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Yeast performance characterisation in different cider fermentation matrices

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Abstract. Nitrogen content management before fermentation is often used in cider production to avoid sluggish fermentations. In addition to enhanced fermentation rates, the proper nitrogen content in the apple must may have an impact on the flavour characteristics of cider. This research aimed to assess yeast performance in two different commercially available musts with similar non-limiting yeast available nitrogen (YAN) content. In addition to fermentation kinetics, volatile ester production by yeast, and sensory properties of the final product were evaluated. The results showed that the fermentation rate and consumption of sugar and nitrogen sources by yeast did not vary between the two different musts. Yeasts consumed more malic acid in the environment of higher initial malic acid content. The content of volatile esters and sensory properties of the final products varied significantly. The occurrence of intense sulfur off-flavour was noted in one of the products.

Key words: cider, fermentation rate, gas chromatography, sensory analysis.

INTRODUCTION

Cider is a beverage made from apples through alcoholic fermentation, although in North-America, the term 'cider' generally refers to cloudy unpasteurized apple juice, and the term 'hard cider' – to a fermented product (Downing, 1995). The alcohol content of cider usually varies between 1.2 to 8.5% by volume (Lea & Piggott, 2003). However, the official alcohol limits are country-specific and defined by local laws. Notably, there are no definitive sensory characteristics for cider since the parameters like that colour, odour, sweetness, and bitterness vary significantly between the apple varieties and regions (Santos et al., 2016). Therefore, in order to obtain a cider with high quality, it is crucial to understand key parameters that contribute to the formation of desirable sensory characteristics during cider fermentation.

The main factors that influence the sensory quality of fermented beverages are the quality of must, the fermentation conditions, and yeast culture (Belda et al., 2017; Laaksonen et al., 2017). One of the primary parameters for successful fermentation is the proper yeast assimilable nitrogen (YAN) content of the must (Swiegers et al., 2008; Seguinot et al., 2018). In apple must, YAN is primarily composed of free amino acids (the so-called free amino nitrogen, FAN) and ammonium ions (Santos et al. 2016; Bourdeau et al., 2018). The contribution of amino acids to YAN can vary across different apple cultivars (Bourdeau et al., 2018).

Even though the average YAN content in apple juice is 120 mg L^{-1}, it can be as low as 30 mg L^{-1} (Drilleau, 1990; Cruz et al., 2002). The lack of initial nitrogen is strongly associated with slow fermentation or incomplete sugar utilisation, i.e. stuck fermentation (Alberti et al., 2014; Boudreau et al., 2018). Nitrogen can be a limiting nutrient in peptide/protein synthesis, sugar transport system, and fermentative activity during the initial stages of alcoholic fermentation (Santos et al., 2016). Initial nitrogen is also essential in terms of aroma generation during fermentation (Carrau et al., 2008; Barbosa et al., 2009; Seguinot et al., 2018) with amino acids acting as metabolic precursors for the biosynthesis of different volatile compounds, e.g. esters (Santos et al., 2016). The lack of nitrogen is often associated with hydrogen sulphide (H_2S) production, although the exact mechanisms of how YAN deficiency influences H_2S formation by yeast are still not clearly defined (Ugliano et al., 2007; Ugliano et al., 2010; Ugliano et al., 2011; Barbosa et al., 2012). Therefore, the management of H_2S in fermented beverages through nitrogen supplementation requires knowledge of both initial YAN and yeast H_2S production characteristics (Ugliano et al., 2011; Barbosa et al., 2012).

Nowadays, the selection of different yeasts (strains of *Saccharomyces cerevisiae* as well as non-*Saccharomyces* yeast species) for fermented beverages production is very diverse. Therefore, the information about yeast performance shared by yeast starter culture manufacturers is of great value for cider producers for choosing the right strain. Today, most yeast manufacturers provide information on the recommended fermentation conditions (e.g. temperature range and optimum temperature, alcohol tolerance), yeast nutrition requirements, expected fermentation duration, and possible sensory properties of the final product. With growing cider production and the increasing number of craft cider producers on the market (The European Cider and Fruit Wine Association, 2018) there is a need for product differentiation in order to gain a competitive advantage while maintaining stable quality. Thus, the proper selection of yeasts along with nutrition management of the fermentation process to ensure sufficient fermentation rate, complex aroma development and avoid off-aroma related defects (e.g., H_2S formation) is becoming more critical.

