The influence of cultivar of apple-tree and yeasts used for fermentation on the concentration of volatile compounds in ciders and their sensory properties

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Summary

The main aim of the study was to evaluate the impact of cultivar of apple-tree and yeasts used for fermentation on the concentration of volatile organic compounds (VOC) in ciders. Among the volatile compounds, amyl alcohols and isobutanol dominated in all beverages. In the group of identified esters, the compound present at the largest concentration was diethylsuccinate. The largest quantities of volatile compounds contained beverages obtained from Rubin apples, while the lowest from the Elise cultivar. Pinocarveol dominated in beverages made from apples of the Rubin and Topaz cultivars, whereas terpinen-4-ol in ciders from the Elise cultivar. In all ciders, relatively large concentration of eugenol and isoeugenol were also observed. The concentration of VOC and terpenoids in beverages largely depended on their concentration in the raw material used for their production. The impact of the yeast strain on their concentration was observed but it was less significant than the impact of apple cultivar. The lowest scores in organoleptic evaluation were obtained for ciders produced by distillery yeasts from apples of Rubin and Topaz cultivars, while the highest scores were obtained for ciders fermented by wine yeasts (Rubin and Topaz cultivars) and cider yeasts (Elise cultivar).

Keywords

yeast; apple-tree cultivar; volatile compound; terpenoid

The original taste and aroma of fermented beverages come mainly from the raw materials used in their production. The main compounds that create aroma of apples are esters, alcohols, terpenoids, aldehydes, ketones, ethers and volatile acids. A particularly important impact on the final sensory sensation has mainly ethylacetate with an ethereal, fruity, sweet, grape and rum-like aroma when present at a low concentration, but unpleasant and nail-polish at higher concentrations. Other important esters are ethylbutyrate associated with apple aroma and methylanthranilate that has a smell of grapes. Some substances are present in low concentrations but they still have a significant impact on the sensory characteristics of apples e.g. phenylopropanoids (eugenol - scent of cloves), terpenoids such as geraniol (scent of roses), limonene (citrus aroma), linalool (rosewood aroma) and myrcene (responsible for apricot, walnut and orange aromas) [1, 2].

The secondary aroma is mainly associated with the synthesis of volatile organic compounds (VOC) by yeasts during ethanolic fermentation. During spontaneous fermentation, most species of native yeasts die out along with the increasing ethanol concentration, creating in such way a niche in which *Saccharomyces cerevisiae*, due to its good resistance to higher concentrations of ethanol, become to dominate [3]. Selection of the right type of yeasts for production of alcoholic beverages has a key impact on the profile of volatile alcoholic beverage compounds. Most yeast species have the ability to convert monoterpenoids. Some of the aroma compounds in the fruits are present in the form of glycosylated precursors that do not affect the taste and scent and only their hydrolysis by the enzyme β-glucosidase releases the volatile substances responsible for the taste and aroma of the drink. Non-*Saccharomyces* yeasts belonging to the genera *Debaromyces*, *Hansenula*, *Candida*, *Pichia* and *Kloeckera* contain this enzyme but it is not encoded by the *S. cerevi-*

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siae genome. Non-*Saccharomyces* yeasts exhibit varying degrees of β-glucosidase activity and may play an important role in the release of volatile compounds from non-volatile precursors [4]. In addition, depending on the yeast strain used for fermentation, different proportions of higher alcohols can be obtained, e.g. *S. cerevisiae* produces most of isoamylalcohol and *S. ludwigii* produces most of isobutanol. Apple wines fermented with *S. cerevisiae* are characterized by high concentrations of *n*-propanol, isoamylalcohol and amylalcohol [5]. On the other hand, 2-phenylethanol is intensively produced during turbulent fermentation by the yeast *S. bayanus*.

Data on the profile of volatile compounds, mainly VOC, in alcoholic beverages were published [6–8]. However, the effect of commercially available yeasts belonging to different species or genera and the impact of cultivars of apple-tree on VOC concentration as well as on the profile of terpenoids in ciders has not yet been analysed. Therefore, the aim of the study was to evaluate the impact of cultivars of apple-tree and commercially available yeasts used for fermentation on the concentration of volatile organic compounds and terpenoids in the ciders. In addition, sensory evaluation of the obtained drinks was carried out.

MATERIALS AND METHODS

Preparation of apple cider by fermentation

Apples of three apple-tree cultivars, namely, 'Topaz', 'Elise' and 'Rubin,' were used in the experiment. The Rubin apple is a fairly modern variety of *Malus domestica* from the Czech Republic, being a cross between the Lord Lambourne and the Golden Delicious. Rubin apples are small to medium in size and round in shape. The bright yellow skin is vibrant, smooth, and is almost completely covered by orange and red striping. It has an intense sweet honeyed flavour with some sharp acidity [9].

The Topaz cultivar is a relative of Golden Delicious and Czech apples Lord Lambourne and James Grieve, which is resistant to common apple-tree diseases such as scab and mildew. Topaz apples are medium in size, pale yellow and overlaid nearly completely with a ruby and orange blush, its yellow base colouring peeking out just slightly at its stem. Its flavour is sweet tart with nuances of spice, a flavour that will mellow during storage [10].

The Elise cultivar comes from Holland. It is characterized by very shapely, spherical and conical fruits of an intensive, dark red colour. It has a green and white juicy flesh of sweet and sour taste [11].

All apples used in experiments were obtained from a pomological orchard of the University of Agriculture, Kraków, located in Garlica Murowana (near Kraków, Poland). The apples were washed in tap water, ground and pressed on a Zottel hydraulic press (35 l). Unclarified and unsulfated 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 industrial application (materials and equipment for fermentation - Erbslöh, Geisenheim, Germany) at 0.2 $g \cdot l^{-1}$. Dry yeasts were hydrated according to the manufacturer's recommendations. The must was not 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 mentioned strains belong to the *Saccharomyces* genus. The selected strain of wild yeasts (*Torulaspora delbrueckii*/*Saccharomyces rosei*) commercially available as 'Wild & Pure' (materials and equipment for fermentation - Erbslöh) were 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.

Analysis of volatile compounds

A sample of 2 ml was placed into a 15 ml headspace vial and 50 μl of internal standard solution (5 $mg \cdot l^{-1}$ ethylnonanoate) was added. Then, the solid phase microextraction (SPME) fibre (85 μm carboxen polydime thylsiloxane; Supelco, St. Louis, Missouri, USA) was placed in the headspace above the sample and the vial was incubated for 30 min at 40 °C. The fibre was subjected to thermal desorption in a gas chromatograph injector at 250 °C.

The chromatographic separation was carried out on a Clarus 580 apparatus (PerkinElmer, Walthman, Massachusetts, USA) with a crossbond dimethyl polysiloxane column (60 m × 0.25 mm, film thickness 1.4 μm; Restek, Bellefonte, Pennsylvania, USA). The carrier gas (He) flow was 2 $ml \cdot min^{-1}$, the temperature programme was 35 °C, 6 min; 8 $°C \cdot min^{-1}$ up to 180 °C, 12 $°C \cdot min^{-1}$ up to 220 °C, 25 min. The detector and dispenser temperature was 250 °C. An HT2800T autosampler (HTA, Brescia, Italy) was used and PerkinElmer

Total Chrom 6.3.2 software (PerkinElmer) was used to process the results.

Analysis of terpenoids and phenylopropanoids

The analysis was carried out according to the procedure described by Matijasevic et al. [12] with modifications. A 40 ml sample was placed into a test tube (50 ml), 100 μl of internal standard solution (5 mg·l^{-1} annetol) and 4 ml of hexane were added and extracted on a rotary shaker (350 cycles per minute, amplitude 3, 1 h). Then, the hexane layer was removed, transferred to the equation vessels and centrifuged (2154 ×*g*, 10 min). The hexane layer was collected and chromatographic analysis was performed.

The separation was carried out on a gas chromatograph HP 5820 (Hewlett-Packard, Palo Alto, California, USA) with a Stabilwax column 30 m × 0.25 mm (Restek). The carrier (He) gas flow was 2 ml·min^{-1}, the temperature programme was 35 °C, 1 min; 4 °C·min^{-1} up to 250 °C; 250 °C, 5 min. The detector and dispenser temperature was 250 °C. An HT3000A autosampler (HTA) was used and the results were processed using Clarity 7.2 software (DataApex, Prague, Czech Republic).

