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The effects of apple variety, ripening stage, and yeast strain on the volatile composition of apple cider

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ABSTRACT

This work examined the degree of influence of apple variety, apple ripening stage, and yeast strain on the volatile composition of apple cider. Four apple varieties grown in Estonia were selected for the study – Antei, Melba, Kulikovskoye, and Orlovski Sinap. The must from the apples at various stages of ripening (unripe, ripe, overripe) underwent alcoholic fermentation using commercially available yeast strains. Gas chromatography - mass spectrometry was employed to assess the differences in volatile composition between the samples. Out of the variables analyzed in this work, apple variety turned out to be the primary attribute influencing the quality and aroma properties of apple cider. The effect of yeast strain and the maturity of the fruit was variety-specific where the volatile profiles of ciders made with Melba variety were the least influenced by the ripening stage of apples and yeast strains used for the fermentation.

1. Introduction

Cider is a fermented alcoholic beverage made from apples. Despite holding a smaller position on a global scale, cider production is common throughout Europe and has also spread to other Western markets (Northern America, Australia) (Nogueira and Wosiacki, 2012). Technological advances made in other parts of the fermented beverage industry strongly influence cider production. Because of that, a limited amount of published information exists regarding the aroma of cider and factors affecting it the most.

No conclusive information is available on the effect of different apple varieties on the volatile composition of cider. However, some apple varieties themselves have been distinguished from one another based on their chemical composition (El Hadi et al., 2013). As far as non-volatile composition is concerned, aside from apparent fluctuations in sugar and acid ratios, the primary varietal differences occur in the phenolic content (Blanco-Gomisa et al., 1998; Wu et al., 2007; Kalinowska et al., 2014). Phenolic compounds, in turn, have been reported to contribute to the odor profiles of alcoholic beverages such as beer, wine, sherry, and whiskey (Vanbeneden et al., 2008).

Some information is available on how the level of apple fruit ripeness impacts the aroma profile of cider. For example, one of the latest researches conducted on this subject by Alberti et al. (2016) has provided some insight into cider aroma based on the ripeness of the apples used. The cider made from senescent apples was 24–52 % (depending on the variety) more abundant in different volatile compounds than the counterparts made from unripe apples.

The yeast used in the production of fermented beverages contributes to the final aroma profile mainly by elevating the levels of higher alcohols and esters (McKay et al., 2011). The relative concentrations of the fermentation products, however, may depend on the strain. Many yeast strains have already been investigated in another fermented beverage – wine. The application of different yeasts in the wine industry and the impact on the sensory properties have been described by Henick-Kling et al. (1998), Soden et al. (2000), Cadez et al. (2002), Becker Whitener et al. (2014), and Synos et al. (2015), to name a few. For example, Synos et al. (2015) have demonstrated the influence of the yeast strain on the formation of aroma compounds in Cabernet icewines. The yeasts differed not only in the diversity of generated odor-active volatile compounds but also in the amounts generated. According to the results, yeast EC1118 displayed the highest amounts of various alcohols, esters, furfural, hexanoic acid, and β-damascenone. Becker Whitener et al. (2014) observed the fermentation of red and white grape musts with non-*Saccharomyces* yeasts. They found that the majority of the investigated non-*Saccharomyces* yeasts provided lower levels of alcohols, esters, and terpenes, except for *Kazachstania gamospora*, which produced more total

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Table 1
Malic acid content and sugar profile of apple musts obtained from different apple varieties at diferent stages of maturity.

Must	Fructose, %	Glucose, %	Disaccharides, %	Malic acid, %
Unripe Melba	4.5 ± 0.1	0.5 ± 0.1	3.8 ± 0.3	1.2 ± 0.1
Ripe Melba	5.3 ± 0.1	0.7 ± 0.1	4.9 ± 0.1	0.9 ± 0.1
Overripe Melba	6.3 ± 0.5	1.2 ± 0.1	5.0 ± 0.1	0.8 ± 0.1
Unripe Antei	5.9 ± 0.2	1.8 ± 0.1	1.3 ± 0.1	0.8 ± 0.1
Ripe Antei	6.5 ± 0.1	1.8 ± 0.1	2.8 ± 0.1	0.6 ± 0.1
Overripe Antei	6.9 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	0.5 ± 0.1
Unripe Orlovski Sinap	5.2 ± 0.3	1.1 ± 0.1	2.4 ± 0.1	1.3 ± 0.1
Ripe Orlovski Sinap	6.4 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	0.8 ± 0.1
Overripe Orlovski Sinap	6.9 ± 0.2	2.0 ± 0.1	3.0 ± 0.1	0.5 ± 0.1
Unripe Kulikovskoye	4.9 ± 0.4	1.1 ± 0.1	1.6 ± 0.1	0.7 ± 0.1
Ripe Kulikovskoye	6.4 ± 0.2	1.3 ± 0.1	2.0 ± 0.1	0.5 ± 0.1
Overripe Kulikovskoye	6.5 ± 0.3	1.6 ± 0.1	1.8 ± 0.1	0.4 ± 0.1