This study aims to explore and compare the performance of five different commercially available wine yeast strains in fermentation of different apple musts. The free amino nitrogen (FAN) content was brought to a similar level in the used apple musts to observe yeast behaviour in different matrices where free amino nitrogen is not a limiting factor. Yeast performance was observed in terms of fermentation efficiency, consumption of nutrients, production of ethyl and acetate esters, and overall sensory properties of the final product, including H_2S.

MATERIALS AND METHODS

Cider fermentation

This study made use of two different apple musts: M1 (Aspall, Suffolk, United Kingdom; Brix 12.8%, pH 3.28, titratable acidity 6.7 g L^{-1} in malic acid equivalents, initial FAN 69.48 ± 2.00 mg L^{-1}) and M2 (Döhler, Darmstadt, Germany; Brix 11.7%, pH 3.18, titratable acidity 3.8 g L^{-1} in malic acid equivalents, initial FAN 51.21 ± 1.84 mg L^{-1}). Free amino nitrogen content in both matrixes was brought to a similar content of 80 ± 2 mg L^{-1} by using organic yeast nutrient (Fermaid K; Lallemand Inc.). The musts (400 mL) were distributed into sterile 500 mL fermentation bottles.

Each bottle was inoculated with a chosen rehydrated yeast starter culture, according to the manufacturer's (Lallemand, Inc.) instructions. The starter cultures used in this study were as follows: Y1 (*Torulaspora delbrueckii*), Y2 (*Saccharomyces cerevisiae*; white wine yeast selected through directed breeding), Y3 (*Saccharomyces cerevisiae var. bayanus*; red and white wine yeast for demanding conditions), Y4 (*Saccharomyces cerevisiae var. bayanus*; sparkling wine yeast), and Y5 (*Saccharomyces cerevisiae*; red, rosé, and white wine yeast selected through evolutionary adaptation).

Inoculated bottles (5×10^6 CFU mL^{-1}) were sealed using screw caps with septums pierced with syringe needles (19G × 1 ½, 1.1 × 40 mm; Terumo Medical Corporation) and coupled with microfilters to vent carbon dioxide. Fermentations were carried out at 18 ± 1 °C by following carbon dioxide production by weighing of the fermentation bottles once per day on a daily basis. Fermentations were considered completed when the mass loss due to carbon dioxide dissipation could not be observed anymore (approx. 336 hours). Samples were withdrawn every second day for subsequent analysis. For each experiment, two parallel fermentations were performed.

Sugar and malic acid content

Sugars and malic acid content during the fermentation was analysed using high-performance liquid chromatograph (Alliance HPLC) equipped with BioRad HPX87H column, RI and UV detectors. Prior to analysis, the samples were diluted 1:10 with MilliQ water and filtered (Whatman Spartan 13; Dassel, Germany). 0.005 M H_2SO_4 solution was used as mobile phase with a flow rate of 0.6 mL min^{-1}. Standard solutions of fructose, glucose, sucrose, and malate were used for calibration curves.

FAN content

The FAN content at different stages of fermentation was assessed using DNFB (dinitrofluorobenzene) method which is based on the reaction of amino groups of free amino acids with 2,4-dinitrofluorobenzene. The reaction derivatives were subsequently measured by spectrophotometry at 420 nm. Standard solutions of glycine with known nitrogen content were used to obtain calibration curve. Results were expressed as mg L^{-1} in glycine equivalents. Three analytical replicates were used for each sample.

GC-TOF-MS analysis of volatile esters

Headspace – solid phase microextraction (HS-SPME) was used for the extraction of volatile compounds. For that, 50 µL of each sample was diluted with 950 µL of distilled water; 2-chloro-6-methylphenol (100 µg L^{-1}) was used as an internal standard for quantitation purposes. Vials were pre-incubated at 45 °C for 5 minutes. An SPME

fiber (30/50 μm DVB/Car/PDMS Stableflex, length 2 cm; Supelco, Bellefonte, PA, USA) was used to extract the volatile compounds from the headspace for 20 minutes.

GCT Premier 6890N gas chromatograph system (Agilent Technologies, Santa Clara, CA, USA) equipped with TOF mass spectrometer (Waters, Milford, MA, USA) and 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 40 °C at a rate of 7.5 °C min^{-1} to a final temperature of 280 °C with an additional holding time of three minutes (total run time 35 min). Mass spectra were obtained at an ionization energy of 70 eV and a scan speed of 10 scans s^{-1}, with a mass scan range of 35 to 350 Da. Two analytical replicates were used for each sample.