Organoleptic evaluation

The evaluation was made using the point method [13]. A qualified sensory panel consisting of 10 people assessed each of the quality factors (colour, clarity, aroma, test) using a 5-point scale in a range from 1 (lowest score) to 5 (highest score). The characteristics assigned to individual grades and weighting coefficients are shown in Tab. 1. Samples for evaluation were administered in 20 ml transparent glasses at room temperature, in triplicate. The result was expressed as the average including weighting factors.

Statistical analysis

There were at least three physical repetitions of each setting. All samples were analysed once but in the case of significant deviation from average 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, San Diego, California, 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.

The principal component analysis (PCA) was performed using Statistica v. 12 software (StatSoft Tulsa, Oklahoma, USA) for two groups of compounds, namely, for volatile organic compounds (VOC), mainly alcohols and esters, and for terpenoids.

RESULTS AND DISCUSSION

Quality parameters of fermented beverages were found to depend mainly on the apple cultivar used. Beverages from the Rubin cultivar met all the requirements for ciders, but the beverages from the Topaz cultivar exceeded the permissible values for total acidity (> 7 g·l^{-1}). Ciders from the Elise cultivar were characterized by quite low content of ethanol (about 5 % vol.) due to the low concentration of sugars in the must (64 g·l^{-1}) [14].

Regarding volatile compounds, diethylsuccinate was a dominant ester in the majority of the tested samples (Tab. 2–4). Use of *S. cerevisiae* for fermentation (Tipico F3, Elegance, Inter Dry F3, Red Ethanol, French Cider Gozdowa) led to a higher concentration of this compound than in samples fermented by *T. delbrueckii* (Wild & Pure). Arslan et al. [15] also showed almost twice as high the concentration of diethylsuccinate in fermented wines using *S. cerevisiae* compared to those feremented with *T. delbrueckii.* Similar results were obtained by Renault et al.

Tab. 1. Parameters of organoleptic evaluation of ciders.

Quality factors	Weighting factor	Point scale				
		5	4	3	2	1
Colour	0.4	light straw to amber			changed compared to the declared one	
Clarity	0.4	full with gloss	full	permissible light opalescence (smoke)	opalescent	turbidity, visible sediment
Aroma	0.8	strong, harmonized, characteristic for the fruits used, attractive	pronounced, harmonized	typical, harmonized, faint	slightly changed	alien, undesirable
Taste	2.4	highly harmonized, intense, pure, attractive	harmonized, intense, pure	typical, without extraneous aftertaste	little harmonized, perceptible extraneous aftertaste	changed, alien, undesirable

Tab. 2. Concentrations of volatile compounds and terpenoids in ciders made from apples of of Rubin cultivar.

Volatile compound	Concentration [mg·l⁻¹]								
	Wild & Pure	Tipico F3	Vetliner F3	Elegance	InterDry F3	Spiriferm Arom	Red Ethanol	Cider Yeast	French Cider Gozdowa
Ethylhexanoate	2.2 ± 0.6^{ae}	2.2 ± 0.6^{ae}	$0.5 \pm 0.0^{bd.}$	1.6 ± 0.1^{ace}	0.3 ± 0.3^{bd}	1.1 ± 0.1^{cd}	0.7 ± 0.2^{d}	1.8 ± 0.6^{e}	0.0 ± 0.0^{b}
Ethyloctanoate	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Ethyldecanoate	0.3 ± 0.0^{a}	0.1 ± 0.1^{b}	0.2 ± 0.0^{b}	0.0 ± 0.0^{c}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.4 ± 0.1^{a}	0.1 ± 0.0^{b}	0.4 ± 0.0^{a}
Ethyldodecanoate	0.8 ± 0.0^{a}	0.4 ± 0.1^{b}	0.8 ± 0.2^{s}	0.4 ± 0.0^{b}	1.2 ± 0.4^{c}	0.8 ± 0.0^{a}	1.3 ± 0.2^{c}	0.4 ± 0.1^{b}	0.1 ± 0.0^{d}
2-Phenylethanol	7.6 ± 0.3^{a}	3.7 ± 0.4^{c}	8.1 ± 0.9^{a}	6.2 ± 1.0^{a}	9.3 ± 0.6^{b}	29.1 ± 0.0^{d}	25.8 ± 0.0^{e}	11.4 ± 2.8^{f}	18.9 ± 2.1^{g}
Isobutanol	58.6 ± 12.9^{a}	47.6 ± 7.4^{a}	56.4 ± 3.7^{a}	54.7 ± 2.3^{a}	51.1 ± 3.0^{a}	55.6 ± 0.4^{a}	50.1 ± 3.0^{a}	99.8 ± 17.2^{b}	56.3 ± 2.8^{a}
Isopropylacetate	19.8 ± 5.4^{a}	21.8 ± 2.1^{ac}	13.8 ± 4.2^{b}	11.9 ± 1.4^{b}	24.8 ± 1.0^{c}	35.5 ± 0.8^{d}	25.1 ± 0.9^{c}	14.2 ± 3.6^{b}	14.2 ± 1.9^{b}
Diethylacetal	0.0 ± 0.0^{a}	0.7 ± 0.1^{bd}	0.2 ± 0.3^{c}	0.3 ± 0.2^{c}	0.6 ± 0.4^{b}	0.9 ± 0.1^{d}	0.7 ± 0.0^{b}	2.2 ± 0.7^{e}	0.3 ± 0.2^{c}
Isoamylalcohol	29.6 ± 5.8^{a}	24.4 ± 0.7^{bc}	26.3 ± 3.3^{c}	32.0 ± 4.0^{d}	28.6 ± 2.7^{a}	22.7 ± 1.0^{b}	30.2 ± 0.7^{ad}	30.3 ± 1.4^{ad}	31.0 ± 2.4^{ad}
Isobutylacetate	4.7 ± 0.7^{ab}	4.6 ± 0.3^{a}	4.5 ± 1.0^{a}	4.5 ± 0.2^{a}	5.2 ± 0.4^{b}	8.0 ± 0.1^{c}	5.8 ± 0.4^{b}	4.7 ± 0.9^{ab}	4.7 ± 0.2^{a}
Isoamylacetate	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.5 ± 0.0^{b}	0.0 ± 0.0^{a}
Hexanol	14.7 ± 0.9^{a}	9.7 ± 1.1^{b}	13.5 ± 1.3^{a}	11.7 ± 0.4^{c}	11.4 ± 0.6^{bc}	16.4 ± 0.4^{d}	15.9 ± 0.5^{d}	13.4 ± 3.9^{a}	12.9 ± 0.1^{ab}
Diethylsuccinate	20.8 ± 5.1^{a}	49.8 ± 6.5^{b}	33.5 ± 2.4^{c}	34.5 ± 9.4^{c}	29.6 ± 6.9^{ac}	28.0 ± 2.2^{d}	32.9 ± 8.7^{c}	11.6 ± 5.7^{d}	32.1 ± 5.4^{c}
2-Phenylethyl acetate	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.8 ± 0.0^{b}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
Terpenoids									
Pinocarveol	0.80 ± 0.14^{abceg}	0.77 ± 0.04^{abceg}	0.64 ± 0.08^{ace}	0.86 ± 0.20^{bcg}	0.78 ± 0.18^{eg}	1.07 ± 0.04^{dg}	1.67 ± 0.10^{f}	0.64 ± 0.04^{ce}	0.90 ± 0.10^{g}
Camphor	0.10 ± 0.03^{abcd}	0.13 ± 0.02^{acd}	0.09 ± 0.03^{bcd}	0.09 ± 0.01^{bcd}	0.09 ± 0.01^{bd}	0.10 ± 0.04^{bcd}	0.10 ± 0.01^{bd}	0.07 ± 0.02^{bcd}	0.08 ± 0.02^{b}
Terpinen-4-ol	0.27 ± 0.11^{ae}	0.85 ± 0.05^{bf}	0.58 ± 0.09^{cef}	0.80 ± 0.05^{b}	0.39 ± 0.07^{e}	0.58 ± 0.02^{cef}	0.74 ± 0.03^{f}	0.06 ± 0.02^{d}	0.75 ± 0.04^{f}
Geraniol	0.13 ± 0.03^{a}	0.18 ± 0.04^{ab}	0.14 ± 0.04^{a}	0.15 ± 0.04^{a}	0.14 ± 0.02^{a}	0.17 ± 0.04^{ab}	0.22 ± 0.02^{b}	0.14 ± 0.03^{a}	0.15 ± 0.03^{a}
Eugenol	0.25 ± 0.18^{a}	0.38 ± 0.03^{a}	0.29 ± 0.05^{a}	0.23 ± 0.13^{a}	0.35 ± 0.05^{a}	0.09 ± 0.01^{b}	0.42 ± 0.04^{a}	0.06 ± 0.03^{b}	0.35 ± 0.04^{a}
β-Damascenone	0.01 ± 0.00^{ace}	0.04 ± 0.02^{bdf}	0.03 ± 0.00^{ade}	0.02 ± 0.00^{acde}	0.03 ± 0.01^{def}	0.03 ± 0.01^{def}	0.02 ± 0.01^{e}	0.01 ± 0.00^{c}	0.04 ± 0.00^{f}
Isoeugenol	0.26 ± 0.05^{acdef}	0.44 ± 0.06^{bce}	0.32 ± 0.05^{cdef}	0.23 ± 0.09^{def}	0.30 ± 0.14^{f}	0.34 ± 0.09^{ef}	0.27 ± 0.07^{f}	0.20 ± 0.09^{df}	0.27 ± 0.03^{f}
β-Ionone	0.05 ± 0.00^{acdefg}	0.06 ± 0.02^{abde}	0.08 ± 0.01^{be}	0.04 ± 0.00^{acdefg}	0.07 ± 0.03^{e}	0.05 ± 0.02^{defg}	0.04 ± 0.01^{fg}	0.04 ± 0.02^{cdfg}	0.04 ± 0.01^{g}

