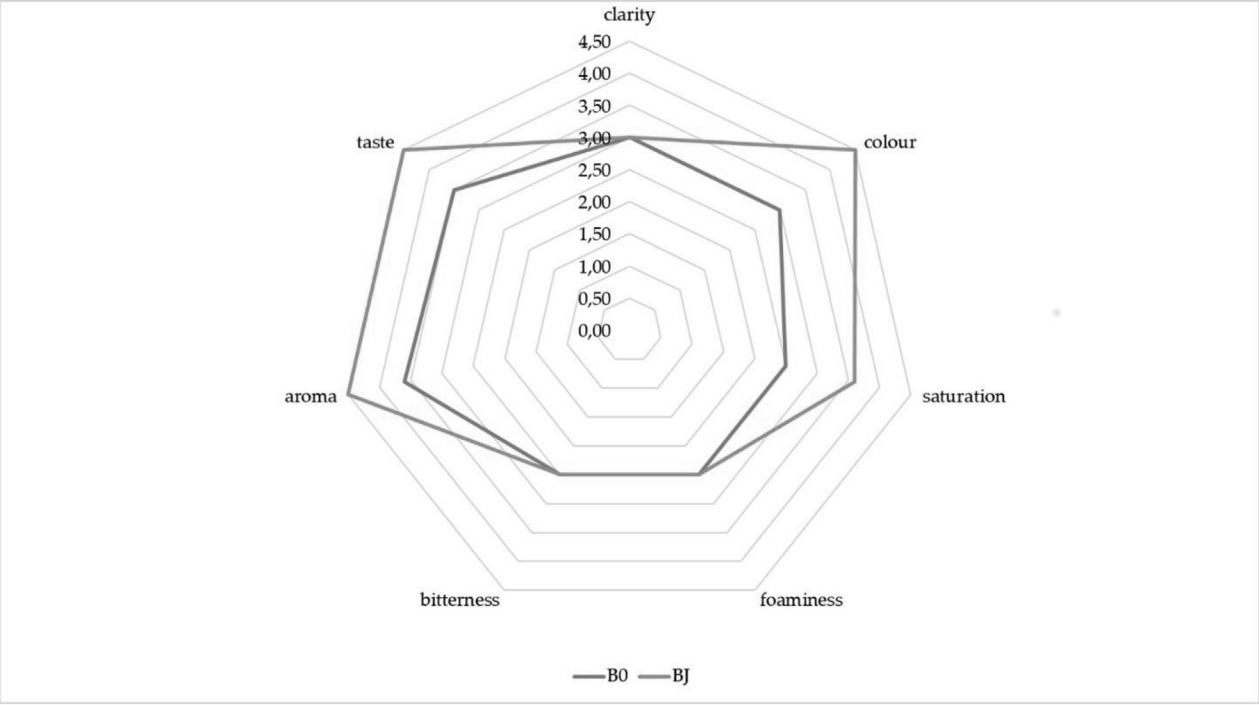

Fig. 2. Mean points scored by consumers to non-alcoholic beers in the sensory assessment.

overall assessment, the control beer was described as light and sufficiently good. In turn, the descriptive analysis of the non-alcoholic Cornelian cherry beer (BJ) demonstrated that consumers described its color as red, raspberry-like or strawberry-like, and also found it cloudy. Its aroma was evaluated as malt-like, intensively fruity (usually raspberry, citrus, strawberry, cherry, red currant), and sour. Its bitterness was evaluated similarly to that of B0 beers, whereas its taste as malt-like, sour, and fruity (cherry, sweet cherry, red currant). Considering the overall acceptability, BJ beer was assessed as good or very good, and described as very drinkable beer, interesting, refreshing, and with pleasant sourness. The consumers pinpointed a drawback of both B0 and BJ beers – namely too low saturation and poor foaminess. Fig. 2 presents points scored by consumers to the non-alcoholic beers in the sensory assessment.

Considering the points scored by consumers for clarity, foaminess, and bitterness, the B0 and BJ beers were evaluated at a similar level. In the case of the other sensory attributes, i.e.: color, saturation, aroma, and taste, at least 1 point more was scored each time to the non-alcoholic Cornelian cherry beer. In the case of this beer, the highest numbers of points were scored for aroma and taste, i.e. 4.5 and 5.0 point on average. Considering the above attributes and their significance, the total number of points the beer could get was 100. B0 received 63 points on average, whereas BJ beer – 82 points on average. Previous studies into the sensory assessment of fruit beers have also demonstrated that consumers noted significant differences in the character of beers of this type (compared to control beers) which concerned their color, clarity, taste, and aroma (Ducruet et al., 2017; Adadi et al., 2017). Sweetness of non-alcoholic beers is often criticized in sensory tests and is usually described by taste tasters as wort-like, malty, and sweet, i.e. as undesirable (Bellut & Arendt, 2019; Bellut et al., 2018; Krebs et al., 2019) Cornelian cherry juice can improve sensorial attributes on that part by increasing sourness and freshness.

4. Conclusions

The special yeast species *Saccharomycodes ludwigii* can be used for

the production of non-alcoholic beers. The addition of juice from fruits of red-colored variety of Cornelian cherry in the brewing process allowed for not only producing sour non-alcoholic beers but also for enriching them in natural antioxidants and, consequently, for increasing their antioxidative potential. The herein described technology enables producing non-alcoholic beers characterized not only by negligible alcohol content but also by good taste, reduced energy value or potential health-promoting properties, due to which they may represent a valuable alternative to other sweetened and less valuable non-alcoholic beverages. Further investigations seem necessary to determine the effect of other physical and chemical factors on the course of the brewing process and on the quality of the final products, mainly with the aim to improve their foaminess and saturation level.

Author Contributions

K.A., J.K.-R., A.K.,: the concept 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

Declaration of Competing Interest

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

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