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How Different Fermentation Type Affects Volatile Composition of Plum Jerkums

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Abstract: Quality of plum jerkum is significantly associated to the profile of volatile compounds. Therefore, we decided to assess the impact of various fermentation types on selected properties of plum jerkums, especially compounds which contribute to the aroma of the finished product. We used the following yeast strains: *S. cerevisiae* S1, *H. uvarum* H2, and Ethanol RED (*S. cerevisiae*). Moreover, we considered spontaneous fermentation. *S. cerevisiae* and *H. uvarum* strains were isolated during the fermentation of Čačanska Lepotica or Węgierka Dąbrowicka (plum cultivars), respectively. As for fermentation type, spontaneous fermentation of *H. uvarum* H2 provided the best results. It could be associated to the fact that plum juices fermented with *H. uvarum* H2 presented the highest concentration of terpenoids, esters, or some higher alcohols. In the current paper, application of indigenous strains of yeasts resulted in the required oenological characteristics, e.g., highest fermentation efficiency and concentration of ethanol was determined in juices fermented with Ethanol RED (*S. cerevisiae*) and also with *S. cerevisiae* S1. Our results suggested that indigenous strains of yeasts present in plums demonstrate great potential for the production of plum jerkums of high quality.

Keywords: plum jerkums; *Saccharomyces cerevisiae*; *Hanseniaspora uvarum*; volatile compounds

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1. Introduction

Oenological parameters, volatile compounds, and overall quality of fruit wines depend on the impact of various factors, such as quality of raw materials, mainly their chemical composition, primary processing techniques, fermentation conditions, further vinification procedures, and possibly aging. Volatile compounds in alcoholic beverages produced with various fruits have a very diverse origin. Some of them naturally occur in fruits and could be exposed to various transformations carried out by microorganisms during fermentation or aging and oxidation. Other aroma compounds are produced by microorganisms, e.g., esters, volatile acids, higher alcohols, carbonyl compounds, and some terpenoids. However, chemical composition of fruits is a key factor to fruit wine quality [1–3].

Plum (*Prunus domestica*) could be used for winemaking or for cider-like alcoholic beverages—plum jerkum is produced in Central England. Plum wine, which is particularly popular in Germany and Pacific Coastal states, has an appealing color and is high quality. Plum could be tart and sweet at the same time which originates from the concentration of sugars and acids. The skin itself might be particularly tart [3].

Fresh plums contain, e.g., ethyl acetate, 2-methyl-l-propyl acetate, propyl acetate, 3-methylbutyl acetate, butyl acetate, pentyl acetate, 3-hexenyl acetate, 1-hexanol, (Z)-3-hexenol, nonanal, linalool, benzaldehyde, γ-octalactone, γ-decalactone, and others which contribute to the aroma of alcoholic beverages [4]. Presence of these substances gives them a fresh, fruity, and floral aroma.

Microorganisms which carry out alcoholic fermentation are either indigenous microorganisms present on fruits or added starter cultures. Initially, in spontaneous fermentation,

musts are dominated by yeast which belong to *Kloeckera*/*Hanseniaspora*, and *Candida* genera. In the middle stages of fermentation when the concentration of ethyl alcohol rises to 3–4%, mainly species of *Pichia* and *Metschnikowia* are present [5]. After that, when ethanol concentration is high, *Saccharomyces* yeast dominates fermentation. However, in the majority of cases, wine producers use starter cultures of *S. cerevisiae*. This approach enables obtaining wine of consistent quality [5]. *Saccharomyces* yeast could produce various volatile compounds—esters, especially ethyl acetate, diethyl succinate, ethyl caproate, ethyl caprylate, methyl anthranilate, aldehydes, ketones, and terpenes, e.g., linalool, linalool oxide, guaiacol, β-ionone, and citral. It also provides high sensory quality of alcoholic beverages [1,6].

