The effect of apple cultivars and yeast strains on selected quality parameters and antioxidant activity of fermented apple beverages

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ABSTRACT
The aim of the study was to evaluate the impact of selected apple cultivars (Rubin, Topaz, Elise) and selected strains of wine, cider, distiller yeast, and wild-type yeast on the dynamics of the fermentation process as well as on the chemical composition and antioxidant properties (AOX) of fermented apple beverages. The quality parameters of the fermented beverages depend mainly on the apple cultivar used. The beverages from the Rubin cultivar met all the requirements for fruit wines, but in the beverages from the Topaz cultivar, the limit values for total acidity were exceeded (>7g/L). The beverages from the Elise cultivar were characterized by low ethanol content and degree of fermentation. The process of fermentation resulted in a reduction of the total polyphenol content (TPC) in the beverages of the Rubin and Topaz cultivars, while in beverages from the Elise cultivar, in an increase of both TPC and AOX.

Abbreviations: AOX - antioxidant activity; TPC - total polyphenol content; FAN - free amino nitrogen; ABTS - diammonium salt of the 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid.

Efecto de distintos cultivares de manzana y cepas de levadura en parámetros de calidad seleccionados y en la actividad antioxidante de bebidas de manzana fermentadas

RESUMEN
El presente estudio se propuso evaluar el impacto de distintos cultivares de manzana seleccionados (Rubin, Topaz, Elise) y de cepas seleccionadas de vino, sidra, levadura de destilador y levadura de tipo silvestre en la dinámica del proceso de fermentación, la composición química y las propiedades antioxidantes (AOX) de bebidas de manzana fermentadas. Los parámetros de calidad de las bebidas fermentadas dependen principalmente del cultivar de manzana que se utilice. Aunque las bebidas elaboradas a partir del cultivar Rubin cumplen con todas las características requeridas para vinos de frutas, se constató que los valores límite de acidez total fueron rebasados (>7g/L) en las bebidas elaboradas a partir del cultivar Topaz. Por otra parte, las bebidas obtenidas del cultivar Elise se caracterizan por su bajo contenido de etanol y su escaso grado de fermentación. Se comprobó que en las bebidas elaboradas a base de los cultivares Rubin y Topaz, el proceso de fermentación produce una reducción en el contenido total de polifenoles (TPC), mientras que en las bebidas obtenidas del cultivar Elise provoca un aumento en el TPC y las AOX.

Introduction
The quality of alcoholic beverages is strictly dependent on the quality of the raw materials, the yeast used and the course of the fermentation process. The fruit should be adequately mature, which guarantees the creation of compounds responsible for taste and aroma (Pischl, 2010). The total extract content, especially sugars, and acidity are parameters of key importance in the production of alcoholic beverages. The appropriate sugar content ensures the required strength of beverages. The ethyl alcohol which is produced has many functions in the beverage, these include: providing the correct flavor, acting as a solvent for many organic compounds, participating in the transformation of volatile components, and also increasing the shelf life of the drink. The proper acidity of the fruit translates into appropriate sensory feelings during consumption, while organic acids contained in the fruit ensure the freshness of the ready-to-drink beverage. Organic acids also help to create the desired fragrance of the drink by forming esters, mainly during the maturation stage (Jordão, Vilela, & Cosme, 2015). So that the fermentation process runs properly, yeast should be provided with elements such as nitrogen, carbon, phosphorus, potassium, and magnesium in an easily absorbable form which allows them to pass through the membrane into the cell (Kotarska, Dziemianowicz, & Czupryński, 2013).

Fresh apples and products from their processing can also be a natural source of antioxidants (Markowski, Mieszczakowska, & Czupryński, 2013). The antioxidant substances responsible for the biological value of these fruits are polyphenols. The main classes of polyphenolic compounds identified in the apple fruit...
are flavanols (catechin and its derivatives), procyanidins, dihydrochalcones (phlorizin and phloretin) and phenolic acids (mainly chlorogenic), and flavonols (quercetin and its glycosides) (Alonso-Salces et al., 2005; Awad, De Jager, & Van Westing, 2000; Khanizadeh et al., 2008). However, it should be noted that some polyphenols present in the fruit undergo transformation during ethanol fermentation. These changes may cause an increase in antioxidant activity (Aksyolu & Karakaya, 2013).