esters than *Saccharomyces cerevisiae*. Less information is known on the effects of different yeast strains on aroma development in cider. The fermentation of industrial apple pomaces with three indigenous yeasts (*Saccharomyces cerevisiae, Hanseniaspora valbyensis,* and *Hanseniaspora uvarum*) was described by Rodríguez Madrera et al. (2015). Suárez Valles et al. (2005) have inoculated Asturian apple juice with three strains of cider making isolates (*S. cerevisiae* r. *cerevisiae* SSA, *S. cerevisiae* r. *uvarum* SSB, *S. cerevisiae* r. *cerevisiae* SSC) and a commercial wine yeast (*S. cerevisiae* r. *bayanus* UVA-PM). The compounds that were found to differentiate the strains were ethyl acetate, acetaldehyde, and isobutanol.

Additional insights need to be gained regarding the impact of cider production at the molecular level so that the industry can understand and control the character of the final product. Given the knowledge, it should be possible to maintain and expand the diversity in the cider market and influence the economics of cider production. The objective of this study was to examine the degree of influence of apple variety, apple ripeness, and yeast strain on the volatile composition of apple cider.

2. Materials and methods

2.1. Chemicals and reagents

Distilled water was obtained using Elix 5 UV Water Purification system (Merck Millipore, Billerica, MA, USA). 2-chloro-6-methylphenol, as an internal standard, was obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cider preparation in a laboratory environment

Four autumn or winter apple varieties, 'Antei', 'Kulikovskoye', 'Melba', and 'Orlovski Sinap' grown in South Estonia, at a private orchard in Valgjärve (58°8′ N, 26°66′ E) were used in the study. Apples were selected at three different stages of ripening: unripe, ripe, and overripe. The estimation of the ripening stage was based on the iodine starch test (Travers et al., 2002). All apples were first harvested at the 'unripe' stage (0 weeks, starch index 1) of their maturity and left to ripen at +4 °C. This is a common practice in Northern-Europe region because some of the

Table 2
Identified volatile compounds.

Compound name	Odor description[b]	RT	RI_{exp}	RI_{lit}[a]	Concentration range in the samples, ppb in IS equivalents
1-propanol	Fermented, fruity, apple, pear	2.60	589	595	1.67–91.47
2-methyl-1-propanol	Wine, whiskey	3.10	627	635	0.00–233.15
1-butanol	Balsamic	3.54	657	660	20.24–411.62
3-methyl-1-butanol	Cognac, banana, fruity	4.85	735	730	0.00–3631.49
2-methyl-1-butanol	Wine, fruity	5.01	743	740	319.31–3541.74
2-hexen-1-ol, (E)	Green, leafy	7.51	861	865	12.61–124.72
1-hexanol	Green, pungent	7.67	868	865	283.49–4659.29
1-octen-3-ol	Earthy, vegetative, mushroom	10.48	990	1000	0.00–83.00
2-ethyl-1-hexanol	Citrus, floral	11.38	1030	1030	0.01–51.97
1-octanol	Citrus, floral, fatty	12.06	1061	1070	0.00–2.87
2-phenylethanol	Floral, rose	13.25	1115	1110	0.00–598.83
Ethyl acetate	Fruity, green	2.74	603	610	10.27–713.71
Methyl-2-methylpropanoate	Fruity, ether	4.02	690	684	0.06–0.92
Ethyl propionate	Fruity, grape, pineapple, rum	4.13	697	705	1.55–109.10
Methyl butanoate	Pungent, fermented	4.38	711	720	1.19–19.85
Ethyl-2-methylpropanoate	Ether, pungent, fruity	5.18	752	760	0.49–24.63
Methyl-2-methylbutanoate	Fruity, ripe, fatty	5.73	780	775	0.53–6.35
Ethyl butanoate	Pineapple, cognac	6.08	798	800	3.24–150.82
Butyl acetate	Solvent, banana	6.58	820	815	0.17–12.26
Ethyl-3-methylbutanoate	Fruity, pineapple, apple, orange	7.37	855	855	0.12–4.90
Isoamyl acetate	Banana, pear	7.76	872	875	3.91–252.74
Ethyl-2-methylbutanoate	Fruity, apple	8.91	891	895	0.22–5.98
Ethyl pentanoate	Fruity, berry, tropical	9.09	930	930	0.07–2.50
Ethyl hexanoate	Fruity, pineapple, banana	10.62	996	1000	10.34–254.60
Hexyl acetate	Fruity, green apple, banana	10.93	1010	1010	0.50–165.57
Hexyl butanoate	Green, fruity, vegetative	14.70	1084	1190	0.00–51.86
Ethyl octanoate	Fruity, wine, banana, brandy	14.80	1189	1190	1.41–837.17
2-phenylethyl acetate	Honey, rose	16.22	1260	1260	0.00–24.34
Ethyl decanoate	Waxy, fruity, apple, grape	18.54	1382	1380	0.01–174.10
3-methylbutyl octanoate	Waxy, fruity, pineapple, coconut	19.73	1449	1450	0.00–2.11
Butanoic acid	Cheesy	6.85	832	840	0.00–27.24
2-methylbutanoic acid	Cheesy, fermented	7.72	870	870	0.00–13.71
Benzaldehyde	Almond, cherry	9.79	960	955	0.00–11.78
3-octanone	Herbal, lavender, mushroom	10.20	978	980	0.00–0.91
Octanal	Waxy, citrus	10.75	1002	1005	0.07–150.04
Phenylacetaldehyde	Honey, rose	11.71	1045	1045	0.00–2.99
Vanillin	Vanilla	18.78	1395	1400	0.00–0.05

