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Chemical and volatile composition of Pálinka produced using different commercial yeast strains of *Saccharomyces cerevisiae*

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A B S T R A C T

Pálinka is Hungarian traditional alcoholic drink, and its quality is strongly depending on applied yeast strain. Unfortunately, all commercial yeast strains used the production of pálinka are selected for oenological purpose, and thus the efficacy and aroma releasing capacity are vary depending on the type and quality of fruit used. In this study, the fermentation efficacy of nine commercial yeast strains of *Saccharomyces cerevisiae* was focused. All strains were able to do alcoholic fermentation of apple juice quite efficiently, and the simple sugars (fructose, glucose and sucrose) were almost exhausted at the end of fermentation. Meanwhile, the alcohol production capacity and yield were no significant differences (around 9.17 *v*/v %–9.43 v/v %), whereas the ability of sugar consumption of strains Uvaferm Danstil A and Fermicru AR2 was stronger than others. The differences in the concentration and composition of volatile compounds were recorded. The highest levels of total volatile compounds were observed in samples fermented with Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses, and Fermicru AR2. Meanwhile total volatile compounds, 2-methyl-1-propanol, 3-methyl-1-butanol, total higher alcohols, ethyl acetate, and total esters were considered as key parameters for describing the profile of fermented apple juices, whereas total fusel alcohols, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and total volatile compounds were characteristic indicators of samples fermented with Uvaferm Danstil A. This work provides very good information of commercial yeast strains for industrial pálinka production.

1. Introduction

Pálinka is a traditional Hungarian spirit drink produced exclusively by the alcoholic fermentation and distillation of any fruit grown in Hungary. It is protected as a geographical indication by the European Union. Therefore, only fruit spirits fermented, distilled and bottled in Hungary can be called “Pálinka”, except some apricot spirits from four provinces (Niederösterreich, Burgenland, Steiermark and Wien) of

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Austria (Liber, 2017; Regulation of EC No 110/2008, n.d.). There are many kinds of pálinka with different distinct characteristics based on specific types of fruits used for fermentation. The most common fruits for processing pálinka are apricot, pear, plum, cherry and apple (Harcsa et al., 2014; László et al., 2016; Pham et al., 2021). The alcohol content of pálinka is not <37.5 % (v/v) but not >86 % (v/v) (Regulation of EC No 110/2008, n.d.).

Apple is one of the popular fruits and it is widely grown in Europe. Apple fruits are rich sources of antioxidant phytonutrients, vitamins and minerals (Muhammad et al., 2019). The total production of apples in Europe was nearly 12.7 million tons per year (Garcia et al., 2019). Hungary is one of seven EU Member States covering >80 % of the whole apple fruit production in Europe, with over 500,000 tons per year (Jortay, 2016); hence apple can be an abundant source supplying for pálinka production. The use of source fruit fosters the growth of spirit industries and enhances the increase in agriculture's value. Each apple variety has unique characteristics that make differences in the flavor and taste of spirit (Rita et al., 2011). Over 300 volatile aroma compounds have been identified in apples, but only a few of them may well be studied (Dixon and Hewett, 2000). Januszek et al. (2020) studied the sensory properties of ten apple spirits produced from different cultivars grown in Poland and reported that the variety and concentration of terpenes such as α-phellandrene, o-cymene, α-terpineol, citric and myrcene play an important role in the formation of the sweet and citrus aroma attribute. The compositions and concentrations of aroma compounds are essential factors in the characteristic sensory profile of apple spirits (Dixon and Hewett, 2000; Espino-Díaz et al., 2016). In the production of spirit, both these factors strongly depend on the activity of yeast applied as well as on the quality of fruits especially on the nitrogen sources, thus study related to these fields can provide significant information in control and improvement of the quality of apple pálinka.

There are four main stages in pálinka production, including mashing, fermentation, distillation, aging and storage (László et al., 2016; Qian et al., 2019). Fermentation is a critical stage because it directly affects the product's yield, flavor and taste. Formerly, the fermentation was carried out by the natural yeast existing in fruit (Molinet and Cubillos, 2020) so called spontaneous fermentation. Nowadays, in the spirit production, commercial yeast cultures are used with ensuring rapid and reliable fermentation, especially reducing the risk of sluggish or stuck compared to natural yeast (Molinet and Cubillos, 2020; Valero et al., 2005). Moreover, applying commercial yeast strains can achieve high ethanol yield without the occurrence of off-flavor and off-taste. It also has significantly contributed to improving the quality of spirit and enhancing the control of the fermentation process (Valero et al., 2005). Unfortunately, most commercial yeast strains were isolated and developed from grapes, thus they are rather using for production of wine and grape distillates. Hence the content of apple fruit is completely different from grape, these yeasts will take different achievement in the apple matrix. No doubt that spirit fermented with different yeast strains will have different flavors and tastes due to particular characteristics of each yeast strain (Li et al., 2011; Oliveira et al., 2005; Pham et al., 2021). In this study, performance of nine commercially available *Saccharomyces cerevisiae* strains (Uvaferm SLO, Uvaferm PM, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses, Fermicru AR2, Oenoferm x-treme F3 and Oenoferm x-thiol F3) in the fermentation of apple juices was focused especially on the chemical and volatile compositions of apple pálinka.

2. Material and methods

2.1. Yeast strains and inoculum preparation

Nine different commercial yeast strains (Table 1) were provided by Kokoferm Ltd. (Gyöngyös, Hungary). These yeast strains were activated by mixing 1.0 g dry yeast with 1.0 g yeast nutrient, namely Uvavital™ (Lallemand Inc., Montréal, QC, Canada), and 100 mL warm water. Then the mixture was incubated in a rotary shaker with a speed of 120 rpm at 28 °C for 2 h for growth. The cell numbers reached approximately 1.5×10^8–3.0×10^8 cells/mL.

Table 1
Commercial yeast strains with their origins and applications.