The chemical composition of apple wines is also closely dependent on the course of the fermentation process. The fermentation process is usually initiated by wild yeasts, which have low tolerance to ethanol as they belong either to the Kloeckera and Hanseniaspora genera or others with low fermentation activity, such as Candida, Pichia or Torulaspora. After 3–4 days, they are replaced by Saccharomyces cerevisiae which continues and completes the fermentation process (Ciani, Beco, & Comitini, 2006). Spontaneous fermentation can bring satisfactory results in the form of a wine with an interesting, rich taste and aroma. In the initial phase, the wild yeasts produce large amounts of glycerol and aromatic substances but do not guarantee the proper course of fermentation and they significantly increase volatile acidity, in contrast to selected noble yeasts. Spontaneous fermentation cannot be conducted at a temperature below 15°C; there are also often problems with the fermentation of musts with a higher concentration of sugars (Bosak, 2008). Selected yeast strains are available on the market and they ferment sugars with the production of ethanol and compounds responsible for the desired bouquet, without creating harmful by-products (Pischl, 2010). Using them, fermentation can be conducted in a specific, intended manner, obtaining a liquor with the required parameters, and doing so in a repeatable fashion, which is important for the industry.

The aim of the study was to assess the impact of apple cultivars and yeast strains on the dynamics of the fermentation process as well as on the selected quality parameters and antioxidant properties of the obtained fermented alcoholic apple beverages.

Materials and methods
Preparation of musts for fermentation

Three cultivars of apples, ‘Topaz’, ‘Elise’ and ‘Rubin’, were used in the experiment. Apple cultivars were selected on the basis of results from preliminary experiments (unpublished data) which assessed the suitability of ten varieties of apples. All apples were obtained from a pomological orchard of the University of Agriculture, located in Garlica Murowana (near Cracow, Poland). The apples were washed, ground, and pressed on a Zottel hydraulic press (35 L). The musts (0.5 L) obtained from particular cultivars were poured into bottles (0.7 L) and then inoculated with the commercial yeast species intended for industry (materials and equipment for fermentation – Erbslöh, Germany) at 0.2 g/L (dry yeasts were hydrated according to the manufacturer’s recommendations). The must had not been sterilized prior to inoculation. In the experiments various yeast types and strains were used; strains of wine yeasts were ‘Tipico F3’, ‘Veltliner F3’, ‘InterDry F3’ and ‘Elegance’; the cider yeasts were ‘Cider Yeast’ and ‘French Cider Gozdowa’; while distillery yeasts were ‘Red Ethanol’ and ‘Spiriferm Arom.’ All these strains belong to the Saccharomyces cerevisiae species. The selected strain of wild yeast (Torulaspora delbrueckii/Saccharomyces rosei), commercially available as ‘Wild & Pure’ (materials and equipment for fermentation – Erbslöh, Germany), was also used. The bottles with inoculated must were closed with fermentation tubes filled with glycerine and allowed to ferment for two weeks (at 20°C). During this time the mass of the settings was periodically checked. The fermented musts were drained from the yeast sludge and aged at 4°C for three weeks.

The content of total extract, sugars, free amino nitrogen and total polyphenols, total acidity and antioxidant activity were determined in the fruit and in the fermented apple musts. In addition, ethyl alcohol and volatile acidity were determined in the fermented samples.

Determination of total acidity, volatile acidity, total extract content, and ethyl alcohol content

The determination of total extract content (i.e., the sum of water-soluble compounds with a density above 1 g/ml), ethyl alcohol content, total acidity, and volatile acidity were conducted in accordance with the methods recommended by the International Organisation of Vine and Wine (OIV, 2014). Total extract content and ethanol content in apple wine were determined by distillation methods using pycnometric density determination, total acidity was determined with the potentiometric method, volatile acidity determined using the titration method, and the total extract of must determined by the refractometric method.