[a] Approximate average value according to NIST database (US Department of Commerce, Gaithersburg, MD, USA).
[b] According to www.thegoodscentscompany.com.

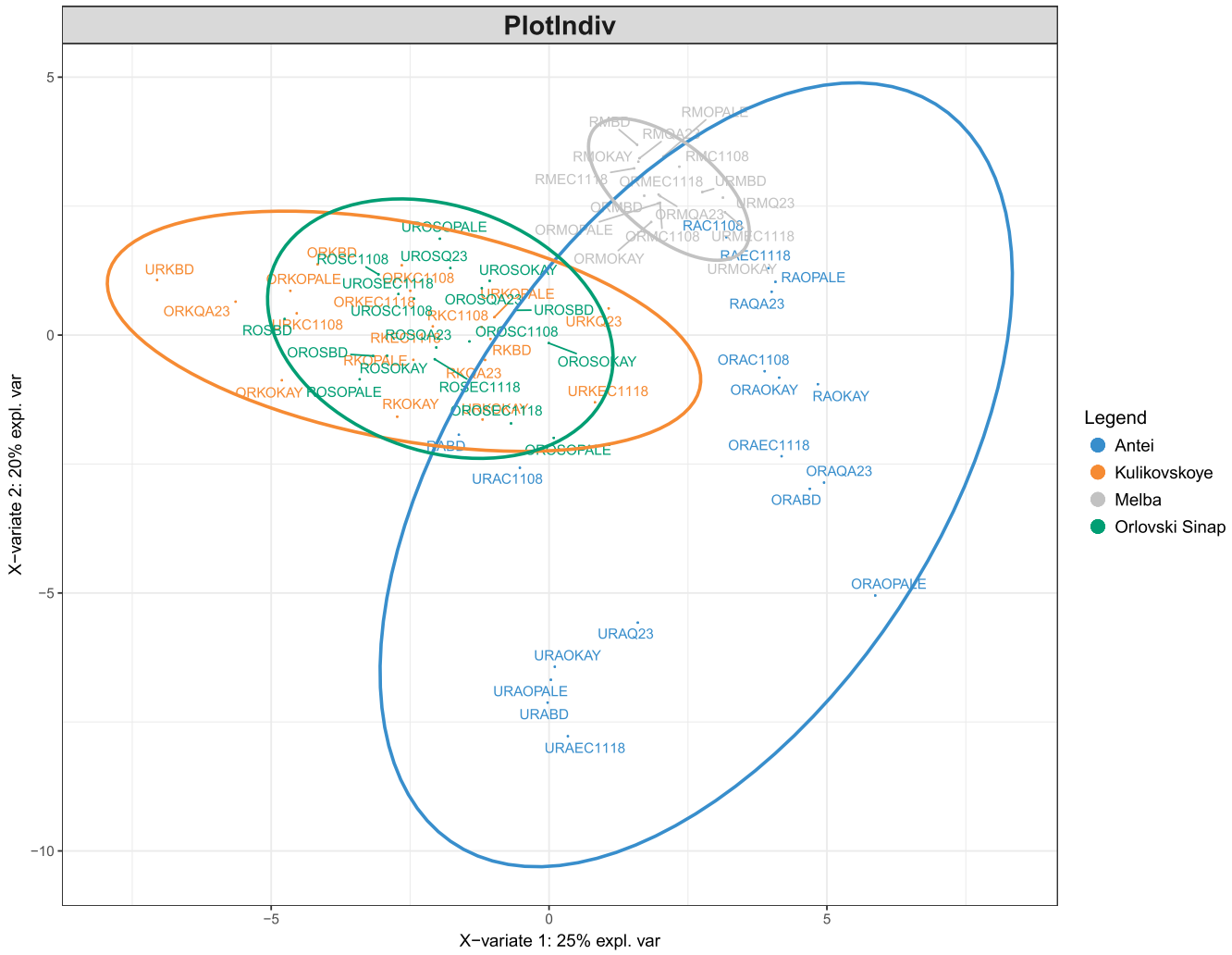

Fig. 1. PLS-DA plot for the cider samples. Samples made with Antei apples are represented with color blue, Kulikovskoye – orange, Melba – gray, Orlovski Sinap – green. Samples are coded according to the preparation: maturity level (UR – Unripe, R – Ripe, OR – Overripe) – apple cultivar (A – Antei, M – Melba, OS – Orlovski Sinap, K – Kulikovskoye) – yeast strain. Thus, sample coded ROSOKAY, for example, represents a cider made with ripe Orlovski Sinap apples and fermented with OKAY commercial yeast strain.

Table 3
Classification error rates of PLS-DA for different cider treatments (Mahalanobis distance, 10 times repetition).

No. of components	Maturity	Variety	Yeast
1 component	0.37	0.44	0.81
2 components	0.31	0.34	0.78
3 components	0.23	0.13	0.79
4 components	0.21	0.16	0.73
5 components	0.17	0.07	0.74

autumn and most winter varieties do not reach their maturity before the first frost. The ripe apples (starch index 3) were collected after 2–8 weeks (depending on the variety: 'Melba' 2 weeks, 'Kulikovskoye' 3 weeks, 'Antei' 6 weeks and 'Orlovski Sinap' 8 weeks) in storage, and the overripe (starch index 5) – after 6–12 weeks (approx. one month after the ripe stage: 6, 8, 10 and 12 weeks, respectively). Apples were washed with tap water and drained. Apples with visible defects (e.g., rotting, mold) were excluded; however, no further selection was made according to the size or appearance. Before pressing, 