Commercial yeast preparation	Strain	Region of isolate	Suggestion of application	Producer
Uvaferm® SLO 10205-06-02 10205-01-02	*S. cerevisiae* E491	Europe	Oenological use	Lallemand Inc. (Montréal, QC, Canada)
Uvaferm® PM 10054-06-02	*S. cerevisiae* E491	Europe	Oenological use	Lallemand Inc. (Montréal, QC, Canada)
Uvaferm® Danstil A	*S. cerevisiae* E342	Europe	Oenological use	Lallemand Inc. (Montréal, QC, Canada)
Fermiblanc® Arom	*S. cerevisiae* SM102	Cognac (France)	Production of aromatic white wine	Oenobrands (Montpellier, France)
Fermicru® AR2	*S. cerevisiae* L0122	Loire-Tal (France)	Production of aromatic white wine	Oenobrands (Montpellier, France)
Viniflora® Melody™	*S. cerevisiae*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*	Europe	Production of red and white wines	Chr. Hansen A/S (Hoersholm, Denmark)
Vin-O-Ferm® Roses	*S. cerevisiae* spp. *Cerevisiae*	Europe	Production of rose wines	OenoBioTech SAS (Chanteloup en Brie, France)
Oenoferm® x-treme F3	Hybrid yeast of two strains: *S. cerevisiae*, *S. cerevisiae* var. *bayanus*	Europe	Production of aromatic wines	Erbslöh (Geisenheim, Germany)
Oenoferm® x-thiol F3	Hybrid yeast of two strains: *S. cerevisiae var. bayanus*	Europe	Production of aromatic white wines and roses	Erbslöh (Geisenheim, Germany)

2.2. Apple juice and fermentation condition

Concentrated apple juice of 70°Brix was purchased from the INNIGHT Company (Budapest, Hungary). It was diluted with tap water to 17.2°Brix, then the pH was adjusted to around pH 3.0 by 3 N phosphoric acid solution.

Fermentation processes were carried out separately in 500 mL Erlenmeyer conical flasks. Each flask contained 300 mL apple juice and 2 % (v/v) pre-cultured and activated yeast strain. After inoculation with relevant yeast, the flasks were mounted by twin bubble airlocks to exclude the air and provide facultative anaerobic condition. The fermentations were conducted statically at 20 °C for 8 days in triplicate, and sampling was carried out daily.

2.3. Measurement of pH and Brix

pH and °Brix values were monitored daily by using a pH meter (Mettler Toledo, Greifensee, Switzerland) and a refractometer (ATAGO, Tokyo, Japan).

2.4. Analysis of alcohol content

Two different methods were used to determine the alcohol content of samples. In the case of metabolism analysis, samples were collected every day to analyse the ethanol content by the high performance liquid chromatography system (HPLC). In other case, the alcohol concentration of fermented mashes was determined by distilling and measuring the density of the distillate. Briefly, 100 mL of the fermented mash was taken out from each fermentation flask; and one drop of silicone oil (Antifoam B Emulsion, Sigma–Aldrich, St. Louis, MO, USA) was added to prevent the mash-foaming during the distillation. The mash was distilled by using a steam injection distillation unit (Büchi K-350, Flawil, Switzerland) for 3 min. The distillate was collected into a 100 mL volumetric flask and diluted to the mark with distilled water. The alcohol content was measured by a digital density meter (Anton Paar DMA 35N, Graz, Austria).

2.5. Analysis of sugars, alcohol and organic acids by HPLC

Samples were centrifuged at a speed of 9168 ×*g* at room temperature for 10 min before the analysis process. The HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with Agilent Hi-Plex H column 7.7 × 300 mm (Agilent Technologies, CA, USA) was used with the following parameters: mobile phase 5 mM H_2SO_4 solution, the flow rate 0.6 mL/min, injected volume 10 μL, the temperature of the column 45 °C, isochromatic elution for 25 min. The sugars, alcohol and organic acids were detected by Refractor Index (RI) and Photodiode Array (PDA) detectors. Standards of sugars (glucose, fructose, sucrose, maltose), alcohol (methanol, ethanol, propanol and butanol) and organic acids (malic, citric, acetic acid, lactic acid, succinic acid, glutaric acid) were used to identify and quantify the components in the samples that were HPLC grade of purity. Internal standards with glucose, fructose, ethanol, acetic and lactic acids were also prepared and injected.

2.6. Analysis of volatile compounds by gas chromatography with flame ionization detection

Analyses of the volatile compounds were carried out using GC-FID (Perichrom 2100, AL-PHA MOS, Toulouse, France). The compounds were separated by CHROMPACK CP-WAX 57CB Wcot (Agilent, Santa Clara, CA, USA) fused silica column (polyethylene glycol stationary phase, 50 m × 0.25 mm i.d. with 0.25 μm film thickness). The temperature program of the oven was as follows: initial 60 °C (isotherm for 6 min), ramp rate (6 °C/min to 83 °C and afterward to 220 °C at a rate of 10 °C/min), temperatures of injector and detector were 210 °C and 220 °C, respectively. The carrier gas was helium at 3 mL/min. 1 μL samples were injected twice with an automatic injecting system. The identification of volatile compounds was made by comparing the retention times of the samples with those of standard compounds injected at the same conditions. The standards of volatile compounds consist of methanol, ethanol, acetaldehyde, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethanol, ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate. Two internal standards with 2-methyl-1-propanol and n-heptanol were also prepared and injected.

2.7. Statistical analysis

The results were expressed as the mean values ± standard deviations. R-studio and R software with version 4.0.0 were used for principal component analysis (PCA), hierarchical cluster analysis (HCA), one-way analysis of variance (ANOVA) and Tukey's test with significant level 5 % (α = 0.05).

3. Results and discussions

3.1. Changes of pH and organic acids during fermentation

The pH increased from pH 3.01 to around pH 3.25 (Fig. 1A) during the fermentation of apple mashes. There was a significant difference in