Content and profile of sugars

The content of sugars was determined by the HPLC method. The analysis of the sugar profile was conducted with the Shimadzu (Japan) NEXERA XR apparatus with an RF-20A refractometric detector. The separation was conducted with an Asahipak NH2P-50 4.6 × 250 mm Shodex column (Showa Denko Europe, Germany) thermostated at 30°C. The mobile phase consisted of an acetonitrile aqueous solution (70%), and the isocratic elution program (0.8 mL/min) lasted 16 min.

Quantitative determinations were made with the use of standard curves prepared for appropriate standards: glucose, fructose, sucrose, and maltose. The tables of results only show the concentrations of sugars with higher amounts than the detection threshold of the method.

Free amino nitrogen (FAN) content

Free amino nitrogen (FAN) was determined with the ninhydrin method. The absorbance of the samples was measured at a wavelength λ = 575 nm (Spedding, Harrison, Ganske, & Dell, 2012).

Total polyphenol content

Total polyphenol content was determined with a spectrophotometric method (UV-VIS Beckman) with Folin-Ciocalteu reagent (Tarko, Duda-Chodak, Sroka, Satora, & Tuszynski, 2010b).
Figure 1. The dynamics of fermentation of must from Rubin apples depending on the yeast strain used.

Figura 1. Dinámicas de fermentación del mosto de manzanas Rubin dependiendo de la cepa de levadura utilizada.

Figure 2. The dynamics of fermentation of must from Elise apples depending on the yeast strain used.

Figura 2. Dinámicas de fermentación del mosto de manzanas Elise dependiendo de la cepa de levadura utilizada.

Figure 3. The dynamics of fermentation of must from Topaz apples depending on the yeast strain used.

Figura 3. Dinámicas de fermentación del mosto de manzanas Topaz dependiendo de la cepa de levadura utilizada.
Table 1. Chemical composition, total polyphenol content, and antioxidant activity of apple must.

|                | Rubin          | Elise           | Topaz          |
|----------------|----------------|-----------------|----------------|
| Total extract  | 125.0 ± 12.6a  | 90.0 ± 3.8b     | 110.0 ± 7.8a   |
| Fructose       | 50.6 ± 0.2a    | 35.8 ± 0.2b     | 35.2 ± 1.1b    |
| Glucose        | 36.0 ± 1.0a    | 28.6 ± 2.4b     | 53.1 ± 3.9b    |
| Total acidity  | 5.05 ± 0.07a   | 5.52 ± 0.08a    | 7.32 ± 0.10a   |
| Free amino nitrogen (FAN) [mg/L] | 72.45 ± 7.97a | 36.51 ± 4.02b | 50.95 ± 5.60b |
| Total polyphenol content [mg of catechin/100 mL] | 28.17 ± 1.30a | 9.29 ± 0.42b | 39.17 ± 1.68b |
| Antioxidant activity [mg of trolox/100 mL] | 74.21 ± 6.75a | 16.47 ± 1.50b | 87.17 ± 7.93a |

Means marked with the same letter in a row are not significantly different at p < 0.05.
Las medias indicadas con la misma letra en una fila no son significativamente diferentes, p < 0.05.

Antioxidant activity

The antioxidant capacity of samples was determined by the ABTS-cation radical scavenge assay (diammonium salt of the 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid; Tarko et al., 2010b).

Statistical analysis

There were at least three physical repetitions of each setting. All samples were analyzed once, but in the case of discrepancies in the results, the analysis was repeated. Results were shown as the arithmetic mean with standard deviation (± SD). The statistical analysis was performed using InStat v.3.01 (GraphPad Software Inc., USA). A single-factor analysis of variance (ANOVA) with post hoc Tukey’s test was applied to determine the significance of differences. The Kolmogorov-Smirnov test was carried out to assess the normality of distribution.

Results and discussion

The effect of apple cultivar and yeast strain on the dynamics of the fermentation process

During the ethanol fermentation process, the change in the mass of the settings takes place due to the release of carbon dioxide. It has been shown that the use of some yeast strains significantly affects the dynamics of fermentation (Figs. 1 – 3). The Rubin apple musts, fermented with the ‘Wild & Pure’ and ‘Cider Yeast’ yeast strains, were characterized by both a longer period of adaptation and slower fermentation (Figure 1). The slowdown in fermentation, in these two cases, extended the process by about three days but did not affect the final fermentation rate. Moreover, such a slowdown may be beneficial due to the smaller amount of heat released and consequently, a lower tendency for excessive temperature increase during the process.