Means marked with the same letter in a row are not significantly different at $p < 0.05$, $n = 3$.

Tab. 3. Concentrations of volatile compounds and terpenoids in ciders made from apples of Elise cultivar

Volatile compound	Concentration [mg·l⁻¹]								
	Wild & Pure	Tipico F3	Vetliner F3	Elegance	InterDry F3	Spiriferm Arom	Red Ethanol	Cider Yeast	French Cider Gozdowa
Ethylhexanoate	1.6 ± 0.3^{ae}	0.5 ± 0.1^{b}	0.9 ± 0.1^{c}	0.2 ± 0.0^{d}	0.4 ± 0.0^{b}	1.4 ± 0.1^{a}	0.7 ± 0.1^{c}	1.9 ± 0.1^{e}	0.4 ± 0.1^{b}
Ethyloctanoate	0.0 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.5 ± 0.0^{b}	0.0 ± 0.0^{a}
Ethyldecanoate	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.0^{b}	0.0 ± 0.0^{a}	0.1 ± 0.0^{c}	0.0 ± 0.0^{a}
Ethyldodecanoate	0.3 ± 0.1^{a}	1.1 ± 0.2^{b}	0.8 ± 0.0^{c}	0.7 ± 0.0^{c}	0.6 ± 0.1^{c}	0.0 ± 0.0^{d}	0.8 ± 0.0^{c}	0.0 ± 0.0^{d}	0.8 ± 0.1^{c}
2-Phenylethanol	2.1 ± 0.1^{ac}	2.1 ± 0.2^{ac}	2.5 ± 0.1^{a}	2.5 ± 0.2	2.3 ± 0.3^{a}	8.6 ± 1.0^{b}	2.6 ± 0.0^{a}	2.0 ± 0.1^{c}	2.7 ± 0.1^{a}
Isobutanol	116.5 ± 8.8^{a}	66.9 ± 2.5^{b}	82.6 ± 3.7^{c}	68.3 ± 4.0^{bd}	72.3 ± 2.9^{bd}	74.7 ± 2.9^{d}	82.6 ± 18.5^{cd}	12.3 ± 1.1^{e}	72.2 ± 2.3^{bd}
Isopropylacetate	12.0 ± 0.5^{a}	16.0 ± 1.3^{b}	9.8 ± 0.5^{c}	10.1 ± 0.4^{c}	17.5 ± 0.1^{b}	33.3 ± 2.0^{d}	9.6 ± 0.9^{c}	21.9 ± 9.8^{b}	11.2 ± 2.4^{ac}
Diethylacetal	18.7 ± 5.9^{a}	7.5 ± 4.1^{bc}	7.0 ± 2.6^{b}	6.5 ± 4.2^{b}	11.6 ± 1.2^{bc}	11.8 ± 3.5^{c}	18.6 ± 5.4^{a}	4.0 ± 1.1^{d}	2.8 ± 1.0^{e}
Isoamylalcohol	23.3 ± 0.9^{a}	19.9 ± 0.0^{b}	23.9 ± 3.7^{a}	23.0 ± 3.1^{a}	20.2 ± 0.4^{ab}	20.2 ± 0.6^{ab}	19.7 ± 0.2^{b}	168.3 ± 4.9^{c}	20.9 ± 0.6^{ab}
Isobutylacetate	2.9 ± 0.3^{a}	3.1 ± 0.2^{ab}	2.9 ± 0.1^{a}	3.3 ± 0.0^{ab}	3.5 ± 0.1^{b}	5.8 ± 0.3^{c}	2.8 ± 0.1^{a}	0.2 ± 0.0^{d}	3.3 ± 0.5^{ab}
Isoamylacetate	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.5 ± 0.6^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	3.9 ± 0.4^{c}	0.0 ± 0.0^{a}
Hexanol	7.2 ± 0.0^{a}	7.7 ± 0.6^{a}	15.3 ± 0.6^{b}	9.6 ± 0.6^{cd}	8.9 ± 0.4^{c}	11.9 ± 1.3^{d}	11.4 ± 1.7^{d}	0.0 ± 0.0^{e}	12.4 ± 1.6^{d}
Diethylsuccinate	19.3 ± 3.3^{a}	46.4 ± 0.9^{b}	29.0 ± 1.4^{c}	42.5 ± 2.2^{b}	33.5 ± 3.7^{c}	18.6 ± 4.5^{a}	42.2 ± 0.6^{b}	2.6 ± 0.1^{d}	36.8 ± 9.9^{bc}
2-Phenylethyl acetate	1.7 ± 0.3^{a}	7.0 ± 1.4^{b}	1.4 ± 0.1^{a}	2.5 ± 0.2^{c}	1.2 ± 0.2^{ad}	0.8 ± 0.3^{d}	3.7 ± 0.6^{e}	0.7 ± 0.0^{d}	11.7 ± 1.2^{f}
Terpenoids									
Pinocarveol	0.18 ± 0.01^{aefg}	0.01 ± 0.01^{b}	0.18 ± 0.07^{aeg}	0.29 ± 0.05^{c}	0.17 ± 0.00^{efg}	0.13 ± 0.00^{ef}	0.12 ± 0.06^{f}	0.12 ± 0.04^{def}	0.20 ± 0.00^{g}
Camphor	0.13 ± 0.01^{ac}	0.07 ± 0.00^{bcd}	0.15 ± 0.04^{a}	0.07 ± 0.01^{bcd}	0.14 ± 0.01^{a}	0.06 ± 0.01^{d}	0.06 ± 0.02^{d}	0.04 ± 0.06^{cd}	0.05 ± 0.03^{d}
Terpinen-4-ol	0.27 ± 0.04^{ac}	0.65 ± 0.17^{bd}	0.50 ± 0.03^{cd}	0.63 ± 0.06^{bd}	0.56 ± 0.03^{d}	0.32 ± 0.07^{c}	0.56 ± 0.08^{d}	0.18 ± 0.01^{a}	0.59 ± 0.08^{d}
Geraniol	0.23 ± 0.04^{ac}	0.17 ± 0.06^{abceg}	0.14 ± 0.04^{bcdefg}	0.18 ± 0.06^{ceg}	0.13 ± 0.01^{efg}	0.12 ± 0.04^{efg}	0.08 ± 0.01^{fg}	0.08 ± 0.01^{defg}	0.13 ± 0.05^{g}
Eugenol	0.36 ± 0.09^{acef}	0.52 ± 0.00^{bcef}	0.47 ± 0.16^{cef}	0.33 ± 0.04^{ae}	0.42 ± 0.02^{ef}	0.30 ± 0.10^{ae}	0.47 ± 0.08^{f}	0.07 ± 0.01^{d}	0.40 ± 0.05^{ef}
β-Damascenone	0.02 ± 0.01^{ace}	0.04 ± 0.00^{bdf}	0.03 ± 0.01^{ad}	0.02 ± 0.02^{ae}	0.01 ± 0.00^{ef}	0.04 ± 0.00^{d}	0.05 ± 0.00^{f}	0.01 ± 0.00^{ce}	0.02 ± 0.00^{ae}
Isoeugenol	0.33 ± 0.08^{a}	0.25 ± 0.09^{a}	0.24 ± 0.09^{a}	0.30 ± 0.12^{a}	0.25 ± 0.01^{a}	0.35 ± 0.00^{a}	0.31 ± 0.11^{a}	0.10 ± 0.04^{b}	0.28 ± 0.10^{a}
β-Ionone	0.06 ± 0.01^{ac}	0.04 ± 0.03^{acd}	0.03 ± 0.01^{acd}	0.04 ± 0.01^{acd}	0.03 ± 0.00^{d}	0.04 ± 0.03^{cd}	0.05 ± 0.00^{b}	0.02 ± 0.01^{bcd}	0.03 ± 0.02^{abd}

