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Analytical Methods

The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples

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ABSTRACT

The aim of this study was to determine the influence of apple variety (Šampion, Idared and Gloster) on the polyphenol profile, volatile composition and sensory characteristics of apple wines. Apples were harvested from the orchard in Garlica Murowana (Poland) and the experiments were conducted on a laboratory scale. Statistically significant differences were detected in the chemical composition of the analyzed wines. The highest antioxidant activity was found in Šampion wines, which was associated with a relatively high concentration of chlorogenic acid and procyanidins. These samples also contained high amounts of acetaldehyde, ethyl acetate and methanol. Idared wines showed a similar polyphenol profile, but they had lower antioxidant capacity and were characterized by a high level of butanol and acetic acid. Gloster wines were distinguished from other samples by a lower concentration of polyphenols and higher concentration of fusel alcohols. During sensory evaluation, wines produced from Idared apples scored the highest value for overall quality.

1. Introduction

The moderate climate of Poland is conducive to high apple production. The average annual harvest of these fruits has been estimated at about 1.5 million tonnes and the industry processes about 700 thousand tonnes. Apples are valuable raw materials for the production of juice, stewed fruits, jellies, dry fruits, concentrates, pomaces, puree and wines. In Poland, for many years, apple wine production has been an important segment of the fruit and vegetable industry. For fermentation, most often late cultivars with smaller fruits and larger ratio of skin surface to flesh are used. The wines produced from this kind of fruit are spicy and more aromatic.

Quantitatively the most important volatiles in apple wine are the higher alcohols, esters, and lower fatty acids (Vidrih & Hribar, 1999). These compounds are formed during fermentation and are mainly derived from raw material, as well as metabolic processes of yeast under anaerobic conditions. Both qualitative and quantitative characterizing aroma-producing compounds in apple wine and their formation during fermentation are desired in order to provide quality control of apple wine.

Phenolic compounds are a second important group of apple wine constituents because they greatly contribute to their sensory properties and other attributes. In particular, polyphenolic compounds have antioxidant activity, free-radical scavenging capacity, coronary heart disease prevention, and anticarcinogenic properties. Furthermore, phenolics are associated with bitterness, astringency, and colour stability, and some of them have been used for detecting adulterations in apple products and could be inhibitors for microbiological growth-avoiding process spoilages (Madrera, Lobo, & Valles, 2006). From the quantitative point of view, there are five major groups of polyphenols found in apple wines: flavan-3-ols, procyanidins, flavonols, dihydrochalcones, and hydroxycinnamic acids and derivatives (Piyasena, Rayner, Bartlett, Lu, & McKellar, 2002; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

The aim of this study was to determine the influence of apple variety on the polyphenolic profile, volatile composition and sensory characteristics of apple wines. Šampion, Idared and Gloster apples, chosen for the research, are among the most popular varieties of dessert apples grown in Poland, constituting over 40% of Polish apple production. The majority of these fruits are destined for consumption. However, in terms of chemical composition and sensory features they represent a valuable raw material for the food processing industry, including the wine industry. Selection of the proper cultivar could result in the production of a beverage with favourable sensory features.

2. Materials and methods

2.1. Yeast, plant material and fermentation

Active wine yeast *Saccharomyces cerevisiae* cv. Johannisberg-Riesling (Culture Collection of the Fermentation Technology and

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Technical Microbiology Department of Agricultural University of Krakow) was used for the fermentation.

Apple musts were obtained from Šampion, Idared and Gloster apples (harvested on 2nd October 2006 from the experimental apple orchard in Garlica Murowana near Krakow) by treatment of clean and crushed fruits with pectinolytic preparation Pektopol PT-400 (0.3 mL kg^{-1}; Pektowin, Jasło, Poland) for 3 h at 28 °C, and pressing the fruit pulp.

Then sucrose (up to 22°Blg) and citric acid (up to 4.5 g/L) were added and the musts were pasteurized for 15 min at 104 °C. Alcoholic fermentation was conducted for 28 days at 25 °C in 3-l glass flasks containing 1.5 L of apple must inoculated with yeast (0.5 g dry weight per litre). The weight losses associated with the liberation of carbon dioxide were measured daily. After the fermentation, the young wine was separated from the sediments by carefully pouring it into another vessel, and was then kept for further clarification for 48 h at 4 °C. Clarified young wines were subjected to the analysis. All samples were done in triplicate.