In the musts of the Elise cultivar of apples (Figure 2), the turbulent fermentation lasted, depending on the yeast strain used, from three to six days, i.e., shorter than in the case of the Rubin (Figure 1) or Topaz (Figure 3) apple-pressed must. This is mainly due to the significantly lower content of fermenting sugars in this must (Table 1). Also, in this case, the musts inoculated with the ‘Wild & Pure’ yeast, and ‘Cider Yeast’ fermented more slowly—complete attenuation was achieved only after about seven days, finally obtaining identical fermentation efficiency as in the other settings (Figure 2).

Effect of apple cultivar and yeast strain on the chemical composition of must and fermented apple beverages

The musts obtained from the studied apple cultivars differed significantly in the content of the total extract and fermentable sugars; this had an effect on the fermentation dynamics. The musts of the Rubin and Topaz cultivars demonstrated high values for the analyzed parameters, whereas the must from the Elise cultivar contained very little fermentable sugars (about 25% less than the other cultivars) and was characterized by a low total extract (Table 1). Noteworthy is the fact that the Topaz apple cultivar was dominated by glucose, while the other cultivars were richer in fructose. Significant differences were also found in total acidity and ammonium nitrogen content. The cultivar most abundant in acids was Topaz, while the Rubin apples contained the highest amount of free amino nitrogen (Table 1). The quality parameters of fruits depend on many factors. In addition to the cultivar of apples, environmental conditions, sun exposure, fertilization method, harvest time, and many more factors are also of great importance (Tarko, Duda-Chodak, Sroka, Satora, & Jurasz, 2010a). The content of the total extract in the Rubin and Topaz cultivars was comparable to the results obtained by other researchers, while the content of reducing sugars was slightly smaller (Ticha et al., 2015; Wu et al., 2007). It was shown, however, that the content of the
### Table 2. Chemical composition, total polyphenol content, and antioxidant activity of beverages from apples of the Rubin cultivar obtained with the various yeast strains.