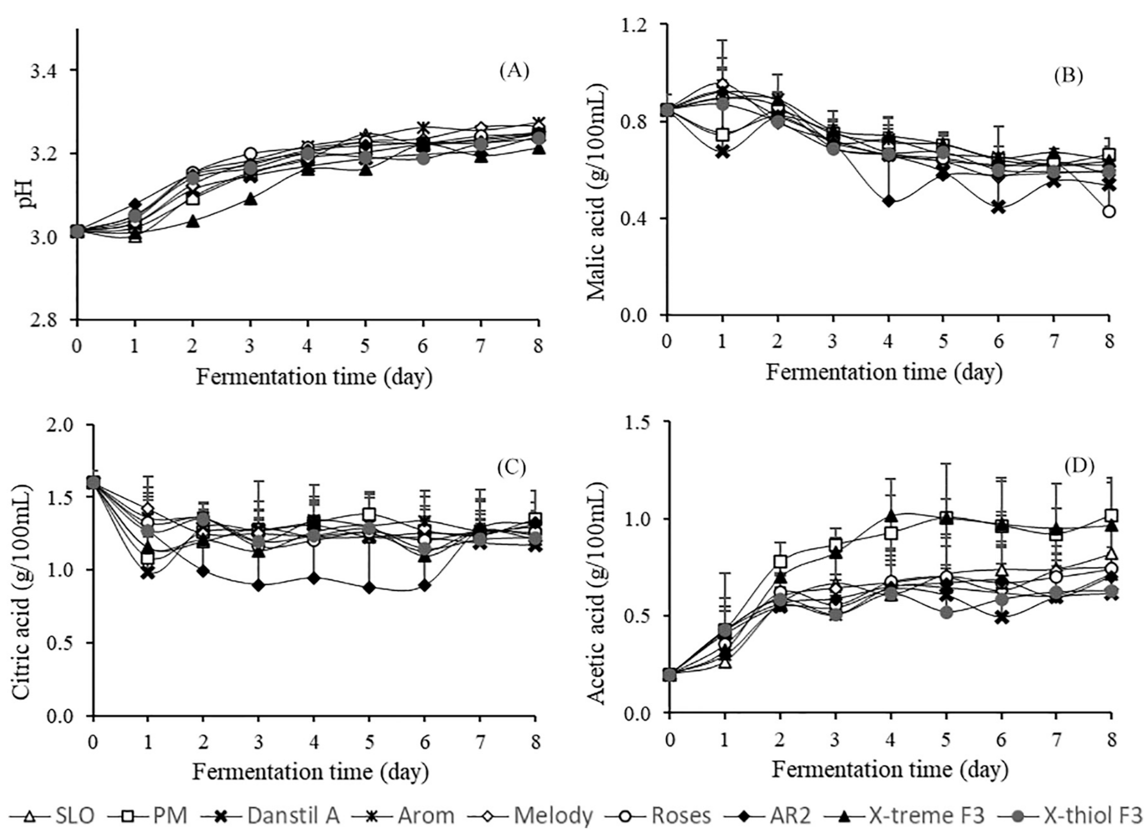

Fig. 1. Changes of pH (A), malic acid (B), citric acid (C) and acetic acid (D) from fermented juices by different yeast strains.

the pH of apple mashes between the initial and the end of fermentation; however, no statistical difference in pH was detected between samples from different yeast strains (Table 2). Similar results were also reported (Chen and Liu, 2014). The increase in pH can be explained by the biosynthesis of organic acids during alcoholic fermentation (Whiting, 1976). Additionally, the metabolic activity of yeast can change the concentration of some acids in raw materials, and thus contribute the increase in pH of mash. Apple contains various organic acid compounds, especially malic acid (Wu et al., 2007). The concentration of malic acid in initial apple juices was 0.85 g/100 mL, and it reduced during fermentation, except samples fermented with Uvaferm SLO, Uvaferm PM and Oenoferm x-treme F3 (Fig. 1B). No significant difference in malic acid content was found among fermented juices (Table 2). Although there was a reduction of malic acid content by different strains, thus it could not be attributed to malolactic fermentation. This could be explained by the low affinity to malic acid substrate of enzyme in *Saccharomyces cerevisiae*, as well as the absence of an active transport system for L-malic acid. Both factors affected the low metabolic efficiency of L-malic acid in yeast cells. Therefore, this reduction was also due to the passive diffusion of L-malic acid to cells of yeast strains (Coloretti et al., 2002). Our results have been in agreement with earlier studies, such as mango wines fermented with different *Saccharomyces cerevisiae* strains (Li et al., 2011) and lychee wines fermented with four commercial yeast strains (Chen and Liu, 2014).

Some previous reports (Berenguer et al., 2016; Chen and Liu, 2014) showed that citric acid content increased during alcoholic fermentation, but the reverse trend was also found (Lee et al., 2012). In our study, citric acid in fermented mashes by strains Fermiblanc Arom, Oenoferm x-treme F3 and Oenoferm x-thiol F3 decreased significantly from 1.60 g/100 mL to 1.30 g/100 mL, 1.21 g/100 mL and 1.22 g/100 mL, respectively. However, it remained steady for other strains (Table 2 and Fig. 1C). A similar trend of citric acid was also found as mango juice fermented by strains of EC1118 and CICC1028 (Li et al., 2011).

Acetic acid content of apple mashes increased during fermentation (Fig. 1D). The concentration of acetic acid reached the highest value in the case of samples fermented with Uvaferm PM (1.02 g/100 mL) and Oenoferm x-treme F3 (0.97 g/100 mL), which differed significantly from the initial samples. The rise of acetic acid content is related to the fermentation and subsequent oxidation of ethanol and acetaldehyde (Satora and Tuszyński, 2010). In addition, acetic acid at high concentrations is undesirable in alcoholic beverages, which may impart a vinegar off-flavor when close to its flavor threshold (200 mg/L) (Li et al., 2011). All acetic acid values of fermented juices were much lower than the threshold level, thus, these mashes did not have an unfavorable flavor note caused by this acid.

3.2. Changes of total soluble solids and reducing sugars

The total soluble solids in juices inoculated with the nine yeast strains reduced rapidly from 17.2 °Brix to 6.0 °Brix after 8 days of fermentation (Fig. 2A). The results indicated a difference in Brix of apple mashes between the initial and the end of fermentation, but no difference in Brix was found among samples fermented with nine different strains. The reduction in Brix is related to the metabolic activity of *Saccharomyces cerevisiae* when it uses sugar for both growth and fermentation to produce ethanol, carbon dioxide and other products (Lambrechts and Pretorius, 2000). The strongest metabolism occurred in the first 4 days, then gradually dropped over the next day and stabilized until the 8th day. In the cases of strains Uvaferm Danstil A and Fermicru AR2, total soluble solid content of fermented juices reduced very speedily compared to other ones.

Glucose and fructose were simple reducing sugars in the initial apple juices and they represented nearly 90 % of total reducing sugars (Table 2). Fructose content was two times higher than glucose content (8.87 g/100 mL and 3.74 g/100 mL). The results were in agreement with ones reported earlier (Wu et al., 2007). The concentration of glucose and fructose decreased rapidly to the minimum levels on the 3rd day, remaining more or less stable until the end of the fermentation process with a final value ranging from 0.22 g/100 mL to 0.27 g/100 mL and from 0.41 g/100 mL to 0.46 g/100 mL, respectively (Fig. 2B, C). In the case of sucrose, a similar trend was also found. Minimum levels reached on the 3rd day, except for sample fermented by Oenoferm x-thiol F3

Table 2
Physicochemical properties, organic acids, reducing sugars concentrations and alcohol content of mashes before and after fermentation.