2.2. Enological parameters analysis

The ethanol content, total extract, sugar-free extract, reducing sugars and sucrose concentrations were determined using official methods (OIV., 2005).

pH and titratable acidity (TA) were determined using Titrator Mettler DL 25 equipped with a printer (Switzerland). Titratable acidity was calculated from the volume of NaOH used for titration and expressed as g/L of malic acid.

2.3. Total antioxidant activity assay (TAA)

Total antioxidant activity was determined using the ABTS method (Miliauskas, Venskutonis, & van Beek, 2004). The $ABTS^{+}$ radical was generated by oxidation of ABTS with potassium persulphate $K_2S_2O_8$ (POCh SA, Gliwice, Poland). Once the radical was formed, 0.1 mL of diluted wine sample was added to 1 mL of $ABTS^{+}$ radical cation and the absorbance was measured at 734 nm with a spectrophotometer (DU-650, Beckman Instruments, Inc., Fullerton, USA). Standard Trolox solutions (40–200 μM) were also evaluated against the radical in order to obtain a calibration curve. Results are expressed as milligrams of Trolox equivalent (TE) per 100 mL of sample.

2.4. Total phenol index (TPI)

The amount of total phenols in wines were determined according to the Folin–Ciocalteu colorimetric method (Waterhouse, 2002). Wine samples were diluted with water (1:4). A 1 ml volume of the standard or sample solution was added to 5 ml of Folin–Ciocalteu reagent (1:10 dilution, Sigma–Aldrich), 50 ml of deionised water and 20 ml of sodium carbonate (20% (w/v)). The reaction mixture was then made up to the mark in a 100 ml volumetric flask and was left to stand for 30 min before measuring the absorbance at 765 nm (spectrophotometer Beckman DU-650). A calibration curve was obtained with gallic acid solutions (concentration range 0.4–5 mg/L; Fluka). The results are expressed as milligrams of gallic acid per litre of wine.

2.5. Polyphenols analysis (HPLC)

HPLC apparatus consisting of Merck-Hitachi L-7455 diode array detector and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan) was employed. Separation was performed on a Synergi Fusion RP-80A 150 × 4.6 mm (4 μm) Phenomenex (Torrance, CA USA) column thermostated at 30 °C. The mobile phase was composed of a solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 36 min 25% B, followed by washing and reconditioning the column. The flow rate was 1 mL/min, and the runs were monitored at the following wavelengths: flavanols at 280 nm, phenolic acids at 320 nm, flavonols at 360 nm and anthocyanidins at 520 nm. Retention times and spectra were compared to those of pure standards within 200–600 nm.

In addition, an enzymatic hydrolysis of flavonol glycosides in citrate buffer solution (pH 5.0) was performed. Afterward, specific enzymes were added: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidinase (Sigma, Steinheim, Germany). The disappearance of single peaks in the chromatogram and formation of the corresponding aglycon was observed using HPLC after 1-h incubation at 38 °C with a specific enzyme.

Results (expressed as mg/100 mL of apple wine) were read from standard curves developed for the corresponding standards: chlorogenic acid, caffeic acid, *p*-coumaric acid, *p*-coumarylquinic acid, (+)catechin, (−)epicatechin, phloridzin, and quercetin glycoside manufactured by the Sigma–Aldrich company. In order to determine the magnitude of error in the selected series, the assays were repeated. A standard error in the HPLC assays was below 10%.

2.6. Volatile compounds analysis (GC-SPME)

Two milliliters of each wine sample was transferred to a 15 mL amber vial having screw caps (Supelco) with a magnetic stirrer and 1 g of NaCl, which was then spiked with 2 μL of internal standard (4-methyl-2-pentanol; Fluka). The SPME device (Supelco Inc., Bellefonte, PA, USA) coated with PDMS (100 μm) fiber was first conditioned by inserting it into the GC injector port at 250 °C during 1 h. For sampling, the fiber was inserted into the headspace under magnetic stirring (300 rpm) for 35 min at 40 °C. Subsequently, the SPME device was introduced in the injector port for chromatographic analysis and was remained in the inlet for 2 min.