20 kg of apples of each variety were randomly selected at each ripening stage. The juice was prepared in two batches of 10 kg using a centrifugal juice press (Vita Pro-Active JE810; Kenwood). The resulting batches were first combined and then distributed immediately into 1 L bottles for fermentation. Malic acid content and sugar profile of the musts are provided in Table 1. The commercial starter cultures used in this study were as follows: Biodiva (*Torulaspora delbrueckii*), C1108 (*Saccharomyces bayanus*), EC1118 (*Saccharomyces cerevisiae*), OKAY (*Saccharomyces cerevisiae*), OPALE (*Saccharomyces cerevisiae*), and QA23 (*Saccharomyces bayanus*). The inoculation and fermentation steps were followed according to the procedure previously described by Laaksonen et al. (2017). The number of cider samples was 144 (4 varieties × 3 ripening stages × 6 yeasts × 2 replicates). A representative sample was taken of each cider and stored at −20 °C in 10 mL plastic tubes.

2.3. Extraction of cider volatiles

The extraction of cider volatiles was carried out using headspace – solid-phase microextraction (HS-SPME). 300 μL of the sample was measured into a 20 mL glass autosampler vial capped with a PTFE/silicone septum and diluted with 700 μL of distilled water. 7.5 ppb of 2-chloro-6-methylphenol was added as an internal standard. Vials were pre-incubated at 45 °C for 5 minutes. SPME fiber (30/50μm DVB/Car/PDMS Stableflex, length 2 cm; Supelco, Bellefonte, PA, USA) recommended by Villière et al. (2012) was used to extract the volatile compounds from the headspace for 20 minutes under stirring at 45 °C.