Means marked with the same letter in a row are not significantly different at $p < 0.05$, $n = 3$.

Tab. 4. Concentrations of volatile compounds and terpenoids in ciders made from apples of Topaz cultivar.

Volatile compound	Concentration [mg·l^{-1}]								
	Wild & Pure	Tipico F3	Vetliner F3	Elegance	InterDry F3	Spiriferm Arom	Red Ethanol	Cider Yeast	French Cider Gozdowa
Ethylhexanoate	2.3 ± 0.1^{a}	2.0 ± 0.3^{ab}	1.9 ± 0.0^{bc}	1.8 ± 0.2^{b}	2.6 ± 0.3^{a}	2.1 ± 0.2^{a}	1.8 ± 0.2^{bc}	2.4 ± 0.3^{a}	2.0 ± 0.5^{ac}
Ethyloctanoate	0.8 ± 0.0^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.5 ± 0.0^{b}	0.5 ± 0.1^{b}	0.9 ± 0.1^{a}	0.4 ± 0.1^{b}	0.7 ± 0.0^{a}	0.5 ± 0.0^{b}
Ethyldecanoate	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.1^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}
Ethyldodecanoate	0.0 ± 0.0^{a}	0.7 ± 0.0^{b}	0.1 ± 0.0^{a}	0.4 ± 0.0^{c}	0.4 ± 0.1^{c}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.0^{c}	0.0 ± 0.0^{a}
2-Phenylethanol	23.5 ± 6.6^{a}	7.9 ± 2.1^{b}	4.6 ± 0.8^{c}	13.9 ± 0.3^{d}	11.0 ± 0.1^{e}	0.0 ± 0.0^{f}	0.0 ± 0.0^{f}	10.6 ± 0.2^{e}	8.7 ± 1.4^{b}
Isobutanol	70.3 ± 5.9^{a}	43.7 ± 3.3^{b}	50.4 ± 2.4^{cd}	52.3 ± 2.2^{c}	40.3 ± 0.8^{b}	51.1 ± 0.5^{c}	41.8 ± 4.6^{bd}	48.5 ± 1.4^{cd}	49.7 ± 2.0^{cd}
Isopropylacetate	39.0 ± 3.2^{a}	44.6 ± 0.8^{b}	18.4 ± 0.3^{c}	18.4 ± 0.7^{c}	45.1 ± 0.9^{b}	50.8 ± 1.2^{d}	24.4 ± 1.4^{e}	17.5 ± 1.1^{c}	17.4 ± 0.6^{c}
Diethylacetal	0.1 ± 0.1^{a}	0.7 ± 0.3^{b}	1.4 ± 0.8^{c}	0.5 ± 0.3^{b}	0.6 ± 0.5^{b}	0.7 ± 0.6^{b}	0.7 ± 0.2^{b}	1.4 ± 0.1^{c}	0.1 ± 0.2^{a}
Isoamylalcohol	25.2 ± 0.6^{a}	30.7 ± 3.7^{bd}	25.4 ± 2.4^{ad}	25.7 ± 2.6^{ad}	29.9 ± 1.0^{bd}	21.3 ± 1.1^{c}	28.9 ± 1.3^{d}	27.0 ± 2.3^{d}	22.8 ± 2.2^{ac}
Isobutylacetate	8.4 ± 0.2^{a}	9.5 ± 0.1^{b}	7.6 ± 0.7^{a}	7.7 ± 0.2^{a}	9.7 ± 0.1^{b}	10.2 ± 0.3^{b}	10.4 ± 0.2^{b}	7.2 ± 0.6^{a}	6.7 ± 0.2^{a}
Isoamylacetate	0.9 ± 0.0^{a}	3.7 ± 0.1^{b}	4.6 ± 0.2^{c}	3.0 ± 1.7^{bc}	3.7 ± 0.1^{b}	0.9 ± 0.1^{a}	1.1 ± 0.0^{a}	3.0 ± 1.7^{b}	0.9 ± 0.2^{a}
Hexanol	16.9 ± 0.8^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	16.7 ± 0.5^{a}	18.8 ± 0.6^{a}	15.6 ± 1.4^{a}	17.0 ± 1.6^{a}
Diethylsuccinate	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	3.5 ± 0.3^{b}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}
2-Phenylethyl acetate	2.5 ± 0.2^{a}	22.6 ± 3.7^{b}	5.6 ± 0.9^{c}	10.0 ± 1.0^{d}	14.8 ± 1.8^{e}	9.8 ± 0.5^{d}	8.6 ± 0.7^{d}	4.4 ± 0.1^{c}	3.4 ± 0.5^{c}
Terpenoids									
Pinocarveol	0.32 ± 0.05^{a}	0.46 ± 0.07^{ab}	0.75 ± 0.08^{bc}	0.74 ± 0.10^{bc}	0.48 ± 0.15^{ab}	0.96 ± 0.31^{c}	2.84 ± 0.50^{d}	0.33 ± 0.04^{a}	0.43 ± 0.06^{ab}
Camphor	0.07 ± 0.02^{abc}	0.04 ± 0.00^{ab}	0.06 ± 0.01^{abc}	0.07 ± 0.01^{ac}	0.04 ± 0.02^{b}	0.07 ± 0.02^{abc}	0.09 ± 0.05^{c}	0.06 ± 0.01^{ab}	0.05 ± 0.00^{ab}
Terpinen-4-ol	0.13 ± 0.02^{acd}	0.31 ± 0.05^{bcef}	0.22 ± 0.03^{cde}	0.34 ± 0.03^{bdf}	0.28 ± 0.11^{ef}	0.17 ± 0.05^{d}	0.28 ± 0.10^{ef}	0.20 ± 0.04^{de}	0.36 ± 0.07^{f}
Geraniol	0.09 ± 0.01^{a}	0.06 ± 0.01^{a}	0.11 ± 0.02^{ab}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.10 ± 0.02^{ab}	0.16 ± 0.09^{b}	0.08 ± 0.03^{a}	0.11 ± 0.04^{ab}
Eugenol	0.09 ± 0.00^{a}	0.14 ± 0.02^{ab}	0.10 ± 0.00^{ab}	0.10 ± 0.04^{ab}	0.15 ± 0.10^{b}	0.13 ± 0.00^{ab}	0.12 ± 0.02^{ab}	0.14 ± 0.00^{ab}	0.23 ± 0.02^{c}
β-Damascenone	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.00^{b}	0.14 ± 0.00^{c}	0.03 ± 0.00^{bd}	0.04 ± 0.01^{d}
Isoeugenol	0.00 ± 0.00^{a}	0.10 ± 0.02^{ac}	0.36 ± 0.02^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.15 ± 0.00^{c}	0.88 ± 0.00^{d}	0.35 ± 0.18^{b}	0.24 ± 0.08^{c}
β-Ionone	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.07 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.15 ± 0.00^{c}	0.00 ± 0.00^{a}	0.02 ± 0.00^{d}

Means marked with the same letter in a row are not significantly different at $p < 0.05$, $n = 3$.