The GC-SPME analysis was performed on a Hewlett Packard 5890 Series II chromatograph system. The tested components were separated on a capillary column HP-INNOVAX (crosslinked polyethylene glycol stationary phase; 30 m × 0.53 mm ID with 1.0 μm film thickness). The detector and injector temperature was 250 °C, and the column was heated using the following temperature program: 35 °C for 5 min at an increment 5 °C/min to 110 °C, then 40 °C/min to 220 °C and maintaining a constant temperature for 3 min. The carrier gas was helium at a 20.0 mL/min flow. Hydrogen flow speed was 33.0 mL/min, and that of air was 400 mL/min.

The qualitative and quantitative identification of volatile substances (acetaldehyde, ethyl acetate, methanol, propanol, isobutanol, butanol, amyl alcohols, pentanol, hexanol, 2-phenylethanol and acetic acid; Sigma–Aldrich) was based on the comparison of retention times and peak surface area read from sample and standard chromatograms.

All tests were carried out three times.

2.7. Sensory analysis

Sensory assessment of apple wine samples was performed using the Buxbaum model of positive ranking (Miličević, Banović, Kovačević-Ganić, & Gracin, 2002). This model is based on four sensorial experiences rated by maximum 20 points. The samples of apple wines were subjected to sensory evaluation by a panel comprising 10 qualified testers, all of them highly experienced in sensory testing.

2.8. Statistical analysis

SPSS 13.0 software was applied for statistical results analyses. Statistically significant differences between results ($p = 0.05$) were evaluated using one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Enological characteristics of apple wines

The fermentation of musts produced from different apple varieties was characterized by diverse kinetics (Fig. 1). A turbulent stage of fermentation appeared first in the Gloster samples (after 24 h), and was observed about a day later in the other fermenting musts. The highest total weight loss, associated with the liberation of carbon dioxide, was also found after Gloster musts fermentation. Fermentation rate of musts inoculated with same microbial culture depends mainly on the bioavailability of nutritious components and presence of compounds that could inhibit yeast growth (Herrero, Garcia, & Diaz, 2003). The relatively high level of free sugar extract in the Gloster apple musts (17.8 g/L; Table 1) could be related to a favourable concentration of nitrogen compounds (polypeptides, amino acids) for yeast growth. In the samples that were characterized by higher amounts of sucrose (natural origin for Gloster as well as added for Idared), higher weight losses were also detected.

The musts that contained more antioxidants and were distinguished by higher antioxidant potential (Table 3) fermented longer and more slowly. Polyphenolic compounds are known to possess antimicrobial activity against a wide range of microorganisms (Ates & Erdogrul, 2003; Cowan, 1999), and the mechanism of their action first of all depends on their chemical structure.

After 4 weeks of fermentation, ethyl alcohol concentration, titratable acidity, antioxidant activity, and the amount of polyphenols and volatile compounds were analyzed in the young apple wines (Tables 1–5).

The fermenting sugars of musts were used almost entirely by the yeast hence the obtained wines could be classified as "dry" (Table 3). A high fermentation degree (near 100%) was obtained in the Gloster wines, which could be a result of a very intense fermentation process.

A similar amount of ethyl alcohol, ranging from 84.1 to 88.4 g/L, was detected in all samples. However, the level of titratable acidity, reported as grams of malic acid per litre of wine, differed significantly among the wines, from 3.32 (Gloster) to 4.31 g/L (Idared). A reduction in the titratable acidity of all fermented wines was noted the greatest decrease (about 1.4 g/L) was found in the samples without added of citric acid. The observed changes in acidity suggest that the strain of *Saccharomyces cerevisiae* used for fermentation showed the capacity to metabolise malic acid, the acid that predominates in apples. It is known that the majority of wine strains of *S. cerevisiae* can assimilate malic acid as the sole carbon or energy source in the presence of glucose or other assimilable carbon sources. However, within *Saccharomyces* species, remarkable differences exist in their ability to decompose malic acid during alcoholic fermentation (from 0 to 3 g/L malic acid) (Redzepovic et al., 2003).

3.2. Antioxidant activity and polyphenolic composition of apple wines

Apple fruits contain relatively high amounts of various polyphenols with antioxidant activity. Most of these compounds pass from the fruits to juices, wines or other products and remain active. However, changes of their profile as well as phenolic degradation during disintegration of raw material, fermentation, and wine aging have been reported (Heinonen, Lehtonen, & Hopia, 1998).