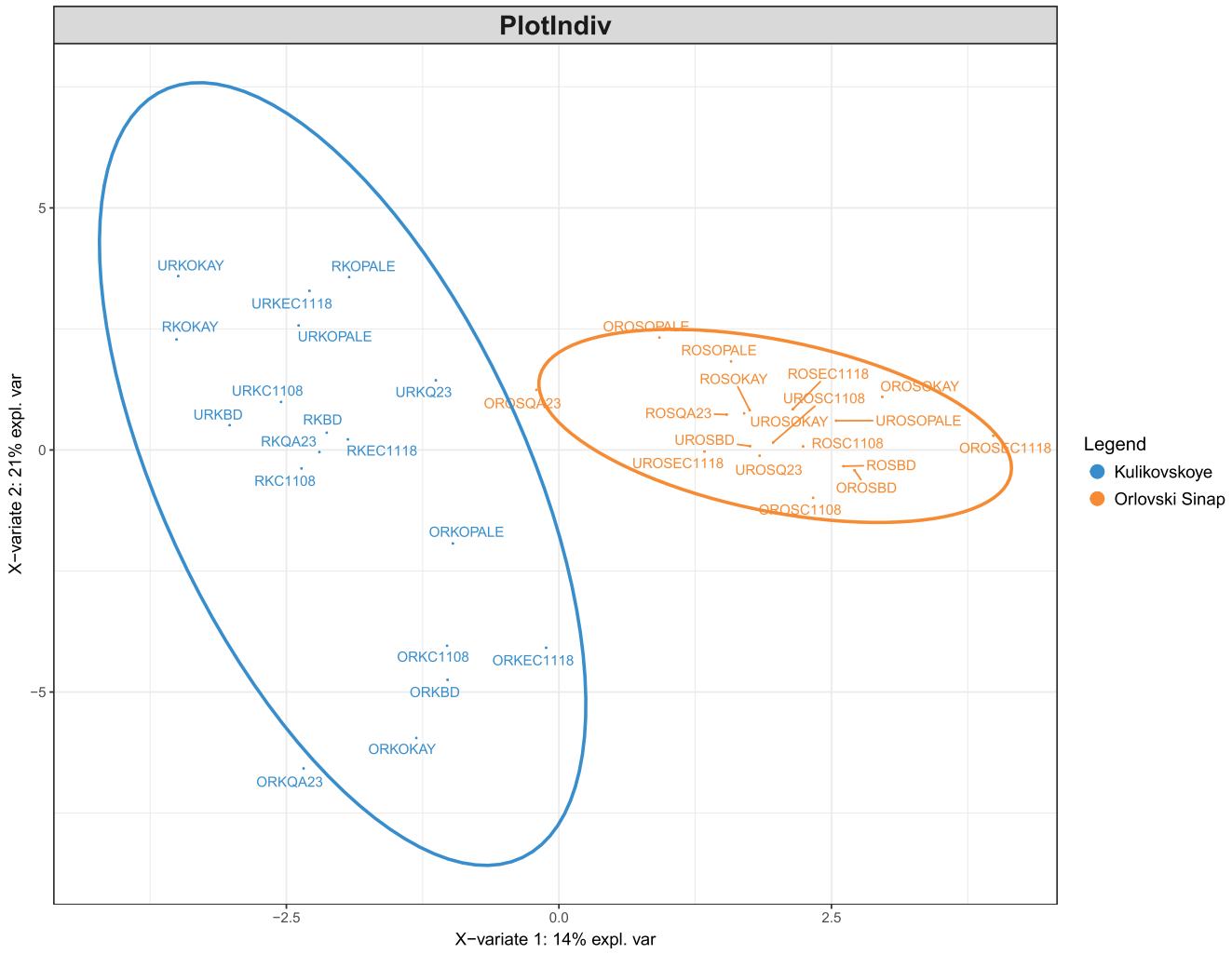

Fig. 2. PLS-DA plot for the cider samples made with Kulikovskoye (color blue) and Orlovski Sinap (color orange) apples. Samples are coded according to the preparation: maturity level (UR – Unripe, R – Ripe, OR – Overripe) – apple cultivar (A – Antei, M – Melba, OS – Orlovski Sinap, K – Kulikovskoye) – yeast strain. Thus, sample coded ROSOKAY, for example, represents a cider made with ripe Orlovski Sinap apples and fermented with OKAY commercial yeast strain.

2.4. GC-TOF-MS analysis of volatiles

Identification and relative quantitation of cider volatiles were performed using a Micromass GCT Premier gas chromatograph system (Waters, Milford, MA, USA) coupled with CombiPAL autosampler (CTC Analytics AG, Lake Elmo, MN, USA). After the SPME procedure, the volatile compounds were desorbed in splitless mode into a GC injection port equipped with a 0.75 mm internal diameter liner at 250 °C for 10 minutes. A DB5-MS column (30 m length × 0.25 mm i.d. × 1.0 μm film thickness; J&W Scientific, Folsom, CA, USA) was used with helium as a carrier gas at a flow rate of 1.0 mL min^{-1}. The oven was programmed to ramp up from 45 °C at a rate of 10 °C min^{-1} to a final temperature of 280 °C with an additional holding time of one minute (total run time 24.50 min). Mass spectra were obtained at ionization energy of 70 eV and a scan speed of 10 scans s^{-1}, with a mass range of 35–350. Each cider sample was analyzed in three analytical replicates.

Non-targeted identification of volatile compounds was carried out using ChromaLynx application (MassLynx software; Waters, Milford, MA, USA) and theoretical calculation of retention indices (RI). Theoretical retention indices were calculated using the retention times of the eluting compounds normalized to the retention times of adjacent n-alkanes. Accurate identification of the compounds was verified by comparing theoretical retention indices to the NIST database (US Department of Commerce, Gaithersburg, MD, USA). Semi-quantitative approach against an internal standard (2-chloro-6-methylphenol) was used for quantitation purposes – the amounts of identified volatile compounds were expressed in internal standard equivalents.