A presence of selected ethyl (medium-chain fatty acid ethyl esters) and acetate esters was monitored across the samples. These two groups of esters are synthesized through different pathways and play a primary role in the perception of desired fruity attributes in fermented beverages (Saerens et al., 2010). A complete list of the compounds of interest and their odour descriptions is provided in Table 1. Accurate identification of the compounds was achieved using respective analytical standards. The concentrations were expressed in μg L^{-1} of internal standard equivalents.

Table 1. A list of selected volatile esters and their odour descriptions

Ester	CAS	Odour description*
Ethyl acetate	141-78-6	Ethereal, fruity, green
Isobutyl acetate	110-19-0	Fruity, ethereal, banana
Isoamyl acetate	123-92-2	Fruity, banana
Butyl acetate	123-86-4	Ethereal, fruity, banana
Hexyl acetate	142-92-7	Fruity, green apple, banana
Ethyl butanoate	105-54-4	Fruity, pineapple, cognac
Ethyl hexanoate	123-66-0	Fruity, pineapple, waxy, green
Ethyl octanoate	106-32-1	Fruity, wine, apricot, banana
Ethyl decanoate	110-38-3	Waxy, fruity, apple, grape
Ethyl dodecanoate	106-33-2	Waxy, floral, soapy

*According to www.thegoodscentscompany.com

Sensory analysis

Descriptive analysis was used in the study to assess the sensory properties of the cider samples. A local sensory panel of 8 highly trained assessors with previous experience in working with cider samples carried out the analysis. There was no pre-session to familiarize assessors with the samples. The working linear scale was established at 0–15, and relative intensities were used. A complete list of assessed attributes and their definitions is provided in Table 2. Additional commentary (e.g., the presence of off-flavours) was encouraged if necessary. Samples were assessed independently by each assessor. Prior to the assessment, all samples were adjusted for sweetness to balance out the sour taste since the secondary malolactic fermentation was not carried out. For that, 3% of diluted sucrose was added. All samples were encoded with a randomized three-digit numerical key.

Table 2. A complete list of sensory attributes and their definitions

Attribute	Description
Odour	
Overall intensity	Overall strength of the perceived odour
Fruity	Strength of all fruity odours (excluding apple)
Cooked apple	Strength of odours characteristic to cooked apples
Apple-like	Strength of odours characteristic to fresh apples
Sweet	Strength of all sweet odours
Sour	Strength of all sour odours
Taste	
Fruity	Strength of overall sensation characteristic to fruits (excluding apples)
Cooked apple	Strength of overall sensation characteristic to cooked apples
Apple-like	Strength of overall sensation characteristic to fresh apples
Sweet	Strength of overall sweet sensation
Sour	Strength of overall sour sensation
Bitter	Strength of overall bitter sensation
Astringency	Strength of overall drying sensation

Data processing

The results of chemical analysis were averaged across biological and analytical replicates. The analysis of variance was performed using ANOVA (R software 3.4.0; Boston, MA, USA), and $p < 0.05$ was considered statistically significant. The results of sensory analysis were statistically evaluated by principal component analysis (PCA) using OriginPro software (OriginLab; Northampton, MA, USA). Prior to the application of PCA, the results were autoscaled.

RESULTS AND DISCUSSION

Fermentation kinetics with different yeasts (Fig. 1a, 2b) did not seem to be dependent on the apple must. Each yeast strain showed similar performance (i.e, fermentation duration, maximum speed of carbon dioxide production) in both matrices. Various authors have previously linked fermentation activity to the nitrogen content of the must (Santos et al., 2016; Boudreau et al., 2017; Lemos Junior et al., 2017; Seguinot et al., 2018). In this study, the nitrogen content of both musts was adjusted to the same value (80 ppm). Thus, the same nitrogen content might be the reason behind the absence of visible differences in fermentation rates between the studied musts M1 and M2.

The composition of initial fermentable sugars in the musts and residual sugars in the samples after the fermentation is summed up in Table 3. Up to 60% of initial sugar content in both apple musts consisted of fructose. The main difference in residual sugar consumption between the musts fermented with different yeasts was due to the different ability of the studied yeasts to consume fructose. For example, residual fructose concentrations in fermentations with *T. delbrueckii* (Y1) were up to 7 times higher than with the other studied strains. In general, sugar consumption patterns for the same strains were quite similar in the two studied musts, and small differences in residual amounts could instead be attributed to the initial Brix%.

Comparing fermentation kinetics and fructose consumption (Fig. 1a, 2b, Table 3) it becomes evident that yeasts with more fructophilic characteristics conduct a more intense fermentation. Thus, intrinsic differences in yeast fructose uptake could be utilised in cider production for process optimisation.