The analysis of antioxidant activity and concentration of individual polyphenol compounds showed statistically significant differences between wines obtained from different apple varieties (Table 3). Šampion wines possessed the highest antioxidant activity (466.1 mg TE/100 mL of sample) among wines, whereas the Gloster samples showed over five times lower activity, which correlated with the antioxidant properties of unprocessed fruits. Šampion apples have 2–3 times higher free-radical scavenging capacity than Gloster apples (Lachman, Šulc, Sus, & Pavlikova, 2006; Łata, 2007). These differences can increase during fruit processing, among other things, as a result of native polyphenol oxidase (PPO) action. The activity of PPO in Šampion apples is much lower than in other apple fruits (Markowski & Płocharski, 2006; Podsędek, Wilska-Jeszka, Anders, & Markowski, 2000).

A positive correlation between antioxidant activity (ABTS) and total polyphenol content ($r = 0.9954$) determined using Folin–Ciocalteu method as well as between antioxidant activity and total polyphenol content determined using HPLC ($r = 0.9989$) was found.

Antioxidant properties of apple products (expressed as a Trolox equivalent) are associated with a free-radical scavenging capacity of polyphenol compounds and some vitamins. According to Lu and Foo (2000), among apple antioxidant compounds, flavonoids

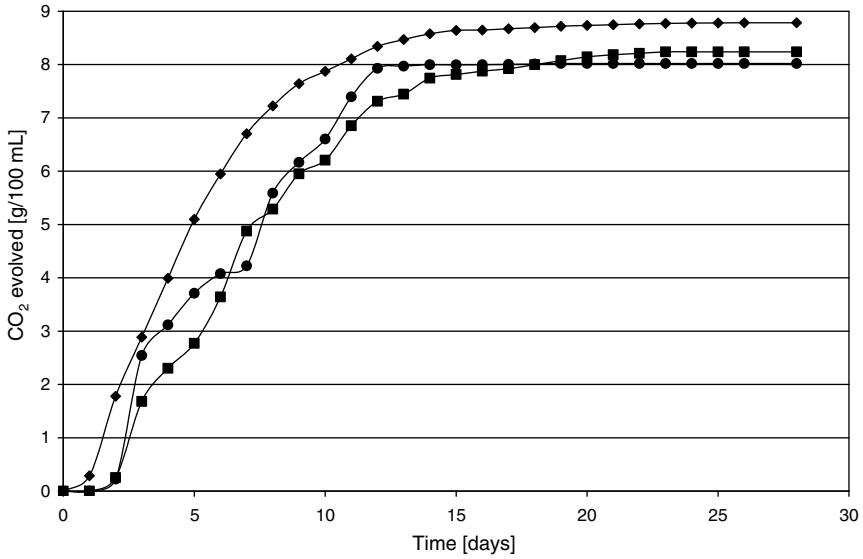

Fig. 1. The fermentation kinetics of Šampion (●), Idared (■) and Gloster (♦) apple must.

Table 1
The chemical composition of apple musts used for wine-making

Apple musts	Extract	Total sugars	Reducing sugars	Sucrose	Sugar-free extract	Titratable acidity[a]	pH
	(g/L)						
Šampion	111.7	104.0	99.6	4.2	7.9	1.49	4.65
Idared	104.7	93.7	91.9	1.8	11.0	2.83	4.03
Gloster	115.0	98.3	75.6	21.6	17.8	4.72	4.69

[a] Expressed as g/L of malic acid.

(epicatechin and its polymers), quercetin, its glycosides and chlorogenic acids show the highest antioxidant activity, while phloridzin and vitamins C and E show much lower activity.

The cinnamic acid derivatives and flavonols represent about 90% of all polyphenol compounds of apples, and their amount can vary significantly depending on the variety of fruit (Markowski & Płocharski, 2006; Podsędek et al., 2000; Robards et al., 1999). The level of individual polyphenols also depends on the vegetation season (Madrera et al., 2006; van der Sluis, Dekker, de Jager, & Jongen, 2001), maturity of fruits (Guyot, Marnet, Sanoner, & Drilleau, 2003), storage time and conditions (van der Sluis et al., 2001), fruit processing (Markowski & Płocharski, 2006), and the presence of peels and seeds during fermentation (Kiczorowska, Kiczorowski, & Bochniarz, 2006).