Parameter	Mash (day 0)	Fermented mash (day 8)								
		SLO	PM	Danstil A	Arom	Melody	Roses	AR2	X-treme F3	X-thiol F3
pH	3.01 ± 0.00^a	3.25 ± 0.03^b	3.25 ± 0.01^b	3.24 ± 0.03^b	3.27 ± 0.01^b	3.27 ± 0.01^b	3.25 ± 0.02^b	3.25 ± 0.01^b	3.21 ± 0.04^b	3.24 ± 0.02^b
Brix	17.20 ± 0.00^a	6.00 ± 0.00^b	6.07 ± 0.06^b	6.00 ± 0.00^b	6.00 ± 0.00^b	6.00 ± 0.00^b	6.00 ± 0.00^b	6.00 ± 0.00^b	6.00 ± 0.00^b	6.00 ± 0.00^b
Organic acids (g/100 mL)										
Acetic acid	0.20 ± 0.02^b	0.82 ± 0.23^{ab}	1.02 ± 0.20^a	0.62 ± 0.23^{ab}	0.71 ± 0.30^{ab}	0.75 ± 0.28^{ab}	0.74 ± 0.20^{ab}	0.70 ± 0.30^{ab}	0.97 ± 0.22^a	0.63 ± 0.19^{ab}
Citric acid	1.60 ± 0.01^a	1.32 ± 0.06^{ab}	1.35 ± 0.19^{ab}	1.17 ± 0.06^{ab}	1.30 ± 0.08^b	1.24 ± 0.22^{ab}	1.26 ± 0.03^{ab}	1.32 ± 0.03^{ab}	1.21 ± 0.17^b	1.22 ± 0.18^b
Malic acid	0.85 ± 0.06^a	0.64 ± 0.04^{ab}	0.66 ± 0.03^{ab}	0.54 ± 0.11^b	0.62 ± 0.02^b	0.58 ± 0.08^b	0.53 ± 0.30^b	0.60 ± 0.10^b	0.64 ± 0.01^{ab}	0.59 ± 0.08^b
Reducing sugars (g/100 mL)										
Glucose	3.74 ± 0.44^a	0.24 ± 0.07^b	0.24 ± 0.06^b	0.32 ± 0.11^b	0.23 ± 0.06^b	0.23 ± 0.07^b	0.23 ± 0.07^b	0.27 ± 0.01^b	0.22 ± 0.06^b	0.25 ± 0.05^b
Fructose	8.87 ± 1.15^a	0.41 ± 0.04^b	0.42 ± 0.04^b	0.44 ± 0.04^b	0.42 ± 0.03^b	0.40 ± 0.04^b	0.41 ± 0.04^b	0.45 ± 0.04^b	0.41 ± 0.01^b	0.46 ± 0.02^b
Sucrose	0.94 ± 0.18^a	0.05 ± 0.00^b	0.05 ± 0.00^b	0.04 ± 0.02^b	0.05 ± 0.00^b	0.04 ± 0.01^b	0.05 ± 0.01^b	0.05 ± 0.01^b	0.05 ± 0.00^b	0.06 ± 0.01^b
Sugar's consumption (%)	n.a.	94.76 ± 1.37^a	94.61 ± 1.25^a	94.14 ± 0.30^a	94.76 ± 1.04^a	94.91 ± 1.32^a	94.75 ± 1.55^a	94.20 ± 0.62^a	94.90 ± 1.20^a	94.20 ± 1.21^a
Alcohol content % (v/v) and yield % (v/v) alcohol/ % total reducing sugar										
Alcohol	n.a.	9.33 ± 0.12^a	9.30 ± 0.10^a	9.43 ± 0.06^a	9.43 ± 0.15^a	9.37 ± 0.06^a	9.20 ± 0.26^a	9.33 ± 0.12^a	9.17 ± 0.06^a	9.37 ± 0.06^a
Yield	n.a.	0.69 ± 0.01^a	0.69 ± 0.01^a	0.70 ± 0.00^a	0.70 ± 0.01^a	0.69 ± 0.00^a	0.68 ± 0.02^a	0.69 ± 0.01^a	0.68 ± 0.00^a	0.69 ± 0.00^a

Key: SLO: Uvaferm SLO, PM: Uvaferm PM, Danstil A: Uvaferm Danstil A, Arom: Fermiblanc Arom, Melody: Viniflora Melody, Roses: Vin-O-Ferm Roses, AR2: Fermicru AR2, X-treme F3: Oenoferm x-treme F3, X-thiol F3: Oenoferm x-thiol F3. Different superscript letters (a, b) in the same row show significant difference according to the Tukey test (p-value <0.05), and "n.a.": not analyzed.