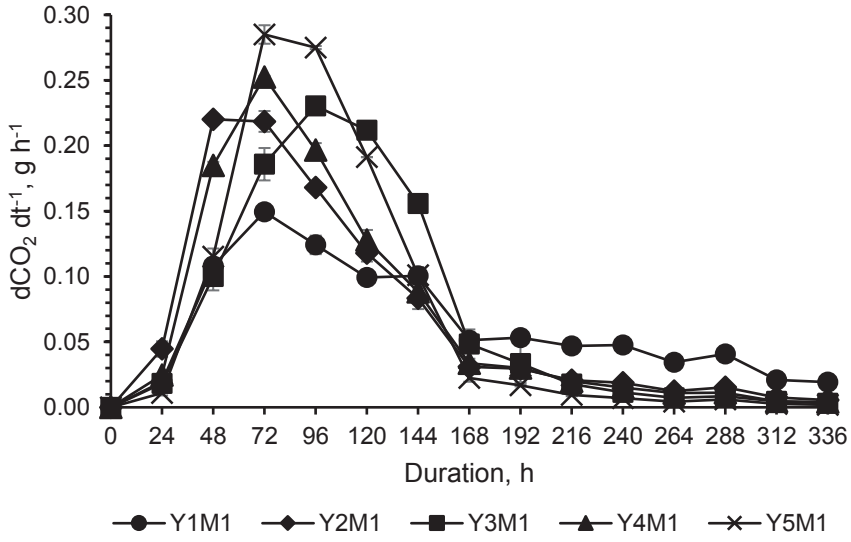

Figure 1a. Fermentation kinetics with different yeasts (Y1-5) in must M1.

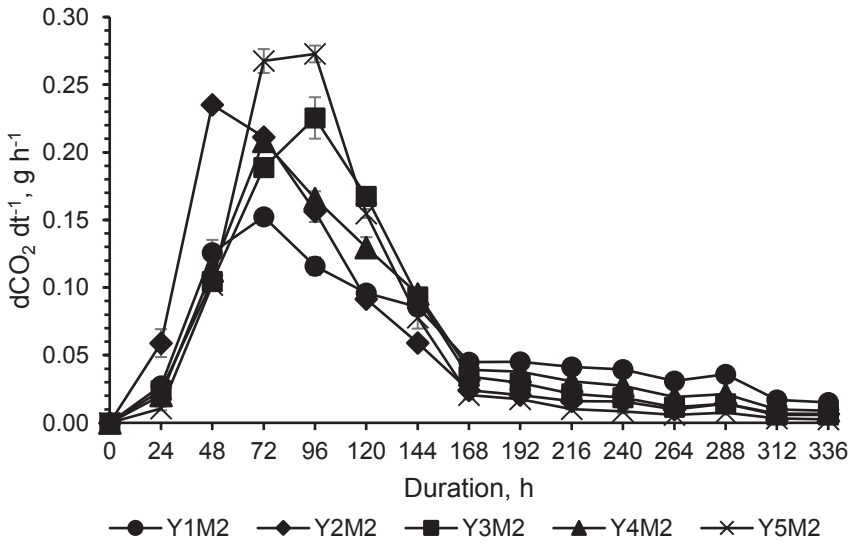

Figure 1b. Fermentation kinetics with different yeasts (Y1-5) in must M2.

Table 3. Composition of initial sugars in the musts (M) and residual sugars at the end of fermentation with different yeasts (YM) and musts ($p < 0.05$)

Amount, g L^{-1}	Glucose	Fructose	Sucrose	TOTAL
M1	25.61 ± 2.18	74.93 ± 2.88	20.35 ± 3.02	120.89 ± 8.08
M2	26.83 ± 3.65	70.69 ± 2.93	16.33 ± 3.94	113.85 ± 10.52
Y1M1	0.67 ± 0.03	8.85 ± 0.94	1.15 ± 0.31	10.67 ± 0.96
Y1M2	1.12 ± 0.33	8.74 ± 0.86	1.27 ± 0.39	11.13 ± 0.79
Y2M1	0.45 ± 0.02	1.81 ± 0.18	0.60 ± 0.06	2.86 ± 0.58
Y2M2	1.29 ± 0.20	3.12 ± 0.29	1.19 ± 0.23	5.60 ± 0.93
Y3M1	0.24 ± 0.05	1.22 ± 0.17	0.53 ± 0.08	1.99 ± 0.12
Y3M2	1.31 ± 0.19	2.17 ± 0.40	1.27 ± 0.27	4.75 ± 0.98
Y4M1	0.22 ± 0.04	2.07 ± 0.18	0.57 ± 0.15	2.86 ± 0.22
Y4M2	1.20 ± 0.25	3.72 ± 0.49	1.15 ± 0.14	6.07 ± 0.81
Y5M1	0.23 ± 0.02	1.11 ± 0.10	0.60 ± 0.03	1.94 ± 0.19
Y5M2	1.36 ± 0.12	1.16 ± 0.17	1.70 ± 0.18	4.22 ± 0.50