Chlorogenic acid was the main polyphenol in all analyzed apple wines, and its concentration ranged from 13.3 (Gloster) to 25.8 mg/L (Šampion). Markowski and Płocharski (2006) found that during processing of apples into apple must the level of this compound can decrease by as much as several times, probably as a result of acid degradation in the presence of PPO (Podsędek et al., 2000). Products obtained from apple varieties characterized by lower polyphenol oxidase activity (Šampion), usually contain a higher amount of chlorogenic acid (Podsędek et al., 2000).

The content of other polyphenol compounds varied widely and depended on the apple variety used for fermentation. Nevertheless, Šampion and Idared wines showed some similarities in polyphenol composition. A shared feature of these samples was a similar concentration of cinnamic acid derivatives and epicatechin, and a small amount of glycosidic derivatives of quercetin. Procyanidins B1 (4.6 mg/L) and B2 (7.4 mg/L) prevailed in wines obtained from Šampion apples, while procyanidin C1 (4.5 mg/L) prevailed in Idared wines. A different polyphenol profile was detected in Gloster wines. Significantly lower levels of cinnamic acid derivatives and procyanidins were found (Table 3). Among polyphenol compounds, epicatechin predominated (6.6 mg/L), with a concentration almost two times higher than other samples. Increased levels of phloridzin (2.2 mg/L) and glycosidic derivatives of quercetin (0.1–1.6 mg/L) were also observed. The higher amounts of epicatechin, quercetin derivatives and phloridzin in wines obtained from Gloster apples might be associated with the small size of fruits used for fermentation. This kind of fruit is characterized by a larger ratio of peel to flesh. It is well known that apple peel contains up to several times higher concentration of glycosidic derivatives of quercetin, epicatechin, phloridzin and phloretin-xyloglucoside (Tsao, Yang, Young, & Zhu, 2003) than apple flesh. All these compounds could be released to the must during pressing and maceration of fruits.

3.3. Volatile composition of apple wines

The amount of volatile compounds formed during fermentation depends mainly on the fermented must composition, fermentation conditions and strain of yeast that was used (Regodon Mateos, Perez-Nevado, & Ramirez Fernandez, 2006). In our experiments, two of the parameters mentioned above were constant hence the chemical composition of the raw material had the greatest influence on the volatile profile of the analyzed wines.

The obtained apple wines were characterized by significantly differentiated volatile composition (Table 4). The highest concentration of methanol (111.7 mg/L), acetaldehyde (20.9 mg/L) and ethyl acetate (15.8 mg/L) was detected in Šampion apple wines. Wines produced from Gloster apples were characterized by over four times lower level of these compounds and the Idared wines contained an intermediate amount of them.

Methanol is formed as a result of pectinesterase action on methoxy groups present in the pectins of crushed fruits. The formation of larger amounts of methanol mainly depends on pectin content and the level of their methylation (apple variety), the activity of original pectinesterase in fruit, and sometimes processing that causes tissue homogenisation (the addition of enzymatic preparations) or the type of yeast strain used for fermentation (Revilla & González-SanJosé, 1998; Tuszyński, 1989).

Acetaldehyde is one of the most important carbonyl compounds in alcoholic beverages, and is produced by yeast during fermentation. The sensory threshold for acetaldehyde ranges from 100 to 125 mg/L. Beyond this level it imparts a sherry type character to the wine which can also be described as green apple, sour and metallic. The flavour threshold of acetaldehyde in cider and apple wines has been established as approximately 30 mg/L. Differences in acetaldehyde production depend on the yeast species or strain used, although factors such as temperature and oxygen concentration affect its production by yeast as well. Sugar is a primary substrate, but metabolism of amino acids such as alanine also contributes to the formation of this compound (Herrero et al., 2003).