H. uvarum could produce increased amounts of volatiles that improve fruit wine quality such as higher alcohols, esters, and carbonyl compounds. Therefore, mixed cultures of *S. cerevisiae* and *H. uvarum* could be used for the production of fruit wine with improved aroma and quality. Among wild yeast, *H. uvarum* strains could synthesize a significant quantity of acetic acid and ethyl acetate which could spoil wine quality when in excess [7].

The current study mostly focuses on assessing the impact of microbial fermentation on chemical composition and the volatile profile of plum jerkums. For the fermentation, spontaneous fermentation, *S. cerevisiae* S1, *H. uvarum* H2, and Ethanol RED (*S. cerevisiae*) were used. *S. cerevisiae* and *H. uvarum* strains were isolated during the fermentation of Čačanska Lepotica or Węgierka Dąbrowicka, respectively. In this study, four cultivars of plums were used: Stanley, Węgierka Zwykła, Węgierka Dąbrowicka, and Čačanska Lepotica. The first three of them are commonly cultivated in Poland and used for processing in the food industry [8], while Čačanska lepotica has great potential for fruit wine production [9].

2. Materials and Methods

2.1. Fermentation of Plum Mashes

Plum musts used for the fermentation were obtained from Węgierka Zwykła, Węgierka Dąbrowicka, Stanley, and Čačanska Lepotica cultivars harvested in Łącko (49°33′30″ N 20°26′06″ E, Małopolska district, Poland). After washing, we manually crushed plums, applied pectynolytic preparation Pektopol PT-400 (0.5 mL/kg) for 12 h at 20 °C, and weighed 2 kg portions which were transferred to 3 L sterile glass flasks. Except for variants designated for spontaneous fermentation, samples were pasteurized at 80 °C for 10 min and inoculated (0.3 g d.w./L of musts) with different types of yeast or fermented spontaneously. We used the *Hanseniaspora uvarum* H2 (MN464119) strain isolated during the second day of fermentation of Węgierka Dąbrowicka fruits; *Saccharomyces cerevisiae* S1 (MN464134) isolated on the 30th day of fermentation of Čačanska Lepotica [10]; and commercial distiller's yeast RED Ethanol (*Saccharomyces cerevisiae*, Starowar, Warsaw, Poland). *H. uvarum* and *S. cerevisiae* were inoculated on slants of Sabouraud agar incubated for 24 h at 28 °C. Then colonies were transferred to 10 mL of Sabouraud broth and incubated for another 24 h at 28 °C. Finally, yeast suspension was transferred to 140 mL of fresh Sabouraud broth and incubated (24 h/28 °C). Yeast suspension was centrifuged (5000 rpm/15 min), supernatant was removed, and the pellet was resuspended in the plum must. We stopped flasks with a plug equipped with the fermentation tube. Then tubes were filled with glycerol. Flasks were stored under a controlled temperature (20 °C). Changes of weight for each flask was recorded each day. Fermentation was carried out until the end of changes in mass loss associated with CO_2 release. When fermentation was complete, samples of fermented musts were collected and stored (−20 °C) for chemical analysis.

2.2. Chemical Composition of Fresh and Fermented Plum Mashes

The concentration of ethanol, reducing sugars, sugar-free extract, total extract, and sucrose concentrations and titratable acidity were determined using officially approved methods described by the International Organization of Vine and Wine (OIV) [11]. Titratable acidity (TA) was calculated from the results obtained with Mettler DL 25 titrator (Mettler Toledo, Greifensee, Switzerland). We used 0.1 M NaOH as a titrant. Samples were

titrated to pH 7.0. Results were shown as grams of malic acid per liter. For the calculation of fermentation efficiency, we applied theoretical correlations which were as follows—1 g of reducing sugars or sucrose could be transformed to 0.511 g or 0.538 g of ethanol, respectively. Ninhydrin method was used for the determination of free amino nitrogen (FAN). Then, 2 mL of diluted (×50) plum musts or jerkums (1 mL) were transferred to caped glass tubes. Then, we added 1 mL of ninhydrin reagent and heated tubes in the boiling water bath for 16 min. After cooling samples, we added 5 mL of dilution reagent. Finally, the absorbance was measured at 575 nm against distilled water with ninhydrin as a blank [12].