A decrease in malic acid concentration was observed to some extent as a result of the fermentation process regardless of the must used (Fig. 2). The consumption of malic acid by yeasts was significantly higher in the must M1 where the initial concentration was approximately two times bigger than in the must M2 (5.02 ± 0.09 g L^{-1} vs 3.03 ± 0.13 g L^{-1}). The most drastic decrease of almost 44% in the malic acid concentration was observed in the case of *S. cerevisiae var. bayanus* (Y3) in M1 must. It has been previously reported that up to 50% of extracellular malic acid can be metabolised by Saccharomyces sp. yeasts during alcoholic fermentation of wine (Barnett & Kornberg, 1960; Delfini & Formica, 2001). The effect of more significant consumption of malic acid at its higher concentration by yeast could potentially be attributed to otherwise low affinity to malic acid of yeast malic enzyme (Mae1p) responsible for its decarboxylation to pyruvate (Volschenk et al., 2003). The K_m of Mae1p enzyme for malic acid is reported to be 50 mM (Boles et al., 1998) or approximately 6.7 g L^{-1} which is close to the initial concentration in M1.

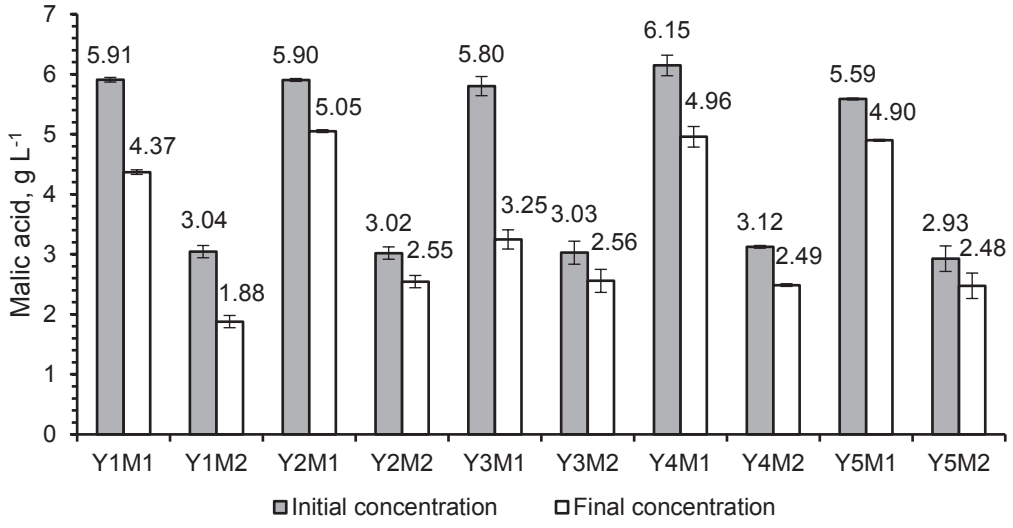

Figure 2. Malic acid consumption by different yeasts (Y1-5) in the two apple musts (M1 and M2) used in the study ($p < 0.05$).

The assimilation of free amino nitrogen (FAN) by the yeast strains used in this study is shown in Fig. 3a, 3b. Notably, complete depletion of FAN was not observed with any of the yeasts regardless of the fermentation matrix. The intense FAN consumption occurred in case of all strains within 2–4 days. The residual concentration of FAN at the end of fermentation was similar regardless of the yeast and must used although differences occurred in the FAN consumption rates. Notably, the consumption rates of FAN by yeasts used in this study did not always correlate with their fermentation activity. However, there might be a potential difference between yeasts with regards to their preference for specific amino acids for synthesis of volatile compounds (Lambrechts & Pretorius, 2000; Santos et al., 2015; Belda et al., 2017; Gobert et al., 2017; Fairbairn et al., 2017).