Ethyl acetate has a significant effect on the organoleptic characteristics of wines it may contribute a pleasant, fruity fragrance to the general wine aroma at concentrations lower than 150 mg/L. Increased ethyl acetate concentrations are indicative of prolonged storage of the raw material and probable acetic bacterial spoilage (Apostolopoulou, Flouros, Demertzis, & Akrida-Demertzi, 2005). The amount of ethyl acetate depends directly on acetic acid con-

Table 2
The chemical composition of young apple wines

Apple wines	Extract	Total sugars	Reducing sugars	Sucrose	Sugar-free extract	Ethanol concentration	Titratable acidity[A]
	(g/L)						
Šampion	18.8	6.3	6.3	0.0	12.5	82.1	3.72[a]
Idared	15.7	1.4	1.2	0.6	14.2	87.8	4.31[b]
Gloster	15.0	0.2	0.2	0.0	14.8	88.4	3.32[c]
Sig.[B]	–	–	–	–	–	ns	***

Values with different superscript roman letters (a–c) in the same column are significantly different according to the Duncan test ($p < 0.05$).
[A] Expressed as g/L of malic acid.
[B] Sig.: significance; *, **, *** – display the significance at 5%, 1% and 0.5% by least significant difference; ns: not significant.

Table 3
The antioxidant activity and polyphenols profile of wines produced from different apple varieties

Apple wines	Antioxidant activity (mg TE/100 mL)	Total polyphenol content (mg GAE/L)	Chlorogenic acid	Caffeic acid-glucoside	*p*-Coumaric acid	*p*-Coumarylquinic acid	(+)catechin	(−)epicatechin	Procyanidin B1	Procyanidin B2	Procyanidin C1	Phloridzin	Phloretin-xyloglucoside	Quercetin-galactoside	Quercetin-glucoside	Quercetin-arabinoside	Quercetin-xyloside	Quercetin-rhamnoside	Total polyphenols
			[mg/L]																
Šampion	466.1^{a} (±6.0)	639.0^{a} (±28.8)	25.8^{a} (±3.4)	2.5^{a} (±0.4)	0.9^{a} (±0.2)	4.3^{a} (±1.1)	3.5^{a} (±0.6)	3.8^{a} (±0.6)	4.6^{a} (±0.5)	7.4^{a} (±0.6)	4.5^{a} (±0.1)	1.2^{a} (±0.2)	0.5^{a} (±0.1)	0.3^{a} (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.1 (±0.0)	0.4^{a} (±0.1)	59.9^{a} (±7.1)
Idared	136.5^{b} (±4.9)	234.1^{b} (±26.2)	17.6^{b} (±0.8)	2.4^{a} (±0.4)	0.7^{a} (±0.2)	3.4^{a} (±0.5)	1.6^{b} (±0.4)	3.5^{a} (±0.7)	1.9^{b} (±0.2)	2.9^{b} (±0.3)	4.5^{a} (±0.5)	1.1^{a} (±0.2)	1.0^{b} (±0.1)	0.2^{a} (±0.1)	0.0 (±0.0)	0.0 (±0.0)	0.1 (±0.0)	0.4^{a} (±0.0)	41.0^{b} (±0.4)
Gloster	88.7^{c} (±4.2)	228.0^{b} (±14.8)	13.3^{c} (±1.2)	0.6^{b} (±0.2)	0.1^{b} (±0.0)	0.3^{b} (±0.0)	1.9^{b} (±0.6)	6.6^{b} (±0.4)	3.5^{c} (±0.5)	5.1^{c} (±0.7)	1.6^{b} (±0.2)	2.2^{b} (±0.3)	1.0^{b} (±0.1)	0.5^{b} (±0.1)	0.1 (±0.0)	0.5 (±0.0)	0.2 (±0.0)	1.6^{b} (±0.2)	39.1^{b} (±1.4)
Sig.[A]	***	***	***	***	***	***	**	***	***	***	***	***	***	*	ns	ns	ns	***	***

Values with different superscript roman letters (a)–(c) in the same column are significantly different according to the Duncan test ($p < 0.05$).
[A] Sig.: significance; *, **, *** – display the significance at 5%, 1% and 0.5% by least significant difference; ns: not significant.