[16], which showed low synthesis of ethyl esters by *T. delbrueckii*. Some apple wines fermented with the yeast *S. bayanus* (e.g. Vetliner F3, Spiriferm Aroma, Cider Yeast – Elise cultivar, Cider Yeast – Rubin cultivar) contained even lower concentrations of this compound than in the case of Wild & Pure (*T. delbrueckii)* yeast fermentation. Diethylsuccinate is formed mainly during wine aging, therefore, in young wines the concentration of this compound may be low.

2-Phenylethylacetate is one of the most important compounds that give alcoholic beverages floral aromas [17]. The concentration of 2-phenylethylacetate was significantly higher in the samples analysed by LOIRA et al. [18] than in the case of ciders analysed in this work, in particular those obtained from apples of Rubin cultivar (from 0.3 $mg \cdot l^{-1}$ to 0.4 $mg \cdot l^{-1}$). A higher concentration of 2-phenylethylacetate was observed in beverages from Elise and Topaz cultivars, the concentrations being greater than 10 $mg \cdot l^{-1}$ with the participation of French Cider Gozdowa yeasts, and greater than 22 $mg \cdot l^{-1}$ using Tipico F3 yeasts.

According to the literature, *S. bayanus* produces a relatively high concentration of 2-phenylethanol and 2-phenylethylacetate [19]. Those findings were not confirmed by us as the use of yeasts containing *S. bayanus* for fermentation did not lead to a significantly higher concentration of this compound in the analysed samples (Tab. 2–4).

Isoamylacetate, associated with pleasant banana and apple notes, with a threshold of perceptibility of 30 $\mu g \cdot l^{-1}$, occurred only in the samples obtained from apple of Topaz cultivar, regardless of which type of fermentation was carried out. In ciders from other apple-tree cultivars, it was absent in many cases. In addition, the presence of this compound was found only in the samples fermented with Cider Yeast *(S. bayanus)*, regardless of the apple-tree cultivar used for production. There is no information in the literature on the increased synthesis of this compound by *S. bayanus* yeasts. The *S. bayanus* yeasts are generally considered to be good producers of esters, being believed to form twice as much of these compounds as *S. cerevisiae* strains [20, 21]. The lowest concentrations of isoamylacetate were found in the beverages fermented with Wild & Pure (*T. delbrueckii)* and this compound was completely absent in samples of Elise and Rubin cultivars fermented with these yeasts. LU et al. [22] reported low concentrations of isoamylacetate in wines, 0.12 $mg \cdot l^{-1}$ for samples fermented with *S. cerevisiae* and 0.01 $mg \cdot l^{-1}$ for samples fermented with *T. delbrueckii*. Similar results were presented by ARSLAN et al. [15], finding a lower concentration of isoamylacetate in wines fermented with *T. delbrueckii* (0.65 $mg \cdot l^{-1}$) than in those fermented with *S. cerevisiae* (1.34 $mg \cdot l^{-1}$).

In wines fermented using *S. cerevisiae* analysed by LU et al. [22] and ARSLAN et al. [15], a higher concentration of ethylhexanoate was found than in those fermented with *T. delbrueckii.* No similar relationship was found in our study as the concentration of ethylhexanoate was highest in ciders obtained with the participation of Wild & Pure yeast (*T. delbrueckii*). In beverages made from Topaz cultivar, slightly higher concentrations of ethylhexanoate were obtained in ciders fermented by *T. delbrueckii* and *S. bayanus* than by *S. cerevisiae*.

In our samples, the presence of ethyloctanoate was demonstrated only in the ciders from apple of Topaz cultivar (Tab. 4) and it was irrespective of the yeast species used for fermentation. However, in the beverages made from Elise cultivar, ethyloctanoate was produced solely by fermentation with Cider Yeast (Tab. 3), while in the ciders obtained from apple of Rubin cultivar the compound was not detected at all (Tab. 2). These results quite differ from previous studies of other authors [15, 22–24] who observed higher concentrations of ethyloctanoate, ethyldecanoate and ethyldodecanoate in wines produced using *S. cerevisiae* than in those produced using *T. delbrueckii.*

In the majority of ciders analysed by us, we found that the highest concentrations among esters had isopropylacetate and isobutylacetate (ciders fermented with Spiriferm Aroma). In ciders made form apples of Topaz and Rubin cultivars, fermented with Wild & Pure yeast, diethylacetal was present at the lowest concentrations, while in the case of apple of Elise cultivar fermented by the same yeasts, it was present at the highest rate (Tab. 2–4). Additionally, the concentration of diethylacetal depended on the apple-tree cultivar, e.g. ciders from apples of Rubin cultivar contained diethylacetal in the range of 0.0–2.2 $mg \cdot l^{-1}$, while those from apples of Elise cultivar contained it in the range of 2.8–18.7 $mg \cdot l^{-1}$. On the basis of our results it can be concluded that the type of yeasts used does not affect the concentration of this compound in fermented beverages.

The concentration of higher alcohols in wines is known to depend essentially on the yeast strain used for fermentation and the conditions of the fermentation process [25]. In the studies carried out by LOIRA et al. [18], the concentration of isoamylalcohol in wines fermented sequentially with *T. delbrueckii* and *S. cerevisiae* was almost twice as high as in the samples analysed in our study (approximately 60 $mg \cdot l^{-1}$ compared to 32 $mg \cdot l^{-1}$ for Elegance yeast, respectively). The exceptions were the beverages obtained from apples of Elise cultivar fermented with Cider Yeast *(S. bayanus)* in which the concentration of isoamylalcohol was approximately 170 mg·l^{-1}. This compound is the main higher alcohol in wines, accounting for up to 50 % of all fusels and it may occur in concentrations up to 290 mg·l^{-1} [26]. Arslan et al. [15] found even higher values of isoamylalcohol (over 120 mg·l^{-1}) using *S. cerevisiae* and *T. delbrueckii* for fermentation.

In our study, the lowest concentration of 2-phenylethanol was observed in ciders obtained from apples of Elise cultivar (Tab. 2–4). However, this compound was absent in two samples obtained from the apple of Topaz cultivar fermented with distillery yeast (Spiriferm Aroma and Red Ethanol). Loira et al. [18] and Lu et al. [22] found significantly higher concentrations of 2-phenylethanol in the analysed wines. These differences may be related to the use of various types of raw material for the production of wines (grapes and durian in the case of wines produced by the above-mentioned authors and apples in the research presented by us). Arslan et al. [15] showed a higher production of 2-phenylethanol by *T. delbrueckii* yeasts than by *S. cerevisiae* yeasts. In our studies, similar results were obtained only for ciders produced from apples of Topaz cultivar.

In our study, higher concentrations of isobutanol and hexanol were found compared to data published by Loira et al. [18] and Lu et al. [22]. The exceptions were samples in which no hexanol was found. This compound comes mainly from the raw material and its level during fermentation usually decreases [27]. At concentrations above 100 mg·l^{-1}, hexanol adversely affects the taste and aroma of alcoholic beverages, giving them an unpleasant grassy, liquorice aroma associated with toothpaste [28]. In wines analysed by Lu et al. [22] obtained by fermentation using *S. cerevisiae*, higher concentrations of isobutanol were found in comparison to those feremented with *T. delbrueckii* yeast fermentation. Renault et al. [16], on the basis of the conducted research, showed a low synthesis of higher alcohols by a *T. delbrueckii* strain. A reverse tendency was observed by Arslan et al. [15], showing a higher concentration of isobutanol in wines fermented using *T. delbrueckii* compared to *S. cerevisiae*. Similar results were obtained in the present study, as a higher concentration of isobutanol was produced by Wild & Pure (*T. delbrueckii)* than by *S. cerevisiae* (Tab. 2–4). According to Torrei et al. [29], the level of individual fusel alcohols in wines can be significantly reduced by using yeast strains with a greater demand for nitrogen compounds.

The content of volatile alcohols and esters in ciders depended mainly on the type of raw material (Fig. 1). The yeast strain used also significantly influenced the volatile component profile, especially in ciders made from the Topaz cultivar, while for the Rubin and Eliza cultivars, the impact of the yeast strain on the composition of volatile fermentation products was much smaller.