2.3. Determination of Sugar and Main Organic Acids Content by High-Performance Liquid Chromatography (HPLC)

Centrifuged (MPW-65R, MPW Med. Instruments, Warszawa, Poland—14,000× g/5 min) musts or jerkums were diluted (×5) with deionized water. Then we used syringe filters (0.45 µm pore density, Sartorius AG, Getinge, Germany) to filtrate obtained dilutions. We analyzed organic acids on a Shimadzu NEXERA XR chromatograph (Kyoto, Japan). That piece of equipment has a pump system, and a UV/Vis detector (monitored at 210 nm). Malic, succinic, acetic, tartaric, lactic, and citric acids (Sigma-Aldrich, St. Louis, MO, USA) were analyzed using Rezex ROA-Organic Acid Aminex HPX-87H column (300 × 7.8 mm) (Rezex, Torrance, CA, USA). Isocrating elutions of samples was carried out at 40 °C with 0.005 M H_2SO_4) at a flow rate of 0.4 mL/min. We analyzed sugar profile with the same piece of equipment, however, we replaced the UV/Vis detector with a refractometer detector RF-20A. Sugars were separated on an Asahipak NH2P-50, 4.6 × 250 mm Shodex column (Showa Denko America, Munich, Germany) at 30 °C. The isocratic elution program (0.8 mL/min) lasted 16 min. We used an aqueous solution of acetonitrile (70%) for elution. We prepared standard curves for each tested compound: fructose, glucose, sucrose, and glycerol.

2.4. Volatile Composition of Fresh and Fermented Plum Mashes Using Solid Phase Microextraction–Gas Chromatography–Mass Spectrometry (SPME–GC–MS) and Gas Chromatography–Flame Ionization Detector (GC–FID) Methods

Analysis of volatile compounds was performed using gas chromatography with mass spectrometry [1,6]. We suspended 0.05 mg of 4-methyl-2-pentanol/L and 0.5 µg of ethyl nonanoate, (Sigma-Aldrich, St. Louis, MO, USA) in 1 mL saturated saline and that solution was used as an internal standard. We transferred 1 mL of juice or jerkums to a 10 mL vial. We conditioned the SPME device (Supelco Inc., Bellefonte, PA, USA, 100 µm polydimethylsiloxane fiber) in the GC injector port at 250 °C for 1 h. After inserting the fiber in the glass vials, samples were stirred (300 rpm/40 °C/30 min) and then fibers were transferred to the injector port Agilent Technologies 7890B chromatograph system (Agilent Technologies, Santa Clara, CA, USA). The system is equipped with an LECO Pegasus HT, High Throughput time-of-flight mass spectrometry (TOFMS) detector. Fibers were held for 3 min in the inlet. Transfers of the fiber were automated with GERSTEL MultiPurpose Sampler (MPS). Volatile compounds were separated on at the Rtx-1ms capillary column (Crossbond 100% dimethyl polysiloxane, 30 m × 0.53 mm × 0.5 µm). The injector and detector were heated to 250 °C, while the separation of compounds was initiated at 40 °C/3 min and then the temperature increased at an increment of 8 °C/min to 230 °C. Finally, samples were held at maximum temperature for 9 min. Helium, which was used as a carrier gas, was delivered at a constant flow (1.0 mL/min), while EIMS electron energy was 70 eV. Temperature of the source and connection parts was 250 °C. Analyte were transferred in the splitless mode. The mass spectrometer detector (MSD) was set to the scan mode from m/z = 40 to m/z = 400. We identified volatiles using mass spectral libraries and linear retention indices (calculated from a series of *n*-alkanes from C6 to C30). Semi-quantitative analysis of substances was assessed from the ratio of relative peak area of each identified component, to relative peak area of adequate internal standard (ethyl nonanoate for esters, anethol for terpenoids, and 4-methyl-2-pentanol for other components). Obtained results were analyzed in the National Institute of Standards and

Technology (NIST) database. This method was validated based on the method described by Antalick et al., 2010 [13].