| Beverages obtained with the various yeast strains | Wild & Pure | Tipico F3 | Vettiner F3 | Elegance | InterDry F3 | Spirifarm Arom | Red Ethanol | Cider Yeast | French Cider Gozdowa |
|-----------------------------------------------|-------------|-----------|------------|----------|-------------|---------------|-------------|-------------|------------------------|
| Ethanol content [%]                          | 7.95 ± 0.14<sup>a</sup> | 7.74 ± 0.05<sup>a</sup> | 7.76 ± 0.12<sup>a</sup> | 7.76 ± 0.05<sup>a</sup> | 7.92 ± 0.09<sup>a</sup> | 7.84 ± 0.05<sup>a</sup> | 7.82 ± 0.05<sup>a</sup> | 7.37 ± 0.46<sup>c</sup> | 7.76 ± 0.15<sup>c</sup> |
| Total extract [g/L]                          | 18.00 ± 0.00<sup>b</sup><sup>c</sup> | 19.73 ± 1.50<sup>c</sup> | 18.00 ± 0.00<sup>b</sup> | 18.00 ± 0.00<sup>b</sup> | 17.13 ± 1.50<sup>d</sup> | 19.73 ± 1.50<sup>d</sup> | 20.60 ± 0.00<sup>d</sup> | 18.00 ± 2.60<sup>c</sup> |
| Fructose [g/L]                               | 4.04 ± 0.23<sup>a</sup> | 4.05 ± 0.01<sup>a</sup> | 3.77 ± 0.13<sup>a</sup> | 3.95 ± 0.49<sup>a</sup> | 4.21 ± 0.33<sup>a</sup> | 3.64 ± 0.38<sup>a</sup> | 3.52 ± 0.62<sup>a</sup> | 3.77 ± 0.15<sup>a</sup> | 4.07 ± 0.33<sup>a</sup> |
| Total acidity [g of malic acid/L]             | 4.96 ± 0.07<sup>a</sup> | 5.61 ± 0.10<sup>a</sup> | 5.38 ± 0.14<sup>a</sup> | 5.92 ± 0.14<sup>a</sup> | 5.32 ± 0.04<sup>a</sup> | 4.98 ± 0.04<sup>a</sup> | 5.47 ± 0.10<sup>a</sup> | 6.03 ± 0.07<sup>a</sup> | 5.76 ± 0.00<sup>a</sup> |
| Volatile acidity [g acetic acid/L]            | 0.17 ± 0.05<sup>a</sup> | 0.25 ± 0.05<sup>a</sup> | 0.31 ± 0.05<sup>a</sup> | 0.50 ± 0.15<sup>a</sup> | 0.23 ± 0.03<sup>a</sup> | 0.27 ± 0.03<sup>a</sup> | 0.32 ± 0.02<sup>a</sup> | 0.50 ± 0.06<sup>a</sup> | 0.38 ± 0.09<sup>a</sup> |
| Free amino nitrogen [FAN] [mg/L]              | 7.77 ± 0.19<sup>b</sup> | 10.76 ± 0.95<sup>b</sup> | 14.99 ± 0.54<sup>b</sup> | 10.90 ± 1.19<sup>b</sup> | 11.99 ± 4.36<sup>b</sup> | 6.13 ± 0.58<sup>b</sup> | 16.44 ± 1.10<sup>b</sup> | 12.81 ± 1.44<sup>b</sup> | 13.49 ± 1.23<sup>b</sup> |
| Total polyphenol content [mg of catechin/100 mL] | 14.97 ± 1.25<sup>c</sup> | 19.23 ± 1.17<sup>c</sup> | 19.45 ± 1.02<sup>c</sup> | 20.49 ± 0.48<sup>c</sup> | 18.67 ± 0.15<sup>c</sup> | 19.39 ± 0.53<sup>c</sup> | 20.52 ± 0.54<sup>c</sup> | 17.31 ± 1.79<sup>c</sup> | 19.44 ± 1.64<sup>c</sup> |
| Antioxidant activity [mg of trolox/100 mL]    | 42.30 ± 4.31<sup>c</sup> | 62.11 ± 6.61<sup>c</sup> | 47.07 ± 12.18<sup>c</sup> | 65.99 ± 2.21<sup>c</sup> | 65.30 ± 0.33<sup>c</sup> | 45.40 ± 3.62<sup>c</sup> | 70.35 ± 4.83<sup>c</sup> | 50.24 ± 8.67<sup>c</sup> | 63.93 ± 3.88<sup>c</sup> |

Means marked with the same letter in a row are not significantly different at p < 0.05.

Las medias indicadas con la misma letra en una fila no son significativamente diferentes, p < 0.05.
extract and sugars in the Elise cultivar was small, which was most probably associated with the harvest before full technological maturity.

The musts used for fermentation contained glucose and fructose (Table 1). After fermentation, the musts were characterized by the use of over 80% reducing sugars irrespective of the apple cultivar, with glucose being used completely (concentration below the detection limit of the method which was 0.2 g/L). This is consistent with studies which indicate that yeasts used for fermentation first use glucose available in the medium, followed by other simple sugars and disaccharides (Ribéreau-Gayon, Dubourdieu, & Donéche, 2006). The content of fructose in fermented beverages ranged from 0.2 g/L to 4.1 g/L, depending on the initial concentration of this sugar in the setting (Tables 2 – 4). The content of total extract and reducing sugars in the fruits (Table 1) translated into the concentration of alcohol in fermented beverages (Tables 2 – 4). Regardless of the yeast used, beverages obtained from Rubin apples had the highest ethanol content.

However, the ethanol concentration in all the beverages obtained was relatively small (4.89% to 7.95%). In the wines obtained from the apples of Szampion, Idared and Gloster cultivars analyzed by Satora, Sroka, Duda-Chodak, Tarko, and Tuszyński (2008), the alcohol content was higher than for the beverages determined in this work and was over 8% v/v.

The yeasts used for the fermentation included wine strains (‘Tipico F3’, ‘Veltliner F3’, ‘InterDry F3’, ‘Elegance’), cider strains (‘Cider Yeast’, ‘French Cider Gozdowa’), distillers strains (‘Red Ethanol’, ‘Spiriferm Arom’) and selected wild yeast cultures (‘Wild & Pure’). In most of the tests, changing the yeast strain used for the fermentation of the must from a given apple cultivar did not result in statistically significant differences in the final ethyl alcohol content.