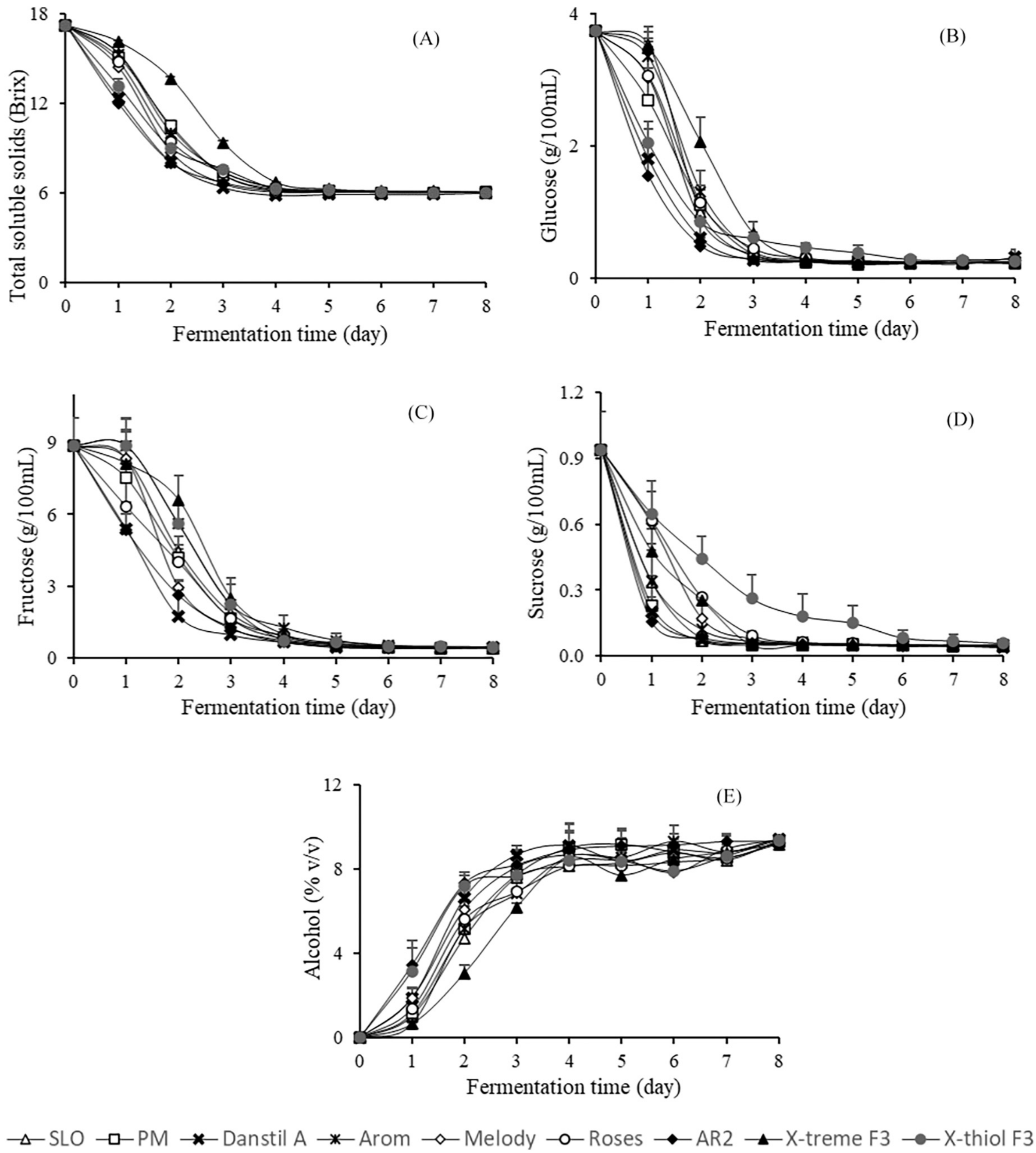

Fig. 2. Changes of total soluble solids (A), glucose (B), fructose (C), sucrose (D) and alcohol (E) from fermented juices by different yeast strains.

(Fig. 2C). The yeast cell exhibited slightly higher preference to glucose than to fructose. It resulted a preponderance of fructose during the later phases of fermentation. Therefore, any yeast with high capability of fructose consumption under stress conditions would potentially be applied in the fermentation industry (Berthels et al., 2004; Tronchoni et al., 2009). All nine yeast strains could consume both glucose and fructose very well. >95 % reducing sugars were utilized during fermentation. As shown in Fig. 2B, C and D, strains Uvaferm Danstil A and Fermicru AR2 consumed reducing sugars more rapidly than other strains. They take this process for only 3 days compared for 4 days in the cases of other strains.

Generally, the amount of sugar consumed is in the correlation with the alcohol produced. Therefore, sugar consumption was proportional to the ability of alcohol production (Fig. 2E). The alcohol formation took place strongly in the first 3 days; then its rate steadily declined on the 4th day. After that, the alcohol content was almost stable until the end of the fermentation (8th day). The results showed that alcohol yield was 0.69 after 8 days of fermentation. Rita et al. (2011) used the commercial *Saccharomyces bayanus* yeast preparation for fermentation of apple juice and after 28 days they got only 0.53 production yield of alcohol. In other reported Kanwar (2016) got much lower yield (0.28) after fermentation of apple juices with six *Saccharomyces cerevisiae* strains for 15 days. Better results (0.51 yield after 30 days) were obtained by Satora and Tuszyński (2010) when they studied the fermentation of plum juice with *Aureobasidium* sp., *Kluyveromyces apiculata* and *S. cerevisiae* K1. In comparison, our result was significantly higher than the results reported so far by other groups. It is also worth to note that our result was obtained after fermentation for only 8 days. Although no significant difference in capacities of alcohol production was detected between the investigated yeast strains. Our results confirm that all commercial yeast strains were highly suitable for alcoholic fermentation of apple juice.

3.3. Changes of volatile compounds

Pálinka has been distinguished by characteristic volatile compounds came from the fruit, volatile compounds generated during fermentation and distillates' maturation. *Saccharomyces cerevisiae* can produce a broad range of aroma compounds, the vital complex flavors of fermented beverages. Table 3 shows the volatile compounds of the fermented apple juice by nine different yeast strains. These compounds differed in concentration, leading to different aromatic profile of the fermented mashes.

Methanol is produced by the enzymatic hydrolysis of pectin, a common component of fruit. A high methanol content has been acknowledged as a characteristic of wines, and red wines direct to accommodate more methanol (120 mg/L–250 mg/L) than white wines (40 mg/L–120 mg/L) (Hodson et al., 2017). It may contribute a mild, bland odor and un-influence on the flavor of beverage drinks because of its very high threshold (10,000 mg/L) (Miller, 2019). However, it is one of the most important elements to observe and manage due to its risks affecting the health of customers (Christoph and Bauer-Christoph, 2007). Methanol concentration in fermented apple mash ranged from 63.70 mg/L to 105.99 mg/L. However, no difference in its content was recorded between samples by different strains meaning the commercial yeast strains do not express any pectin-methyl esterase activities.

Acetaldehyde is a vital carbonyl compound found in alcoholic beverages with a pleasant fruity odor at low concentrations, but at a higher value, it give a pungent, irritating odor taste (Li et al., 2011). It is a metabolic byproduct regarding alcoholic fermentation and also product of chemical and enzymatic oxidation of ethanol (Lachenmeier and Sohnius, 2008; Liu and Pilone, 2000). The concentration of acetaldehyde ranged from 3.86 mg/L to 31.07 mg/L, and the lowest acetaldehyde content was recorded in the sample by Viniflora Melody. Our results are in the acetaldehyde levels (0.5 mg/L–286 mg/L) produced by *Saccharomyces cerevisiae* (Liu and Pilone, 2000).