2.5. Statistical analysis and data processing

The results of GC-TOF-MS analysis were statistically evaluated by partial least square discriminant analysis (PLS-DA) (mixOmics package, R software 3.4.0; Boston, MA, USA). For evaluation of the classification power of each treatment, cross-validation of PLS-DA results was carried out by calculating classification error using Mahalanobis distance (10 times repetition) – the low numerical value of classification error signifies the statistical importance of the clustering seen on the plot.

The correlation of cider samples with identified volatile compounds was observed using principal component analysis (PCA) (factoextra package, R software 3.4.0; Boston, MA, USA). Each volatile compound is represented on the PCA biplot by a vector. The length of any given vector illustrates the level of correlation between the compounds and the samples – the longer the vector, the stronger the correlation. Before the application of PCA and PLS-DA, the quantitation results were autoscaled.

3. Results and discussion

In total, 37 volatile compounds were identified in the cider samples (Table 2). Partial least square-discriminant analysis (PLS-DA) was applied to evaluate the influence of each treatment (apple variety,

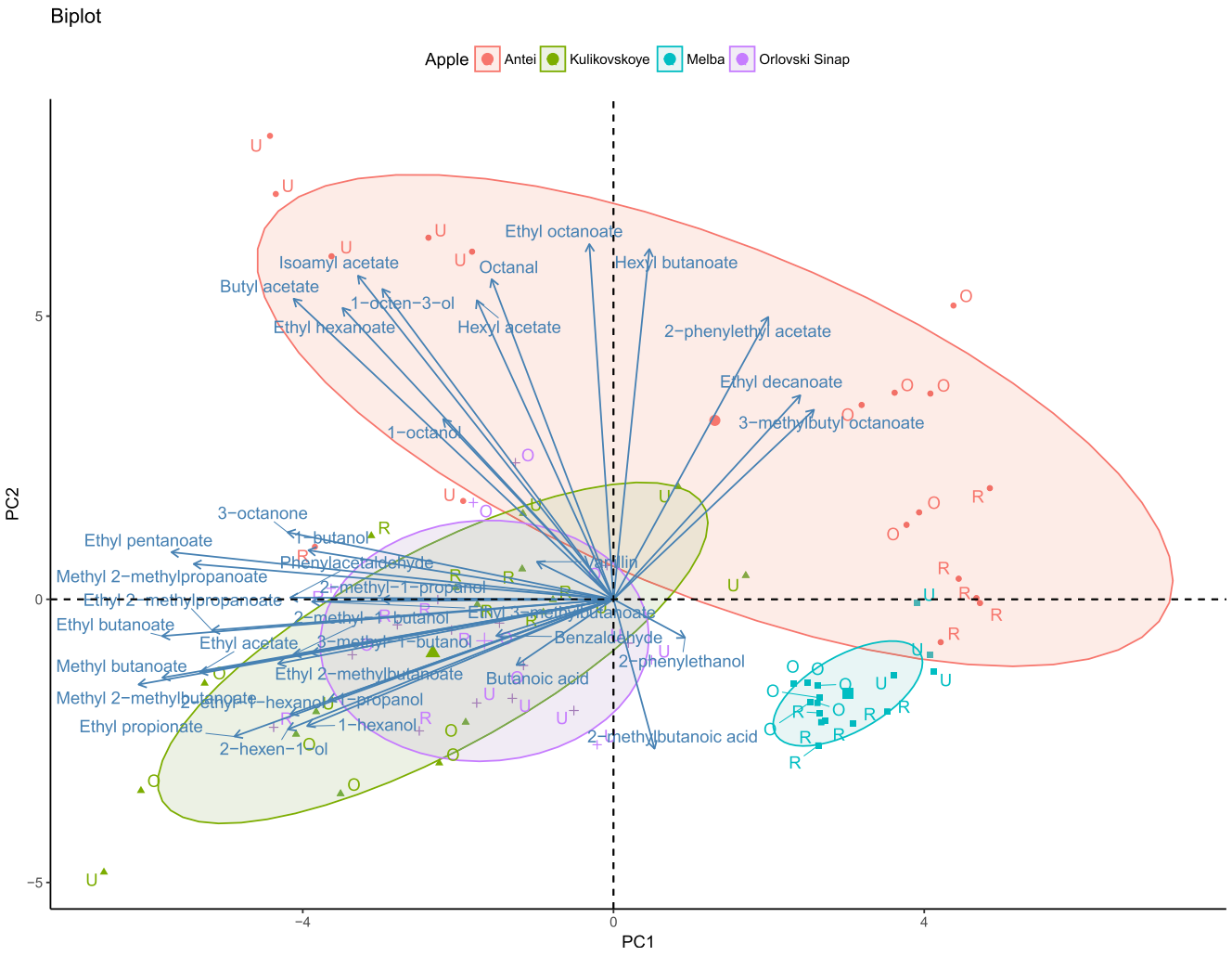

Fig. 3. Principal component analysis biplot. Samples made with Antei apples are represented with color red, Kulikovskoye apples – color green, Melba apples – color blue, Orlovski Sinap apples – color purple. Letter U represents samples made with underripe apples, letter R – ripe apples, letter O – overripe apples.