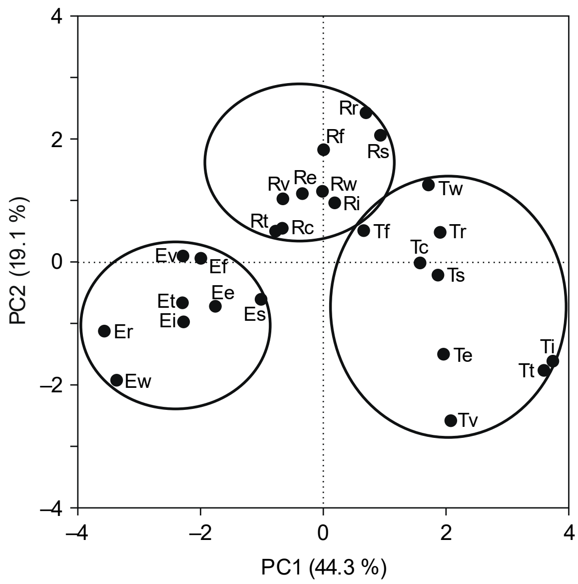

Fig. 1. Principal component analysis based on concentrations of volatile organic compounds in ciders.

Two principal components explained 63 % of the variance. Cultivars (designated by capital letters): E – Elise, R – Rubin, T – Topaz. Yeast strains (designated by lowercase letters): c – Cider Yeast, e – Elegance, f – French Cider Gozdowa, i – InterDry F3, r – Red Ethanol, s – Spiriferm Arom, t – Tipico F3, v – Vetliner F3, w – Wild & Pure.

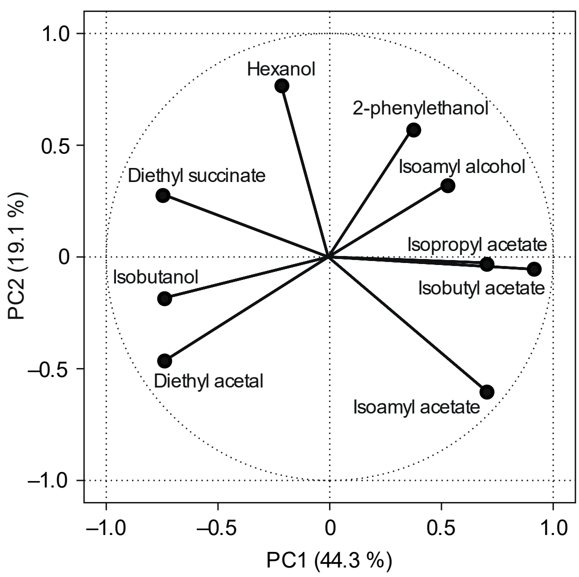

Fig. 2. Principal component analysis correlation circle for volatile organic compounds.

In the case of the load factor chart (VOC), a correlation was found for the analysed acetates, while an inverse correlation was observed between isobutylacetate and isobutanol concentrations (Fig. 2).

It has been shown instudy of Riekstina-Dolge et al. [30], that apples contain low amounts of alcohols, mainly hexanol, which are also present in ciders. In addition, apple fruit contains esters, some of which (hexylacetate and 2-methylbutylacetate) can also be found in ciders, while others (butyl acetate and dimethylpropyl butyrate) are absent in ciders. The profile of volatile compounds in alcoholic beverages depends largely on the cultivar of apple-tree, on the yeast strains used, on the ripening stage of apples and on the extent of malolactic fermentation (transformation of malic acid into lactic acid by lactic acid bacteria) [31–33].

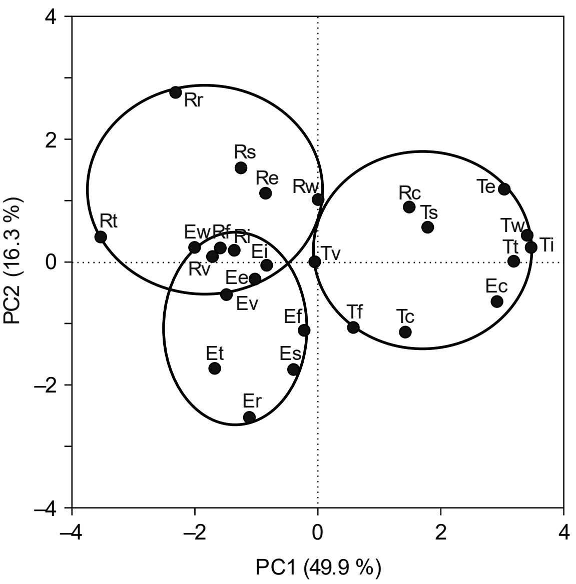

Fig. 3. Principal component analysis based on concentrations of terpenoids in ciders.

Two principal components explained 63 % of the variance. Cultivars (designated by capital letters): E – Elise, R – Rubin, T – Topaz. Yeast strains (designated by lowercase letters): c – Cider Yeast, e – Elegance, f – French Cider Gozdowa, i – InterDry F3, r – Red Ethanol, s – Spiriferm Arom, t – Tipico F3, v – Vetliner F3, w – Wild & Pure.

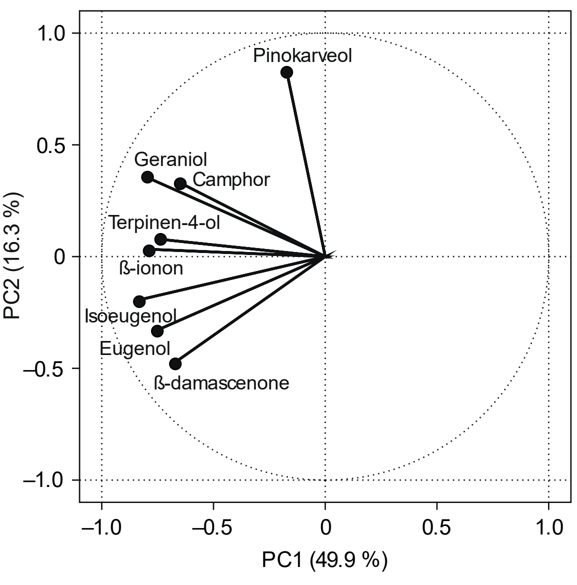

Fig. 4. Principal component analysis correlation circle for terpenenoids.

Ciders obtained from apples of Rubin and Topaz cultivars were characterized by a significantly higher concentration of pinocarvone (Tab. 2, 4), compared to Elise cultivar (Tab. 3). In the case of the former cultivar, the compound constituted from 30 % to even 50 % of all terpenoids. The highest concentration of pinocarvone was found in ciders fermented with distillery yeasts (Spiriferm Aroma and Red Ethanol). In all analysed samples, camphor occurred in a similar, low concentration (not greater than 0.15 mg·l^{-1}).

The highest concentration of terpinen-4-ol was in samples obtained from apples of Rubin cultivar. In most cases, the highest concentration of this compound occurred in beverages fermented with Tipico yeast *(S. cerevisiae)* and the lowest in the samples fermented with Cider Yeast *(S. bayanus)* as well as the wild yeast Wild & Pure *(T. delbrueckii)*. This compound can be produced by yeasts from linalool and nerol [34].

Geraniol was present in all analsed samples in concentrations up to 0.23 mg·l^{-1} (Elise cultivar fermented with Wild & Pure (*T. delbrueckii)* yeasts). King and Dickinson [34] demonstrated the ability of these yeasts to produce geraniol from nerol. The lower concentration of geraniol in some of the samples could be related, inter alia, to the ability to reduce geraniol to citronellol by *S. cerevisiae* or to the formation of linalool from both geraniol and nerol [34].

Eugenol, associated with the scent of carnations, is considered to be the characteristic compound for apples and drinks made from them. The highest concentration of this compound was found in the ciders from apples of Eliza cultivar, while the lowest in apples from Topaz cultivar. Isoeugenol was present in the lowest concentration in the beverages obtained from apples of Rubin and Elise cultivars fermented with Cider Yeast (Tab. 2, 3).

Among all analysed terpenoids, those which occurred in the lowest concentration were β-ionon (violet, sweet aroma) and β-damascenon (smell of honey, stewed apple with fruity and floral notes). The highest concentrations of these terpenoids

Tab. 5. Results of organoleptic evaluation of ciders.