Fusel alcohols with a pleasant odor play an essential role in the aroma profile of fruit spirit. However, they may negatively affect a strong, pungent flavor and taste (Spaho, 2017). During the fermentation, higher alcohols are produced through the conversion of branched-chain amino acids. For instance, 3-methyl-1-butanol, 2-methyl-1-butanol and 2-methyl-1-propanol can be created by the Ehrlich pathway from the catabolism of the respective L-isoleucine, L-leucine and L-valine (Blombach and Eikmanns, 2011). Our results demonstrated that total of 8 higher alcohols were identified from the samples fermented with different yeast strains, principally including isoamyl alcohols such as 3-methyl-1-butanol and 2-methyl-1-butanol. The 3-methyl-1-butanol

Table 3
Volatile compounds of fermented apple mashes with different yeast strains.

Code	Volatile compound (mg/L)	SLO	PM	Danstil A	Arom	Melody	Roses	AR2	X-treme F3	X-thiol F3
M	Methanol	93.88 ± 9.72^{a}	100.31 ± 14.23^{a}	100.80 ± 9.67^{a}	90.54 ± 9.10^{a}	105.99 ± 9.37^{a}	83.09 ± 21.27^{a}	69.79 ± 21.66^{a}	98.83 ± 20.98^{a}	63.70 ± 21.60^{a}
A	Acetaldehyde	23.24 ± 6.93^{ab}	14.08 ± 7.00^{abc}	20.39 ± 6.19^{abc}	20.48 ± 8.36^{abc}	3.86 ± 3.85^{c}	7.02 ± 4.16^{bc}	31.07 ± 9.73^{a}	18.09 ± 2.91^{abc}	7.17 ± 2.94^{bc}
Higher alcohols										
H1	1-Propanol	15.72 ± 0.85^{cd}	25.35 ± 0.69^{abc}	13.50 ± 2.56^{c}	17.26 ± 2.52^{bcd}	16.19 ± 2.40^{cd}	16.55 ± 2.31^{cd}	26.63 ± 4.74^{ab}	29.31 ± 4.51^{a}	20.15 ± 6.04^{abcd}
H2	2-Propanol	1.92 ± 0.74^{d}	15.68 ± 1.84^{b}	7.10 ± 2.49^{cd}	30.41 ± 4.66^{a}	11.28 ± 0.46^{cb}	7.63 ± 2.07^{cd}	32.35 ± 2.61^{a}	9.58 ± 1.61^{cb}	8.90 ± 2.45^{cb}
H3	1-Butanol	0.11 ± 0.08^{a}	0.02 ± 0.03^{a}	0.12 ± 0.16^{a}	0.12 ± 0.07^{a}	0.15 ± 0.03^{a}	0.16 ± 0.21^{a}	0.26 ± 0.17^{a}	0.09 ± 0.16^{a}	0.01 ± 0.02^{a}
H4	2-Butanol	nd.	0.32 ± 0.08^{a}	0.33 ± 0.44^{a}	0.18 ± 0.29^{a}	0.47 ± 0.82^{a}	0.26 ± 0.17^{a}	0.82 ± 1.04^{a}	0.48 ± 0.51^{a}	0.35 ± 0.57^{a}
H5	2-Methyl-1-propanol	32.88 ± 3.40^{abcd}	24.14 ± 2.59^{d}	38.06 ± 2.74^{ab}	39.28 ± 4.15^{a}	31.17 ± 1.80^{abcd}	29.58 ± 3.27^{bcd}	34.78 ± 0.65^{abc}	23.75 ± 1.68^{d}	26.05 ± 6.35^{cd}
H6	2-Methyl-1-butanol	26.11 ± 2.32^{b}	20.52 ± 2.29^{b}	38.40 ± 2.30^{a}	28.09 ± 2.29^{b}	24.63 ± 2.95^{b}	29.03 ± 2.14^{ab}	27.35 ± 4.58^{b}	25.41 ± 4.33^{b}	22.71 ± 4.78^{b}
H7	3-Methyl-1-butanol	128.88 ± 9.85^{a}	93.45 ± 9.38^{c}	141.89 ± 6.52^{a}	134.04 ± 2.66^{a}	123.66 ± 2.26^{ab}	137.91 ± 1.82^{a}	124.41 ± 10.13^{ab}	103.84 ± 6.07^{bc}	91.92 ± 9.94^{c}
H8	2-Phenylethanol	0.01 ± 0.01^{a}	nd.	nd.	nd.	0.01 ± 0.01^{a}	nd.	0.01 ± 0.02^{a}	nd.	nd.
TH	Total higher alcohol	205.62 ± 12.79^{bcd}	179.48 ± 11.81^{de}	239.41 ± 1.23^{ab}	249.37 ± 15.63^{a}	207.56 ± 1.13^{bcd}	221.14 ± 3.07^{abc}	246.61 ± 6.30^{a}	192.46 ± 8.52^{cde}	170.10 ± 25.30^{e}
Esters										
E1	Ethyl acetate	20.40 ± 2.12^{b}	30.99 ± 2.51^{a}	30.95 ± 3.72^{a}	33.27 ± 3.81^{a}	32.66 ± 2.98^{a}	31.29 ± 2.70^{a}	30.89 ± 2.54^{a}	28.05 ± 4.53^{ab}	28.08 ± 2.17^{ab}
E2	Ethyl formate	nd.	1.74 ± 0.27^{a}	1.24 ± 0.48^{a}	nd.	0.22 ± 0.15^{b}	0.22 ± 0.15^{b}	nd.	0.46 ± 0.10^{b}	nd.
E3	Ethyl lactate	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.04^{a}	nd.	nd.	nd.	nd.	nd.	nd.
E4	Ethyl hexanoate	0.35 ± 0.11^{a}	0.55 ± 0.16^{a}	0.43 ± 0.14^{a}	0.74 ± 1.27^{a}	0.96 ± 1.66^{a}	0.30 ± 0.12^{a}	0.02 ± 0.04^{a}	0.29 ± 0.30^{a}	0.63 ± 0.84^{a}
E5	Butyl acetate	nd.	nd.	nd.	0.11 ± 0.01^{a}	0.01 ± 0.02^{a}	0.02 ± 0.03^{a}	nd.	0.03 ± 0.06^{a}	nd.
E6	Propyl acetate	nd.	nd.	0.03 ± 0.05^{a}	0.01 ± 0.01^{a}	nd.	nd.	nd.	nd.	nd.
E7	Isoamyl acetate	0.41 ± 0.16^{a}	0.29 ± 0.17^{a}	1.05 ± 1.09^{a}	0.68 ± 0.69^{a}	1.16 ± 0.11^{a}	0.85 ± 0.76^{a}	0.43 ± 0.25^{a}	0.46 ± 0.29^{a}	0.18 ± 0.16^{a}
TE	Total ester	21.17 ± 2.04^{b}	33.58 ± 2.31^{a}	33.72 ± 3.59^{a}	34.80 ± 1.88^{a}	35.01 ± 1.82^{a}	32.70 ± 1.65^{a}	31.35 ± 2.70^{a}	29.30 ± 4.09^{a}	28.89 ± 2.06^{a}
T	Total volatile compound	226.79 ± 10.86^{cde}	213.05 ± 13.77^{de}	273.13 ± 3.88^{ab}	284.17 ± 14.01^{a}	242.58 ± 1.88^{bcd}	253.83 ± 4.69^{abc}	277.96 ± 7.67^{ab}	221.76 ± 12.60^{cde}	198.99 ± 26.70^{e}