maturity level, and yeast strain) on the volatile composition of the samples. For that, one treatment at a time was taken as a predicted variable with the other two acting as replicates. The best visual separation of the samples was achieved when using apple variety as a predicted variable (Fig. 1). Statistical significance of the separation was observed using classification power of each treatment. Based on calculated PLS-DA classification error rates, apple variety also showed the best classification capacity followed by maturity and then yeast strain (Table 3). Since there was no clear grouping of the samples based on the maturity level of apples or yeast strains used for fermentation, no conclusive evidence could be drawn on their significance in terms of the volatile composition of the final product. Based on the grouping according to the apple variety, cider samples obtained with Melba apple variety were found to possess similar volatile profiles due to the proximity of the samples on the plot. Thus, the profiles of ciders made with Melba were influenced the least by apple maturity and yeast strain. This may indicate either a specific dominant aroma profile of Melba apple variety making it difficult to influence the cider by using different yeast strains or a lack of certain nutrients (e.g., amino acids) to be used as metabolic precursors in the formation of volatile compounds. As a contrast to the samples made with Melba variety, Antei variety showed a wide spread of the samples on the plot allowing additional subclustering based on the maturity level of apples. The samples made with unripe apples formed a clear subcluster, whereas the samples made with ripe and overripe samples were more similar to each other. The clusters formed by the samples made with Kulikovskoye and Orlovski Sinap varieties overlapped due to similarities in volatile composition. New PLS-DA analysis was carried out with these samples to get a better insight on clustering patterns (Fig. 2). As seen from Fig. 2, better separation of the samples on the plot was achieved. The samples made with Orlovski Sinap variety were relatively similar to each other. However, with the samples of Kulikovskoye variety, a specific clustering based on the maturity level of apples could be observed – the samples made with overripe apples were separate from the samples made with unripe and ripe apples. In terms of the effect of the maturity level of apples, the samples made with Kulikovskoye variety resulted differently to Antei variety where the samples from unripe apples were more different from the other stages of maturity.

According to overall results of PLS-DA analysis, ciders were grouped based on the apple variety first and only then based on the maturity level or yeast type. These results indicate that the apple variety has a significant influence on the technological aspects of cider production. As was shown with Melba variety, the extent to which any given yeast could potentially influence the volatile composition of the final product will depend on the apple variety used. Similarly, the degree of influence of the maturity stage of apples on the volatile composition of the final product is closely related to an apple variety picked for processing.

To evaluate the correlations between volatile compounds and apple variety, principal component analysis (PCA) was carried out (Fig. 3). According to the PCA biplot, ciders from Melba apple variety had the least diverse volatile composition; the samples formed a tight cluster with

Table 4
Statistical importance (p-values) of the identified volatile compounds in differentiating the sampling according to variety, maturity level, and yeast used. The compounds with significant statistical importance across each viewed variable are marked in **bold**.