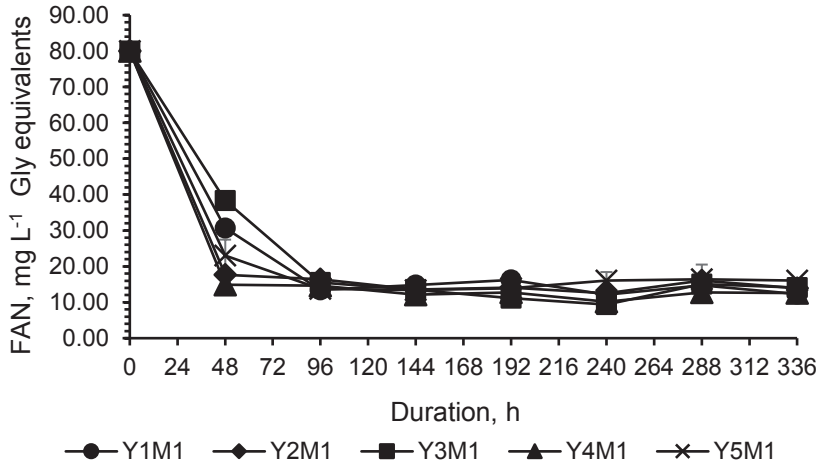

Figure 3a. Free amino nitrogen consumption by yeasts (Y1-5) during cider fermentation in the must M1.

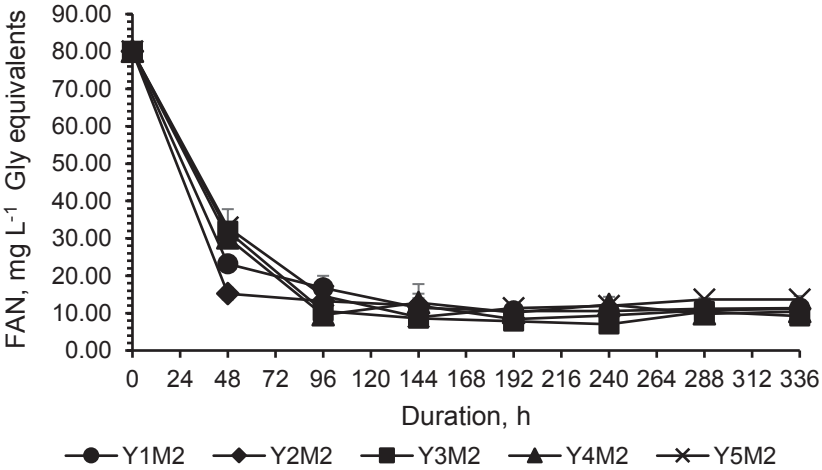

Figure 3b. Free amino nitrogen consumption by yeasts (Y1-5) during cider fermentation in the must M2.

In total, the concentration of 10 ethyl and acetate esters was monitored in the cider samples produced with five different yeasts and two musts (Table 4). Isobutyl acetate, butyl acetate, and ethyl dodecanoate were not detected in any of the analysed samples. *T. delbrueckii* (Y1) showed the lowest production of esters except isoamyl acetate. Others have previously noted significant production isoamyl acetate in *T.delbrueckii/S.cerevisiae* mixed inoculation during winemaking trials (Herraiz et al., 1990; Zhang et al., 2018). In addition to *T. delbrueckii*, *S. cerevisiae var. bayanus* (Y4) also produced considerably more (up to 3.5 times) isoamyl acetate in comparison to the others. The formation of three esters – ethyl acetate, isoamyl acetate, hexyl acetate depended on the matrix used. Both ethyl acetate and isoamyl acetate were produced in considerably (up to 7 and 2 times, respectively) higher concentrations by most strains in the must M2. Hexyl acetate, on the other hand, had higher relative concentrations in the samples made with the must M1. All three aforementioned esters are yeast metabolites produced during fermentation process. Ethyl acetate is formed in anaerobic glucose metabolism from acetyl-CoA with glutamate, methionine and cysteine precursors in its synthesis pathway (Nordström, 1962). Isoamyl acetate can either be created from amino acids (leucine, valine) or de novo synthesised from isoamyl alcohol (Eden, et al., 1996; Plata et al., 2003). Hexyl acetate originates from C6 alcohols and aldehydes (e.g., hexanol, 2-hexenol, 2-hexenal) (Dennis et al., 2012). Thus, in terms of volatile composition development of the final product, other intrinsic properties of the fermentation matrix than the YAN content should also be taken into account. The amount of ethyl esters depended on the particular yeast-must combination. For example, Y2 (*S.cerevisiae)* and Y4 (*S. cerevisiae var. bayanus)* favoured the production of ethyl decanoate in M1; Y3 (*S. cerevisiae var. bayanus)* and Y5 (*S. cerevisiae*)– in M2.