Key: SLO: Uvaferm SLO, PM: Uvaferm PM, Danstil A: Uvaferm Danstil A, Arom: Fermiblanc Arom, Melody: Viniflora Melody, Roses: Vin-O-Ferm Roses, AR2: Fermicru AR2, X-treme F3: Oenoferm x-treme F3, X-thiol F3: Oenoferm x-thiol F3. Different superscript letters (a, b) in the same row show significant difference according to the Tukey test (p-value <0.05), and "nd.": not detected.

generally contributes the alcoholic, banana-like, sweetish and malty flavor for spirits (Miller, 2019). The concentration of this compound fluctuated in the range of 91.92 mg/L–141.89 mg/L. The largest quantities were found in the samples fermented with Uvaferm SLO, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses and Fermicru AR2. In the case of Uvaferm Danstil A, the highest value (38.40 mg/L) of 2-methyl-1-butanol was recorded that gives alcoholic, malty and fruity odor (Miller, 2019). In the cases of other isoamyl alcohols, the concentration of 2-methyl-1-propanol and 1-propanol reached 23.75 mg/L–39.28 mg/L and 13.50 mg/L–29.31 mg/L, respectively. The highest concentration of 1-propanol was observed in the samples of Uvaferm PM, Fermicru AR2 and Oenoferm x-treme F3, and the lowest values in the samples of Uvaferm SLO, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody and Vin-O-Ferm Roses. Meanwhile the 2-methyl-1-propanol assigns the malty, ethanolic odor, whereas the pleasant, alcoholic and ripe fruit odor is generally sensored in the presence of 1-propanol (Miller, 2019). It worth to note that relatively high amounts of 1-propanol were detected in all samples. The quantities of the other fusel alcohols were significantly lower in the fermented apple juices. The sum of 2-propanol, 1-butanol, 2-butanol and 2-phenylethanol was accounted for <10 % of the total higher alcohols, except from the samples fermented by Fermiblanc Arom and Fermicru AR2 yeast strains. The highest amounts of 2-propanol were found in the samples of Fermiblanc Arom (30.41 mg/L) and Fermicru AR2 (32.35 mg/L), while the lowest amount was detected in sample fermented by Uvaferm SLO (1.92 mg/L). Meanwhile the concentration of 1-butanol varied from 0.01 mg/L to 0.26 mg/L, whereas value of 2-butanol was in a range of 0.18 mg/L - 0.82 mg/L. However, the 2-butanol was not detected in the Uvaferm SLO sample. Very low concentration of 2-phenylethanol was detected only in some fermented juices such as those by Uvaferm SLO, Viniflora Melody and Fermicru AR2. Generally, the 2-phenylethanol positively affects the flavor of alcoholic beverages and is originated from *L*-phenylalanine amino acid via the metabolic reaction of yeast during carbonic anaerobiosis (Tešević et al., 2009; Kovács et al., 2017). Overall, the largest contents of total higher alcohols were measured in the samples fermented by Uvaferm Danstil A (239.41 mg/L), Fermiblanc Arom (249.37 mg/L), Vin-O-Ferm Roses (221.14 mg/L) and Fermicru AR2 (246.61 mg/L). Still, the smallest amounts were observed in Uvaferm PM (179.48 mg/L), Oenoferm x-treme F3 (192.46 mg/L) and Oenoferm x-thiol F3 (170.10 mg/L) samples. Remarkable that these levels of fusel alcohols are still acceptable and sensorially assigned as pleasant characteristic flavor in pálinkas.

During alcoholic fermentation, esters are formed mainly via the esterification of alcohols with fatty acids, in which ethyl esters factor the largest group in the number and concentration of aroma components found (Lambrechts and Pretorius, 2000). The most abundant ester in the fermented apple juices was ethyl acetate (Table 3). Similar result was also found in the study by Chen and Liu (2014). Low concentrations of ethyl acetate give an ethereal, fruity, sweetish taste which may mash the harsh flavor in alcoholic beverages. However, it contributes to the vinegar odor at high amounts, a negative flavor for spirits (Miller, 2019; Nsengumuremyi et al., 2019). Our results showed that ethyl acetate ranged from 20.40 mg/L to 33.27 mg/L, and the lowest concentration was reported in sample by Uvaferm SLO. Ethyl formate supplies a fruity flavor for spirits (Miller, 2019). The highest content of this compound was observed in the sample of Uvaferm PM (30.99 mg/L) and Uvaferm Danstil A (30.95 mg/L). However, it was not detected in samples fermented by strains of Uvaferm SLO, Fermiblanc Arom, Fermicru AR2 and Oenoferm x-thiol F3. Ethyl hexanoate expresses apple, fruity and sweetish aroma, and isoamyl acetate performs banana and apple odor (Miller, 2019). Their presence contributed positive influences on the flavor of spirits (Tešević et al., 2009). Meanwhile, concentration of ethyl hexanoate ranged from 0.02 mg/L to 0.96 mg/L, whereas the concentration of isoamyl acetate increased from 0.18 mg/L to 1.16 mg/L. Still, there was no significant difference in these concentrations between the samples. Ethyl lactate has artificial strawberry, raspberry odor; butyl acetate produces a banana, sweet, fruity aroma, and propyl acetate supports a sweetish, perfumed flavor (Miller, 2019). They were present in very low concentrations in the fermented apple juices leading to their appearance among these samples being very different. Specifically, ethyl lactate was detected in Uvaferm SLO, Uvaferm PM and Uvaferm Danstil A samples, whereas butyl acetate presented in samples fermented with Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses and Oenoferm x-treme F3, as well as propyl acetate was only found in samples of Uvaferm Danstil A and Fermiblanc Arom. The total esters in samples varied from 21.17 mg/L to 35.01 mg/L, in which the lowest content was recorded in Uvaferm SLO sample. In general, total volatile compounds ranged from 198.99 mg/L to 284.17 mg/L. The highest concentrations of total volatile compounds were recorded in samples by Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2, whereas the lowest contents were found in samples by strains Uvaferm SLO, Uvaferm PM, Oenoferm x-treme F3 and Oenoferm x-thiol F3.