Only beverages produced with cider yeast (‘Cider Yeast’) were characterized by a lower alcohol concentration and a higher amount of remaining extract compared to other strains. The content of ethanol was determined by the concentration of sugars in the must. Although different yeast strains are characterized by different adaptability to the conditions, as demonstrated by the analysis of fermentation dynamics (Figs. 1 – 3), the sufficiently long fermentation time allows almost complete use of available sugars, regardless of the yeast strain. The research of Ribéreau-Gayon (Ribéreau-Gayon et al., 2006) indicates that alcohol in a concentration above 2% negatively affects the quality of yeast, causing slower metabolism and weakening of cell viability. It should be noted, however, that all strains used in this work were yeasts selected specifically for the production of alcoholic beverages and therefore tolerate high ethanol concentrations.

The acidity of the musts used in the experiment met the requirements for fruit intended for the production of alcoholic beverages (>3.5 g/L), although the values obtained were slightly lower than those found in the studies by Wojdyło, Oszmiański, and Bielicki (2010). According to the research conducted by Suszyna (2007), the total acidity of the apples of the Elise, Idared, Jonagold, Pinowa, Ligol, and Szampion cultivars, in the period from August to the end of October, amounted to approximately 6.58 g/kg. The quality parameters of fermented apple beverages are not covered by EU standards and regulations. However, the requirements for these beverages are governed by the regulations of individual member states. In turn, according to the legal regulations in force in Poland (Dz.U., 2013), the total acidity in fermented fruit drinks should be 3.5 g to 7 g of malic acid per liter, and volatile acidity should not exceed 0.9 g/L. In beverages prepared from the Rubin cultivar, regardless of the yeast strain used to conduct fermentation, all samples showed normative acidity (Table 2). Similarly, the beverages obtained from the Elise cultivar showed normative acidity apart from three exceptions—it was too small in beverages obtained with the use of ‘Wild & Pure’ yeast, ‘Veltliner F3,’ and ‘Red Ethanol’ (Table 3). On the other hand, in the beverages obtained from the Topaz apples, the total acidity in all cases exceeded the limit values (Table 4), which was probably the result of the high acidity of the raw material used for fermentation. Among the 81 analyzed samples of fermented beverages, only one—obtained from the Topaz apples fermented with the ‘Spiriferm Arom’ yeast—had too much volatile acidity at 1.02 g/L (Table 4).

It is worth mentioning that even in the samples meeting the normative requirements, a significant influence of yeast strains on the general and volatile acidity of beverages was demonstrated. The smallest differences between the total acidity of fermented beverages with different yeast strains were observed in the Topaz cultivar (Table 4). The yeast strain ‘Cider Yeast’ should be especially noted: the beverages obtained with its presence were characterized by particularly high acidity, often higher than in fermented beverages using distillers yeast, wine yeast or wild yeast. The beverages obtained with the use of selected wild yeast (‘Wild & Pure’) were characterized by the lowest total and volatile acidity, sometimes even below the requirements of the legal standard (Dz, 2013).

The nitrogen fraction of apples includes the amino acids asparagine, glutamine, aspartic acid, glutamic acid, and serine, which constitute 86% to 95% of total amino acids, and can be easily assimilated by yeasts. The fruits harvested from extremely high fertilized trees can contain up to five times higher concentration of nitrogen compounds than usual. The content of nitrogen in apple can be low (<75 mg/L) or high (>150 mg/L) and depends on the age of the orchards, area, and the kind and amount of fertilizers used (Alberti, Giovanetti Vieira, Françoise Drilleau, Wosiacki, & Nogueira, 2011; Nogueira & LeQuéré, 2003). In the analyzed musts, the content of free amino nitrogen was small and ranged from 36 mg/L to 72 mg/L (Table 1). After fermentation, as a result of consumption by metabolically active yeast, the content of free amino nitrogen in all analyzed samples was several times lower than in the initial must. These results corresponded to total acidity—the higher the FAN content, the lower the total acidity of the wine.