Compound name	p-value		
	Maturity	Variety	Yeast
1-propanol	**0.001860**	**0.000050**	0.624665
2-methyl-1-propanol	**0.004188**	**0.048784**	**0.018511**
1-butanol	0.433735	**0.000000**	0.775817
3-methyl-1-butanol	**0.005499**	**0.000004**	0.700366
2-methyl-1-butanol	**0.001963**	**0.000000**	0.849864
2-hexen-1-ol, (E)	0.067941	**0.020647**	0.308530
1-hexanol	**0.007116**	**0.028643**	0.390312
1-octen-3-ol	**0.002924**	**0.000270**	0.676520
2-ethyl-1-hexanol	**0.020197**	**0.000000**	0.719560
1-octanol	0.183813	**0.000117**	0.541413
2-phenylethanol	**0.000015**	**0.000206**	0.583034
Ethyl acetate	0.093975	**0.000004**	0.165060
Methyl-2-methylpropanoate	0.083557	**0.000088**	0.237205
Ethyl propionate	0.589052	**0.000162**	**0.003749**
Methyl butanoate	0.220832	**0.000000**	0.948923
Ethyl-2-methylpropanoate	**0.038091**	**0.000005**	**0.027933**
Methyl-2-methylbutanoate	0.169946	**0.000000**	0.899196
Ethyl butanoate	0.185900	**0.000007**	0.991370
Butyl acetate	**0.034334**	**0.000061**	0.677873
Ethyl-3-methylbutanoate	**0.014082**	**0.000005**	**0.001300**
Isoamyl acetate	**0.009240**	**0.000662**	0.546437
Ethyl-2-methylbutanoate	0.618731	**0.000052**	0.999389
Ethyl pentanoate	0.481229	**0.000000**	0.446490
Ethyl hexanoate	0.099934	**0.000735**	0.747280
Hexyl acetate	0.122128	**0.001101**	0.556462
Hexyl butanoate	0.469554	**0.000000**	0.825437
Ethyl octanoate	0.183897	**0.000000**	0.797699
2-phenylethyl acetate	0.125416	**0.000008**	0.384849
Ethyl decanoate	0.054354	**0.000007**	0.620213
3-methylbutyl octanoate	0.237256	**0.000014**	0.313501
Butanoic acid	0.108986	0.236146	**0.000084**
2-methylbutanoic acid	**0.016010**	**0.001982**	**0.009141**
Benzaldehyde	**0.038750**	**0.007941**	0.890327
3-octanone	**0.000041**	**0.015216**	0.881535
Octanal	0.102392	**0.000253**	0.446443
Phenylacetaldehyde	**0.006131**	**0.000000**	0.837806
Vanillin	0.188694	0.235916	0.670147

a substantial similarity in the volatile profiles which corresponds to the results obtained using PLS-DA approach. Ciders made with Kulikovskoye, and Orlovski Sinap varieties had similar but the most diverse volatile compositions with different alcohols, aldehydes, and esters contributing to the aroma. As per PLS-DA results, subclustering of the samples made with unripe Antei apples was also observed on the upper left corner of Fig. 3. These samples had higher contents of different acetates like isoamyl acetate, hexyl acetate, and butyl acetate.

The influence of apple variety, maturity level, and yeast strain on the relative content of identified volatile compounds was observed using p-values (Table 4). According to the results, most of the compounds that distinguished the samples from one another were associated with apple variety, which corresponded to the conclusions made based on the results of PLS-DA. The apple variety and juice obtained from it can be viewed as a nutritional base for fermentation that directly affects the volatile composition and properties of cider. The difference between the apple varieties could, for example, come at the expense of initial nitrogen content. According to Santos et al. (2015), the initial content of nitrogen-containing compounds in apples is mainly composed of amino acids, especially aspartic acid, glutamic acid, asparagine, serine, and proline. The content of amino acids and yeast assimilable nitrogen can, in turn, be tied to the production of desired volatile compounds (e.g., esters) (Lambrechts and Pretorius, 2000; Bell and Henschke, 2005; Belda et al., 2017). Most of the alcohols identified in the samples, some esters (butyl acetate, ethyl-3-methylbutanoate, and isoamyl acetate), 3-methylbutanoic acid, 3-octanone, benzaldehyde, and phenylacetaldehyde were associated with the maturity stage of the apples used in processing. Some of the compounds (2-methylbutanol, 3-methylbutanol and hexanol) were previously detected by Sapers et al. (1977) in ripe McIntosh apples as indicators of ripeness. The mentioned compounds could have either originated from the biochemical changes during ripening or formed from specific precursors developed during ripening (e.g., 3-methylbutanol can be utilized as a precursor in the formation of isoamyl acetate) (Eden et al., 1996; Osorio and Fernie, 2013). The number of volatiles significantly influenced (significance level of more than 95%) by the yeast strain used for fermentation was the lowest when compared to the other treatments. Compounds 2-methyl-1-propanol, ethyl propionate, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, butanoic acid, and 2-methylbutanoic acid were found to be associated with the yeast strain used for fermentation and are products of metabolic activity. For example, 2-methyl-1-propanol is a product of amino acid (valine) catabolism (Bigelis et al., 1983). Acids (butanoic acid, 2-methylbutanoic acid) are formed as a by-product of either fatty acid metabolism or oxidation of intermediate compounds in amino acid catabolism (Alexandre and Charpentier, 1998). Fatty acid esters are reported to be the result of enzymatic activity during lipid biosynthesis (Suomalainen, 1981).

4. Conclusions

Apple variety was the primary attribute influencing the volatile composition of apple cider. The effect of yeast strains and the maturity of apples was highly variety-specific. Ripe and overripe apples imparted mostly similar aroma profiles; however, with Kulikovskoye variety, the cider from overripe apples differed the most from the others. The volatile profiles of the samples made with Melba variety were the least influenced by the maturity level of apples and yeast strains used for the fermentation.

Declarations

Author contribution statement

Julia Rosend: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rain Kuldjärv: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Sirli Rosenvald: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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