3.4. Principal component analysis

Volatile compounds with differed most between the strains were applied for principal component analysis and hierarchical cluster analysis, including acetaldehyde (A), 1-propanol (H1), 2-propanol (H2), 2-methyl-1-propanol (H5), 2-methyl-1-butanol (H6), 3-methyl-1-butanol (H7), total higher alcohol (TH), ethyl acetate (E1), ethyl formate (E2), total ester (TE) and total volatile compounds (T). The results from PCA (principal component analysis) explained 73.9 % of the variability of the primary compounds in two components of PC1 (49.8 %) and PC2 (24.1 %). Aroma compounds enormously contributing to PC1–2 (>10 % contribution) consisted of total volatile compounds, 2-methyl-1-propanol, 3-methyl-1-butanol, total higher alcohols, ethyl acetate and total esters. It suggested that these variables were suitable for describing fermented apple juices' profile.

In Fig. 3A, the data points of yeast strains were distributed separately, which showed a significant difference in volatile compounds between tested samples produced by different yeasts. Besides, it also showed that samples fermented with strains Viniflora Melody, Vin-O-Ferm Roses, and Uvaferm Danstil A were represented by various volatile compounds, especially Uvaferm Danstil A sample with more variety and higher levels of aromas. Meanwhile ethyl acetate, 2-propanol and total esters were described for sample by strain Viniflora Melody, whereas 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were expressed in tested samples by strain Vin-O-Ferm Roses. Total higher alcohols, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and total volatile compounds were characterized for fermented juices by strain Uvaferm Danstil A.

In Fig. 3B, the result of hierarchical cluster analysis (HCA) identified groups of samples with similar characteristics. Fermented juices from nine commercial yeast strains could be divided into four groups, namely group 1 of Uvaferm PM, Oenoferm x-treme F3, Oenoferm x-thiol F3, group 2 of Fermiblanc Arom, Fermicru AR2, group 3 of only Uvaferm SLO, and group 4 of Viniflora Melody, Vin-O-Ferm Roses, Uvaferm Danstil A. The same statements were also found in the sample of group 3. Juices fermented by strains Viniflora Melody, Vin-O-Ferm Roses, and Uvaferm Danstil A had similar features due to being characterized by many other volatile compounds. In addition, fermented juices made by strains Viniflora Melody and Vin-O-Ferm Roses were different from samples by strain Uvaferm Danstil A. It is probably caused by higher concentrations of volatile compounds produced strain Uvaferm Danstil A.

4. Conclusions

The fermentation rate, alcohol yield, components and concentrations of volatile compounds during apple juice fermentation depends on the commercial yeast strains. *Saccharomyces cerevisiae* strains of Uvaferm Danstil A and Fermicru AR2 exhibited stronger fermentation ability

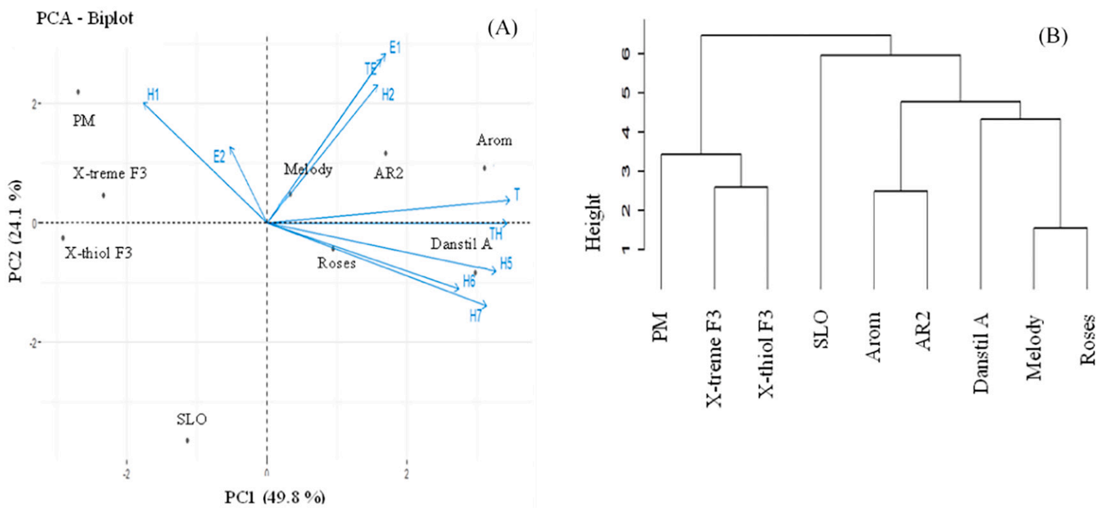

Fig. 3. Principal component analysis (A) and hierarchical cluster analysis (B).

through the conversion rate of sugar to alcohol and shorter fermentation time. Although there was no significant difference in ethanol production capacity of different yeast strains. The highest concentrations of total volatile compounds were observed in samples fermented by Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2. In addition, the total volatile compounds, 2-methyl-1-propanol, 3-methyl-1-butanol, total higher alcohols, ethyl acetate and total esters were key parameters for describing the profile of fermented apple juices. Moreover, the distribution of samples by these yeast strains showed that volatile compounds described clearly the characteristics of tested samples by strains Viniflora Melody, Vin-O-Ferm Roses and Uvaferm Danstil A, especially by Uvaferm Danstil A with higher levels of total higher alcohols, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and total volatile compounds. In summary, the strain Uvaferm Danstil A had strongest fermentation ability and produced much more fruity aromas than other strains, thus, it could be selected as a good candidate organism for pálinka production. Additionally, our work also provided the basic information for selecting suitable yeast strains applied in the spirit industry to enhance the ethanol yield and flavor of pálinka.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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