**Influence of the apple cultivar and yeast strain on antioxidant activity and polyphenol content in musts and fermented apple beverages**

Numerous studies have evaluated the polyphenolic composition of apple fruit and showed a considerable variation, depending mainly on the cultivar and origin of the fruit. It was, for example, shown the quercetin glucoside
The fermentation process significantly affected both the antioxidant potential and the content of polyphenols in beverages (Tables 2 – 4), with the direction and extent of the changes depending on the apple cultivar and the yeast strain used for fermentation. An exception was beverages obtained from apples of the Rubin cultivars, which, regardless of the yeast strain used, showed a reduction in both the total polyphenol content and antioxidant activity when compared to the initial must (Table 2). Despite the fact that in all beverages obtained from the Topaz apples the polyphenol content decreased, their antioxidant activity significantly depended on the yeast strain used for fermentation. In the samples fermented with the ‘InterDry F3’, ‘Spiriferm Arom,’ and ‘Red Ethanol’ yeast, the antioxidant potential increased significantly; whereas in the beverages for the production of which wild yeast and ‘Tipico F3’ wine yeast were used, the potential significantly decreased (Table 4). The fermentation of must from the Elise cultivar caused a significant increase in both the content of polyphenols and antioxidant activity of the beverages (Table 3).

It is worth noting that irrespective of the apple cultivar, the beverages fermented with the use of ‘Red Ethanol’ yeast were characterized by a greater antioxidant potential than those fermented with the other distillers yeast strain—‘Spiriferm Arom.’ It was also shown that the use of wild yeast for fermentation, irrespective of the apple cultivar, resulted in a relatively low content of polyphenols in fermented beverages and low antioxidant potential, compared to the other tested yeast strains. Among the wine yeasts, the concentration ranged from 0.69 mg/100 g (Vrhovsek, Rigo, Tonon, & Mattivi, 2004) to 2.4 mg/100 g (Lee, Kim, Kim, Lee, & Lee, 2003), while epicatechin concentration ranged from 5.18 mg/100 g to 7.42 mg/100 g (Arts, Van De Putte, & Holliman, 2000; Lee et al., 2003; Vrhovsek et al., 2004). The polyphenol compounds present in apples, however, differ in antioxidant properties; due to this criterion, they can be arranged in the order of epicatechin gallates > caffeic acid > epicatechin > chlorogenic acid > catechin > rutin > ferulic acid (Bandoniene & Murkovici, 2002). Kahle, Kraus, and Richling (2005) observed that the total content of polyphenols was 154 mg/L to 178 mg/L in dessert apple juice, but as much as 261 mg/L to 970 mg/L in cider apple juices. In the apple must analyzed in this work, the polyphenol content was significantly lower and ranged from 9.29 mg catechin/100 ml to 39.17 mg catechin/100 ml (Table 1). Although the antioxidant activity of apples is not high, due to the frequent consumption of these fruits, they are among the raw materials with significant anti-radical potential. The Topaz apples were characterized by the highest antioxidant activity, among those studied, which correlated with the content of polyphenolic compounds (R = 0.98). Polyphenols are produced by plants as a defense against pests and infections; therefore, in mature fruits (the Topaz and Rubin cultivars) there are more polyphenols than in the Elise apples, which were harvested before they reached full maturity. These fruits contain only a few simple sugars (the main nutrient of microorganisms) and polyphenolic compounds.

The tests showed that the proper selection of a yeast strain is important for the preservation and even improvement of the antioxidant properties of fermented beverages. The fermentation process significantly affected both the antioxidant potential and the content of polyphenols in beverages (Tables 2 – 4), with the direction and extent of the changes depending on the apple cultivar and the yeast strain used for fermentation. An exception was beverages obtained from apples of the Rubin cultivars, which, regardless of the yeast strain used, showed a reduction in both the total polyphenol content and antioxidant activity when compared to the initial must (Table 2). Despite the fact that in all beverages obtained from the Topaz apples the polyphenol content decreased, their antioxidant activity significantly depended on the yeast strain used for fermentation. In the samples fermented with the ‘InterDry F3,’ ‘Spiriferm Arom,’ and ‘Red Ethanol’ yeast, the antioxidant potential increased significantly; whereas in the beverages for the production of which wild yeast and ‘Tipico F3’ wine yeast were used, the potential significantly decreased (Table 4). The fermentation of must from the Elise cultivar caused a significant increase in both the content of polyphenols and antioxidant activity of the beverages (Table 3).
‘InterDry F3’ and ‘Elegance’ strains proved particularly beneficial in the context of antioxidant properties of beverages, especially in beverages obtained from the apples of the Rubin and Topaz cultivars (Tables 2 and 4).