Table 4
The volatile composition of wines produced from different apple varieties

Apple wines	Acetaldehyde	Ethyl acetate	Methanol	Propanol	Isobutanol	Butanol	Amyl alcohols	Pentanol	Hexanol	2-Phenylethanol	Total fusel alcohols	Acetic acid
	[mg/L]											
Šampion	20.9^{a} (±7.0)	15.8^{a} (±1.5)	111.7^{a} (±1.6)	18.6^{a} (±1.5)	90.5^{a} (±5.0)	19.2^{a} (±0.8)	314.7 (±19.3)	0.7 (±0.3)	5.1 (±0.3)	38.5^{a} (±2.4)	487.3^{a} (±26.0)	76.0^{a} (±6.9)
Idared	4.5^{b} (±0.7)	9.9^{b} (±0.3)	70.4^{b} (±0.7)	39.7^{a} (±1.7)	169.6^{b} (±0.9)	22.1^{a} (±0.1)	348.8 (±6.7)	2.3 (±1.4)	6.1 (±2.4)	49.4^{b} (±2.3)	638.0^{b} (±12.0)	124.0^{b} (±18.3)
Gloster	2.2^{b} (±0.2)	8.2^{b} (±2.0)	41.8^{c} (±2.2)	133.8^{b} (±10.5)	209.4^{b} (±20.8)	14.3^{b} (±3.2)	347.0 (±20.1)	2.1 (±0.2)	2.2 (±0.8)	56.4^{c} (±2.7)	746.5^{b} (±70.4)	36.0^{c} (±7.2)
Sig.[A]	*	***	***	***	***	**	ns	ns	ns	***	**	***

Values with different superscript roman letters (a)–(c) in the same column are significantly different according to the Duncan test ($p < 0.05$).
[A] Sig.: significance; *, **, *** – display the significance at 5%, 1% and 0.5% by least significant difference; ns: not significant.

Table 5
Sensory analysis of tested apple wines – Buxbaum model of positive ranking

Apple wines	Assessment characteristics				Total (max 20 pts)
	Colour (max 2 pts)	Clearness (max 2 pts)	Odour (max 4 pts)	Taste (max 12 pts)	
Šampion	2.0 (±0.0)	1.9 (±0.3)	2.9^{ab} (±0.9)	7.5 (±1.7)	14.3 (±2.5)
Idared	2.0 (±0.0)	1.9 (±0.3)	3.5^{a} (±0.5)	8.6 (±2.4)	16.0 (±2.4)
Gloster	2.0 (±0.0)	1.9 (±0.3)	2.4^{b} (±0.7)	7.6 (±3.6)	13.9 (±4.1)
Sig.[A]	ns	ns	**	ns	ns

Values with different superscript roman letters (a)–(c) in the same column are significantly different according to the Duncan test ($p < 0.05$).
[A] Sig.: significance; *, **, *** – display the significance at 5%, 1% and 0.5% by least significant difference; ns: not significant.

centration an increase in the level of ethyl acetate causes a decrease in the concentration of acetic acid (Lilly, Lambrechts, & Pretorius, 2000).

Wines obtained from Idared apples were distinguished by a high content of acetic acid (124.0 mg/L). In other samples the amount of these compounds was several times lower.

Collectively, acetic acid and ethyl acetate are responsible for volatile acidity (VA) in wine. Carboxylic acids form a fairly large group of volatile compounds in wines. Their qualitative and quantitative composition mainly depends on the yeast strain applied and, to a lesser extent, on the raw material used. Acetic acid dominates among the organic acids detected in alcoholic beverages (75–85%). The amount of acetic acid produced is associated with sugar and nitrogen compound concentration, pH value and temperature during fermentation. The content of this compound in wines increases with sugar concentrations above about 20% (w/v). Acetic acid formation is also higher below pH 3.2 and at pH values more neutral than pH 4. Nitrogen compounds have a variable effect on acetic acid biosynthesis. Some compounds (ammonium, glutamic acid, glutamine, asparagine) inhibit the formation of acetic acid, while others (methionine, valine) promote it when sufficient nitrogen compounds are available for growth. When the nitrogen supply is insufficient, an increase in sugar concentration favours the production of this acid (Radler, 1994).