Table 4. Relative concentration (in μg L^{-1} of IS equivalent) of selected esters at the end of fermentation with different yeasts (Y1-5) and apple musts (M1 and M2) ($p < 0.05$)

	Y1M1	Y2M1	Y3M1	Y4M1	Y5M1
Ethyl acetate	93.33 ± 18.86	95.00 ± 16.50	53.33 ± 0.00	456.67 ± 4.71	350.00 ± 4.71
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl acetate	3,970.00 ± 381.84	2,646.67 ± 405,41	2,160.00 ± 341.50	4,933.33 ± 150.85	1,640.00 ± 18.86
Butyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.
Hexyl acetate	n.d.	213.33 ± 37.71	156.67 ± 4.71	120.00 ± 18.86	580.00 ± 107.50
Ethyl butanoate	115.56 ± 15.40	248.89 ± 30.79	253.33 ± 18.86	222.22 ± 30.79	120.00 ± 18.86
Ethyl hexanoate	253.33 ± 56.57	1,484.44 ± 253.45	1,564.44 ± 320.37	1,366.67 ± 250.51	2,862.22 ± 348.03
Ethyl octanoate	24.44 ± 3.85	1,733.33 ± 188.56	1,960.00 ± 320.56	1,066.67 ± 149.27	6,680.00 ± 358.27
Ethyl decanoate	n.d.	66.67 ± 18.86	186.67 ± 37.71	n.d.	328.89 ± 55.51
Ethyl dodecanoate	n.d.	n.d.	n.d.	n.d.	n.d.

Table 4 (continued)

	Y1M2	Y2M2	Y3M2	Y4M2	Y5M2
Ethyl acetate	255.56 ± 56.70	466.67 ± 18.86	358.33 ± 21.21	1,273.33 ± 122.57	516.67 ± 14.14
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl acetate	7,730.00 ± 183.85	3,084.44 ± 111.02	2,826.67 ± 270.64	7,533.33 ± 546.83	2,222.22 ± 114.96
Butyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.
Hexyl acetate	n.d.	n.d.	n.d.	n.d.	26.67 ± 0.00
Ethyl butanoate	166.67 ± 24.04	266.67 ± 37.41	228.89 ± 19.25	226.67 ± 56.57	186.67 ± 37.71
Ethyl hexanoate	66.67 ± 18.86	1,048.89 ± 181.52	1,813.33 ± 226.27	4,160.00 ± 37.71	3,040.00 ± 263.99
Ethyl octanoate	n.d.	4,231.11 ± 858.46	2,106.67 ± 462.65	19,866.67 ± 1,395.36	7,680.00 ± 1,244.51
Ethyl decanoate	n.d.	106.67 ± 0.00	95.00 ± 16.50	623.33 ± 61.28	266.67 ± 57.61
Ethyl dodecanoate	n.d.	n.d.	n.d.	n.d.	n.d.

According to the ANOVA, statistically significant differences ($p < 0.05$) were obtained for most of the sensory attributes assessed with the exception of overall odour intensity ($p = 0.61$), 'apple-like' in odour ($p = 0.08$), 'apple-like' in taste ($p = 0.10$), sourness in odour ($p = 0.19$), and astringency ($p = 0.98$). The results of sensory analysis with the exception of statistically insignificant parameters were then subjected to principal component analysis (PCA). The obtained PCA biplot is presented in Fig. 4. The Principal Component 1 accounted for 54.54% of the differences between the samples; the Principal Component 2 – for 17.18%. Based on the biplot, ciders produced with the apple must M2 possessed strong correlation with fruitiness, sweetness, and 'cooked apple' characteristic. However, the samples made with the apple must M2 contained more residual sugar at the end of fermentation than the samples made with M1 (Table 3). This might have partially contributed to an enhanced perception of sweetness and fruitiness. The ciders made with the must M1 strongly correlated with sourness, which corresponds well with its higher titratable acidity and higher malic acid content in finished ferments. Correlation with other parameters in these samples is negative, which means the intensities were considerably lower in comparison with the samples made with M2.