Polyphenols in the raw material usually occur in the form of glycosides (Lee et al., 2003), which are hydrolyzed during fermentation to free sugars (partly used by yeasts) and aglycones, which usually have greater antioxidant activity than their corresponding glycosides (Bouayed, Hoffmann, & Bohn, 2011). This explains the increase in the antioxidant potential of the must after fermentation. It should also be mentioned that the fruits characterized by incomplete maturity contain higher amounts of tannins in their composition, whose hydrolysis leads to an increase in the concentration of catechins. The Elise cultivar showed incomplete technological maturity during the performance of this work, which explains the significantly increased total polyphenol concentration and antioxidant activity. In addition, apple fruit contains relatively large amounts of procyanidins which belong to the complex forms of polyphenols. Their hydrolysis to monomers also contributes to an increase in the total polyphenol content (determined by the Folin-Ciocalteu method) and the antioxidant activity of fermented beverages (Bouayed et al., 2011). The increase in antioxidative potential and the total polyphenol content of some fermented beverages studied in this work may also be due to the fact that some yeasts strains, e.g., from the Saccharomyces cerevisiae species, are able to synthesize polyphenol compounds, somehow de novo, from amino acids. Phenylalanine and tyrosine are precursors of p-coumaric acid, from which kaempferol and quercetin may then be formed (Eichenbergera et al., 2017; Rodriguez, Chen, Khoomrungb, Özdemira, & Borodina, 2017a; Rodríguez et al., 2017b).

On the other hand, a decrease in polyphenol content and/or antioxidant activity was observed in some fermented beverages. This phenomenon occurs, among others, due to the binding of polyphenolic compounds (adsorption) responsible for the antioxidant potential by the cell walls of dead yeast cells. After fermentation, these cell walls sediment and are separated from the beverages, which mature at a reduced temperature to harmonize the taste and smell. It was shown that the reduction in the concentration of polyphenol compounds caused by such binding may reach even 50% of the initial value (Hainal, Ignat, Volf, & Popa, 2011).

Another explanation for the lower antioxidant activity after fermentation is the possibility of degradation of polyphenolic compounds to less active derivatives. For instance, phenolic acids have a lower antioxidant potential than the flavonoids from which they are formed (Bandoniene & Murkovic, 2002). As a result of such transformations, a relatively high concentration of polyphenolic compounds is observed, but the antioxidant activity of beverages decreases.

Conclusions

The quality of alcoholic beverages is strictly dependent on the quality of the raw material used. The Elise apple cultivar used in the research was collected before their full technological maturity, so that the musts obtained from them contained very few sugars, and as a consequence produced a small alcohol content. The fermented beverages obtained from the Rubin cultivar met all the requirements for these type of alcoholic beverages, whereas in the Topaz beverages, the limit values for total acidity were exceeded. A significant influence of the yeast strains used in the fermentation on the total and volatile acidity of alcoholic beverages was observed. The beverages obtained with the use of the ‘Cider Yeast’ were characterized by a higher total acidity than those obtained with the other yeast strains. The Topaz apples were characterized by the highest antioxidant activity, among those studied, which correlated with the content of polyphenolic compounds. In the beverages made from the Rubin and Topaz cultivars, a reduction in the total polyphenol content was observed after fermentation, whereas in the case of the Elise cultivar, an increase in the polyphenol content and antioxidant activity occurred, dependent on the yeast strain used. The use of wild yeast for fermentation, regardless of apple cultivars, resulted in a relatively low content of polyphenols in fermented beverages and a low antioxidant potential. The most favorable quality parameters of the fermented beverages were obtained after fermentation with the ‘InterDry F3’ and ‘Elegance’ wine yeast strains and the ‘Red Ethanol’ distillers yeast strains.

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