Higher alcohols are quantitatively the largest group of flavour compounds in alcoholic beverages, and are a secondary product of alcoholic fermentation. It has been reported that concentrations below 300 mg/L add a desirable level of complexity to wine, whereas concentrations that exceed 400 mg/L can have a detrimental effect (Lambrechts & Pretorius, 2000). The amount of fusel alcohols in the analyzed wines was inversely proportional to other volatile compounds' concentration. The Gloster wines contained a relatively high level of higher alcohols (746.5 mg/L), while Šampion wines had a significantly lower level (487.3 mg/L). This tendency was associated with higher concentrations of isobutanol (209.4 mg/L), propanol (133.8 mg/L) and 2-phenylethanol (56.4 mg/L) in wines obtained from Gloster fruits. These values exceeded by several times those determined in other samples. The analyzed wines did not show statistically significant differences (Table 4) in concentration of amyl alcohols, which ranged from 314.7 to 348.8 mg/L. Higher alcohols are formed during fermentation from keto acids produced either catabolically, involving degradation of an amino acid (valine, leucine, isoleucine, threonine, phenylalanine) via the so-called Ehrlich pathway, or anabolically via the biosynthesis route from the carbon source. Ammonium and amino acid deficiencies in must lead to increased formation of higher alcohols. Amino acids in must are among the most important factors influencing fusel alcohol formation. They are able to alter the yield of higher alcohols in several different ways. Despite the fact that low levels of amino acids were present with respect to the quantity of corresponding higher alcohols formed (via the Ehrlich reaction), the amino acids may play a role in controlling the pathways of their own formation and thus influence the anabolic formation of higher alcohols (Lambrechts & Pretorius, 2000; Lilly, Bauer, Styger, Lambrechts, & Pretorius, 2006). The content of ammonium compounds as well as amino acids in apple musts may vary widely (Kuneman, Braddock, & McChasney, 1988; Moreno-Arribas, Pueyo, & Polo, 1996), which directly determines the amounts of higher alcohols that are produced during fermentation of different apple varieties.

3.4. Sensory evaluation of apple wines

The results of the sensory evaluations of the apple wine samples are given in Table 5. Sensory evaluation was performed by applying the method of positive rating according to Buxbaum's model (Miličević et al., 2002). Statistically significant differences ($P < 0.05$) were not found in the examined aroma apple wine characteristics.

The Šampion and Idared wines received respective scores of 14.3 and 16.0 (Table 5), equivalent to a grade of slightly above "medium". The wines obtained from Gloster apples received the lowest score (13.9), which was associated with weak apple aroma and flavour, and very intense, solvent aroma. The low quality of these wines was probably due to the low titratable acidity (3.32 g/L) and relatively high concentration of higher alcohols (746 mg/L). Differences in major volatile compounds appeared to be related to the sensory attributes of wine. It is generally known that the production of high amounts of acetaldehyde and fusel alcohols have a negative effect on wine quality, and production of moderate amounts of ethyl acetate has a positive effect on wine quality (Regodon Mateos et al., 2006).

The wines produced from Šampion and Idared apples were characterized by pleasant and well-harmonized taste and aroma. Their sensory characteristics were also strongly influenced by relatively high amounts of polyphenol compounds, particularly procyanidins. These compounds are responsible for the sensory sensation known as astringency, which is a result of binding and precipitation of the mucus proteins in the saliva. An increase of polymerisation rate of procyanidins is accompanied by more intense astringent taste (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998).

4. Conclusions

Wines produced using fruits of three different dessert apple varieties were characterized by significant variation in antioxidant activity as well as content of polyphenol and volatile compounds. On the basis of results obtained by other Polish authors, it could be affirmed that the profile of the analyzed wines was strongly affected by the composition of fruits used for fermentation. The amount of fermenting sugars, nitrogen and polyphenol compounds in fruits directly determines the content of cinnamic acid derivatives, flavonols, carbonyl compounds, methanol, ethanol and higher alcohols in wine, as well as indirectly affecting its sensory characteristics.

The analyzed dessert varieties of apples appear to be a good raw material for wine-making. The relatively low total acidity may be a disadvantage, but this could be improved by blending with wines showing higher total acidity or by using yeast strains that are able to synthesize malic acid.

However, it should be mentioned that the fruits' chemical composition strongly depends on the environmental and agricultural conditions, orchard location, presence of pests, age of trees, etc. Changing these conditions results in a wine characterized by a different chemical and sensory profile to that of our samples. At the same time, further studies conducted on an industrial scale are needed, because different apple must volume, fermentation vessels, yeast strain and/or fruit processing technology can significantly change the chemical composition of produced apple wines.

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