In the course of sensory analysis, the ciders also received additional commentary by the panel members. According to this, all samples made with the must M1 had an off-flavour described as 'animalic' and 'sulfur'. The occurrence of the off-flavour of significant intensity in the ciders made with M1 could also mask the fruitiness of these ciders. Based on the description, the most likely source of this off-flavour was proposed to be hydrogen sulphide. Indeed, the overproduction of hydrogen sulphide is regarded as one of the main challenges in cider production (Boudreau et al., 2017). The accumulation of hydrogen sulphide during fermentation can be related to multiple different factors such as susceptibility of yeast strain to produce it as well as nutritional composition of the environment (e.g., YAN and vitamins content) (Boudreau et al., 2017). The difference in initial YAN content was not the case in this study as it was

brought to the same level with Fermaid nutritional supplement prior to the start of fermentation. However, the difference in intrinsic amino acid and/or vitamin composition in the apple must could still be a key factor in off-flavour production. For example, Bohlscheid et al. (2011) have noted that biotin and pantothenic acid deficiency in the fermentation environment could result in excessive production of hydrogen sulphide by yeasts.

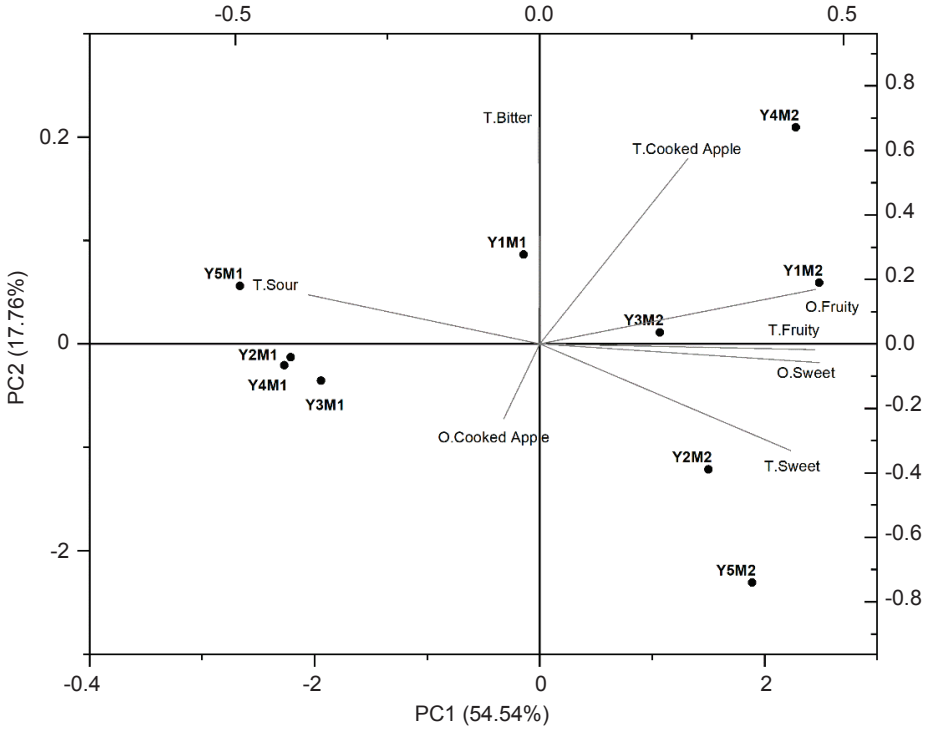

Figure 4. Grouping of the samples on the PCA biplot according to statistically significant ($p < 0.05$) sensory properties perceived at the end of fermentation with each combination of yeast (Y1-5) and fermentation matrix (M1 and M2).

CONCLUSIONS

Yeast performance in the fermentation of cider from the apple musts with similar nitrogen content was studied in this work. The differences in yeast fermentation activity, consumption of nutrients (sugars, malic acid, nitrogen), production of selected volatile esters and development of sensory properties were characterised. Based on the results the fermentative activity of yeasts and consumption of certain nutrients like fructose and nitrogen did not depend on the must. Malic acid consumption was found to depend on the initial malic acid content in the environment. The main differences between the two apple musts used for the fermentations were related to the content of volatile esters and sensory properties of cider. Despite the similar level of initial YAN content in the musts, the yeast performance in terms of the development of sensory properties was not the same. Lower volatile ester formation and synthesis the off-flavour was noted with one of the musts used for the experiments. Thus, initial nitrogen content adjustment did not

guarantee a good quality of the finished product. Since the sensory properties are the driving force behind consumer behaviour, a proper approach to off-flavour management should be implemented. Further research is required to establish the factors/combination of factors that could allow for simultaneous reduction of off-flavour production risks and an increase in the production of volatile esters in cider.

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