Yeast strains	Cultivars		
	Rubin	Elise	Topaz
Wild & Pure	3.36 ± 0.68^{bc}	3.20 ± 0.53^{bc}	3.83 ± 0.92^{ab}
Tipico F3	3.17 ± 0.86^{c}	3.27 ± 0.81^{bc}	3.82 ± 0.80^{ab}
Vetliner F3	3.68 ± 0.85^{bc}	3.05 ± 0.55^{c}	4.08 ± 0.57^{a}
Elegance	3.30 ± 0.85^{bc}	2.91 ± 0.59^{c}	3.85 ± 0.60^{ab}
InterDry F3	3.57 ± 0.99^{bc}	3.27 ± 0.72^{bc}	3.88 ± 0.90^{ab}
Spiriferm Arom	3.33 ± 0.90^{bc}	2.94 ± 0.77^{c}	3.60 ± 0.74^{b}
Red Ethanol	2.80 ± 0.86^{c}	3.05 ± 0.66^{c}	3.33 ± 1.04^{bc}
Cider Yeast	3.48 ± 0.86^{bc}	3.48 ± 0.89^{bc}	4.05 ± 0.56^{a}
French Cider Gozdowa	3.46 ± 0.86^{bc}	3.43 ± 0.84^{bc}	3.93 ± 0.08^{ab}

5-point scale specified in Tab. 1. Means marked with the same letter are not significantly different at $p < 0.05$, $n = 3$.

were found in beverages from apples of Topaz cultivar fermented with Red Ethanol yeasts (Tab. 4). As a result of the analyses carried out as part of this work, slightly higher total concentrations of terpenoids were found in samples fermented using *S. cerevisiae* yeasts than in those fermented using *S. bayanus* or *T. delbrueckii.* No similar relationship was found for volatile esters and higher alcohols.

The concentration of terpenoids in the ciders depended mainly on the apple-tree cultivar used for their production (Fig. 3). The type of yeast strain used was also important. Particular attention should be paid to samples fermented with Cider Yeast, which were similar in terpenoid content, regardless of the apple cultivar used to make the must. The load factor chart analysis (Fig. 4) showed that terpenoids can be divided into two independent main groups. The first consists of eugenol, isoeugenol and β-damascenone, while the second one consists of geraniol, camphor, terpinen-4-ol and β-ionon. The analysed ciders showed a correlation between the concentrations of individual terpenoids belonging to the above-mentioned groups.

Cider samples were evaluated for organoleptic properties and were found to be characterized by a light straw colour, typical for the used raw material and, in most cases, marked by high clarity (Tab. 5). Single samples showed slight turbidity due to the presence of proteins of a high molecular weight, which are often produced during fermentation [35].

The evaluation of aroma and taste determined the final result of each trial, distinguishing between more or less acceptable ones. The lowest marks in the organoleptic evaluation were obtained for samples from apples of Rubin and Topaz cultivars fermented with distillery yeasts Red Ethanol *(S. cerevisiae)* and from the Elise cultivar fermented with the wild yeast Wild & Pure (*T. delbrueckii)*. The highest overall scores were obtained for ciders fermented using Vetliner yeasts *(S. bayanus)* (for Rubin and Topaz) and Cider Yeast *(S. bayanus)* (for Eliza). It can therefore be concluded that the use of the *S. bayanus* strain for fermentation allowed beverages of high quality to be obtained.

Different observations were made by Eglinton et al. [36] at analyses of Chardonnay grape musts, presenting the *S. cerevisiae* strain as responsible for typical fruit flavours (ester, pineapple, peach and lemon) and *S. bayanus* as slightly worse, which introduced yeast, nut and aldehyde notes. Taking into consideration results on all three apple-tree cultivars used for cider production (Tab. 5), we can, in total, give the highest value to beverages fermented with Cider Yeast *(S. bayanus)* and French Cider Gozdowa *(S. cerevisiae)*. The very good general evaluation of samples obtained from apples of Elise cultivar fermented with Cider Yeast may be related to the levels of ethyloctanoate and isoamylacetate, which were not found in ciders produced using other yeasts.

CONCLUSIONS

The concentration of volatile organic compounds depends primarily on the type of yeast used for fermentation. Among the analysed volatile compounds, alcohols dominated, mainly isobutanol and amylalcohols. In the group of identified esters, the component with the largest concentration was diethylsuccinate. The largest quantities of volatile compounds contained beverages obtained from apples of Rubin cultivar, while the lowest were those from the Elise cultivar. The apple-tree cultivar was of great importance in shaping the profile of terpenoid compounds. Pinocarveol dominated in beverages made from apples of Rubin and Topaz cultivars, whereas terpinen-4-ol dominated in ciders from apples of Elise cultivar. In all beverages, relatively great concentrations of eugenol and isoeugenol were also determined. Beverages made from apples of Rubin and Topaz cultivars were rated higher than those of Elise cultivar. Fermentation of beverages using wine yeasts and cider yeasts resulted in higher marks in organoleptic evaluation than when using wild-type or distillery yeasts.

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REFERENCES

1. Xu, Y. – Fan, W. – Qian, M.: Characterization of aroma compounds in apple cider using solvent-assisted flavor evaporation and headspace solid-phase microextraction. Journal of Agricultural and Food Chemistry, *55*, 2007, pp. 3051–3057. DOI: 10.1021/jf0631732.
2. Mosciano, G: Organoleptic characteristics of flavor materials. Perfumer and Flavorist, *19*, 1994, pp. 79. ISSN: 0272-2666.
3. Satora, P. – Tuszyński, T.: Influence of indigenous yeasts on the fermentation and volatile profile of plum brandies. Food Microbiology, *27,* 2010, pp. 418–424. DOI: 10.1016/j.fm.2009.12.005.
4. Jolly, N. P. – Varela, C. – Pretorius, I. S.: Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Research, *14*, 2014, pp. 215–237. DOI: 10.1111/1567-1364.12111.
5. Díaz-Montaño, D. – Ramírez Córdova, J.: The fermentative and aromatic ability of *Kloeckera* and *Hanseniaspora* yeasts. In: Satyanarayana, T. – Kunze, G. (Eds.): Yeast biotechnology: diversity and applications. Dordrecht : Springer, 2009, pp. 281–305. ISBN: 978-1-4020-8291-7. DOI: 10.1007/978-1-4020-8292-4_14.
6. Vidrih, R. – Hribar, J.: Synthesis of higher alcohols during cider processing. Food Chemistry, *67*, 1999, pp. 287–294. DOI: 10.1016/S0308-8146(99)00136-3.
7. Verstrepen, K. J. – Derdelinckx, G. – Dufour, J. P. – Winderickx, J. – Thevelein, J. M. – Pretorius, I. S. – Delvaux, F. R.: Flavor active esters: adding fruitiness to beer. Journal of Bioscience and Bioengineering. *96*, 2003, pp. 110–118. DOI: 10.1016/S1389-1723(03)90112-5.
8. Furdíková, K. – Ševcech, J. – Ďurčanská, K. – Hronská, H. – Malík, F.: Influence of different nutrition conditions on main volatiles of wine yeasts. Journal of Food and Nutrition Research, *53*, 2014, pp. 304–312. ISSN: 1336-8672 (print), 1338-4260 (online). <http://www.vup.sk/download.php?bulID=1625>
9. Piestrzeniewicz, C. – Sadowski, A. – Dziuban, R.: Performance of ‚Rubin‘ apple trees on nineteen rootstocks after four years. Latvian Journal of Agronomy, *9*, 2006, pp. 98–102. ISSN: 1691-3485.
10. Konopacka, D. – Kaczmarek, U. – Matulska, A. – Wawrzyńczak, A – Kruczyńska, D. – Rutkowski, K. P.: The comparison of sensory quality and processing potential of 'Topaz' apples grown in organic orchards and orchards managed in integrated production system. Journal of Fruit and Ornamental Plant Research. *20*, 2012, pp. 51–61. DOI: 10.2478/v10290-012-0015-7.
11. Kowalczyk, W. – Wrona, D.: Growth and bearing of apple cultivar 'Elise' on eighteen vegetative rootstocks in "V" planting system. Acta Scientiarum Polonorum Hortorum Cultus, *10*, 2011, pp. 125–135. ISSN: 2545-1405. <http://www.hortorumcultus.actapol.net/pub/10_2_125.pdf>
12. Matijasevic, S. – Todic, S. – Beslic, Z. – Rankovic Vasic, Z. – Zunic, D. – Atanackovic, Z. – Vukosavljevic, V. – Cirkovic, B.: Volatile components of grape brandies produced from Muscat table grapevine (*Vitis vinifera* L.) cultivars. Bulgarian Journal of Agricultural Science, *19*, 2013, pp. 783–791. ISSN: 1310-0351.
13. PN-A-79120-02:1990 Wina i miody pitne – ocena organoleptyczna. (Wines and meads – an organoleptic assessment.) Warsaw : Polish Standard Organization, 1990.
14. Tarko, T. – Kostrz, M. – Duda-Chodak, A. – Semik-Szczurak, D. – Sroka, P – Senczyszyn, T.: The effect of apple cultivars and yeast strains on selected quality parameters and antioxidant activity of fermented apple beverages. CYTA – Journal of Food, *16*, 2018, pp. 892–900, DOI: 10.1080/19476337.2018.1503616.
15. Arslan, E. – Çelik, Z. D. – Cabaroğlu, T.: Effects of pure and mixed autochthonous *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on fermentation and volatile compounds of Narince wines. Foods, *7*, 2018, article 147. DOI: 10.3390/foods7090147.
16. Renault, P. – Miot-Sertier, C. – Marullo, P. – Hernández-Orte, P. – Lagarrigue, L. – Lonvaud-Funel, A. – Bely, M.: Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. International Journal of Food Microbiology, *134*, 2009, pp. 201–210. DOI: 10.1016/j.ijfoodmicro.2009.06.008.
17. Ledauphin, J. – Guichard, H. – Saint-Clair, J. F. – Picoche, B. – Barillier, D.: Chemical and sensorial aroma characterization of freshly distilled calvados. 2. Identification of volatile compounds and key odorants. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 433–442. DOI: 10.1021/jf020373e.
18. Loira, I. – Vejarano, R. – Bañuelos, M. A. – Morata, A. – Tesfaye, W. – Uthurry, C. – Villa, A. –

Cintora, I. – Suárez-Lepe, J. A.: Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality, LWT - Food Science and Technology, *59*, 2014, pp. 915–922. DOI: 10.1016/j.lwt.2014.06.019.

19. Masneuf-Pomarède, I. – Bely, M. – Marullo, P. – Lonvaud-Funel, A. – Dubourdieu D.: Reassessment of phenotypic traits for *Saccharomyces bayanus* var. *uvarum* wine yeast strains. International Journal of Food Microbiology, *139*, 2010, pp. 79–86. DOI: 10.1016/j.ijfoodmicro.2010.01.038.
20. Favale, S. – Pietromarchi, P. – Ciolfi, G.: Metabolic activity and interactions between two strains, *Saccharomyces cerevisiae* r.f. *bayanus* (SBC2) and *Saccharomyces cerevisiae* r.f. *uvarum* (S6u), in pure and mixed culture fermentation. Vitis, *46*, 2007, pp. 39–44. ISSN: 2367-4156.
21. Muratore, G. – Asmundo, C. N. – Lanza, C. M. – Caggia, C. – Licciardello, F. – Restuccia, C.: Influence of *Saccharomyces uvarum* on volatile acidity, aromatic and sensory profile of Malvasia delle Lipari wine. Food Technology and Biotechnology, *45*, 2007, pp. 101–106. ISSN: 1330-9862. <http://www.ftb.com.hr/images/pdfarticles/2007/January-March/45-101.pdf>
22. Lu, Y. – Chua, J. Y. – Voon, M. K. W. – Huang, D. – Lee, P. R. – Liu, S. Q.: Effects of different inoculation regimes of *Torulaspora delbrueckii* and *Oenococcus oeni* on fermentation kinetics and chemical constituents of durian wine. South African Journal for Enology and Viticulture, *38*, 2017, pp. 273–285. DOI: 10.21548/38-2-2330.
23. Hernández-Orte, P. – Cersosimo, M. – Loscos, N. – Cachoa, J. – Garcia-Moruno, E. – Ferreira, V.: The development of varietal aroma from non-floral grapes by yeasts of different genera. Food Chemistry, *107*, 2008, pp. 1064–1077. DOI: 10.1016/j.foodchem.2007.09.032.
24. Viana, F. – Gil, J. V. – Genovés, S. – Vallés, S. – Manzanares, P.: Rational selection of non-*Saccharomyces* wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiology, *25*, 2008, pp. 778–785. DOI: 10.1016/j.fm.2008.04.015.
25. Molina, A. M. – Swiegers, J. H. – Varela, C. – Pretorius, I. S. – Agosin, E.: Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Applied Microbiology and Biotechnology, *77*, 2007, pp. 675–687. DOI: 10.1007/s00253-007-1194-3.
26. Boulton, R. B. – Singleton, V. L. – Bisson, L. F. – Kunkee, R. E. (Eds.): Principle and practices of winemaking. New York : Chapman & Hall, 1996. ISBN: 978-0834212701.
27. Satora, P. – Semik-Szczurak, D. – Tarko, T. – Bułdys, A.: Influence of selected *Saccharomyces* and *Schizosaccharomyces* strains and their mixed cultures on chemical composition of apple wines. Journal of Food Science, *83*, 2018, pp. 424–431. DOI: 10.1111/1750-3841.14042.
28. Ferreira, V. – Hernández-Orte, P. – Escudero, A. – López, R. – Cacho, J.: Semipreperative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. Journal of Chromotography A, *864*, 1999, pp. 77–88. DOI: 10.1016/S0021-9673(99)01004-3.
29. Torrea, D. – Fraile, P. – Garde, T. – Ancín, C.: Production of volatile compounds in the fermentation of Chardonnay musts inoculated with two strains of *Saccharomyces cerevisiae* with different nitrogen demands. Food Control, *14*, 2003, pp. 565–571. DOI: 10.1016/S0956-7135(02)00146-9.
30. Riekstina-Dolge, R. – Kruma, Z. – Karklina, D. – Seglina, D.: Influence of different yeast strains on the production of volatile compounds in fermented apple juice. In: Annual 17th International Scientific Conference Proceedings "Research for Rural Development 2011". Jelgava : Latvia University of Agriculture, 2011, pp. 113–139. ISSN: 1691-4031.
31. Pello-Palma, J. – Mangas-Alonso, J. J. – Dapena de la Fuente, E. – González-Álvarez, J. – Díez, J. – Gutiérrez Álvarez, M .D. – Abrodo, P. A.: Characterization of volatile compounds in new cider apple genotypes using multivariate analysis. Food Analytical Methods, *9*, 2016, pp. 3492–3500, DOI: 10.1007/s12161-016-0521-7.
32. Rosend, J. – Kuldjärv, R. – Rosenvald, S. – Paalme, T.: The effects of apple variety, ripening stage, and yeast strain on the volatile composition of apple cider. Heliyon, *5*, 2019, E01953. DOI: 10.1016/j.heliyon.2019.e01953.
33. Zhao, H. – Zhou, F. – Dziugan, P. – Yao, Y. – Zhang, J. – Lv, L. – Zhang, B.: Development of organic acids and volatile compounds in cider during malolactic fermentation. Czech Journal of Food Sciences, *32*, 2014, pp. 69–76, ISSN: 1805-9317. <https://www.agriculturejournals.cz/publicFiles/127_2013-CJFS.pdf>
34. King, A. – Dickinson, R. Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae, Torulospora delbrueckii* and *Kluyveromyces lactis*. Yeast, *16*, 2000, pp. 499–506. DOI: 10.1002/(SICI)1097-0061(200004)16:6<499::AID-YEA548>3.0.CO;2-E.
35. Musmanno, R. A. – Di Maggio, T. – Coratza, G.: Studies on strong and weak killer phenotypes of wine yeasts: production, activity of toxin in must, and its effect in mixed culture fermentation. Journal of Applied Microbiology, *87*, 1999, pp. 932–938. DOI: 10.1046/j.1365-2672.1999.00954.x.
36. Eglinton, J. – McWilliam, S. – Fogarty, M. – Francis, L. – Kwiatkowski, L. – Hřj, P. – Henschke, P.: The effect of *Saccharomyces bayanus*-mediated fermentation on the chemical composition and aroma profile of Chardonnay wine. Australian Journal of Grape and Wine Research, *6*, 2000, pp. 190–196. DOI: 10.1111/j.1755-0238.2000.tb00178.x.

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