Determination of the selected volatiles was analyzed using gas chromatography (Hewlett Packard 5890 Series II chromatograph system; Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector as described by Satora and Tuszyński [3]. We used HP-INNOWax capillary column (30 m × 0.53 mm ID with 1.0 µm film thickness, crosslinked polyethylene glycol stationary phase; Agilent, Santa Clara, CA, USA) for the separation of volatile compounds. The temperature of both detector and injector was set to 250 °C. Initial temperature of the column was 35 °C (5 min) and then it was increased to 110 °C at an increment of 5 °C/min, and then it was increased to 220 °C at an increment of 40 °C/min. The last temperature was sustained for 3 min. We used helium as a carrier gas (20.0 mL/min). Hydrogen was delivered at a 33.0 mL/min flow speed, while for air it was 400 mL/min. Volatiles were identified and quantified (acetaldehyde, acetone, methanol, propanol, isobutanol, butanol, pentanol, hexanol, phenylethanol, amyl alcohols, and ethyl acetate, Sigma-Aldrich) by the comparison of obtained surface peak area to those obtained for standards.

2.5. Statistical Analysis

Each experiment was carried out in three physical replicates. All analyses were carried out for each replicate. We used R 3.6.1 (Vienna, Austria) for statistical analysis. The Shapiro–Wilk test was applied to assess the normality of the data distribution. Multivariate analysis of variance (MANOVA) was carried out prior to the post hoc Tukey test.

3. Results and Discussion

3.1. Chemical Composition of Fresh Plum Musts and Jerkums

Musts obtained from Węgierka Dąbrowicka and Stanley demonstrated the highest concentration of total extract and total sugars which constituted about 80% of the total extract. However, in comparison to other cited studies, concentrations of total sugars in our samples were slightly low and ranged from 113.2 (Węgierka Zwykła) to 123.8 g/L (Węgierka Dąbrowicka) (Table 1), whereas in other studies it reached 153.6 g/L [14] or 127.5 g/L [4], respectively. Sugar content in plum influences consumer perception of maturity of consumed fruits and its concentration depends on the fruit cultivar, climatic conditions, and harvesting time [4,15].

Table 1. Chemical composition of fresh plum musts.

Plum Cultivars	Total Extract	Total Sugars	Reducing Sugars	Sucrose	Sugar-Free Extract	Titratable Acidity	Citric Acid	Malic Acid	Succinic Acid	Free Amino Nitrogen
				[g/L]					[mg/L]	
Węgierka Zwykła	146.0 ^{c} (±2.0)	113.2 ^{b} (±4.3)	42.6 ^{ab} (±2.3)	51.4 ^{a} (±2.0)	26.7 ^{b} (±4.2)	9.06 ^{b} (±0.39)	1.12 ^{b} (±0.07)	6.13 ^{ab} (±0.24)	0.56 ^{b} (±0.11)	119.6 ^{c} (±11.4)
Węgierka Dąbrowicka	155.7 ^{b} (±1.5)	123.8 ^{a} (±5.7)	45.9 ^{a} (±2.6)	47.9 ^{ab} (±2.9)	37.8 ^{a} (±4.2)	12.22 ^{a} (±0.23)	1.00 ^{b} (±0.04)	6.41 ^{ab} (±0.58)	1.13 ^{a} (±0.14)	135.6 ^{bc} (±4.6)
Stanley	161.7 ^{ab} (±6.4)	121.4 ^{a} (±2.6)	40.3 ^{ab} (±4.3)	56.7 ^{a} (±2.2)	37.7 ^{a} (±9.0)	9.81^{b} (±0.47)	1.50 ^{a} (±0.17)	7.26 ^{a} (±0.29)	0.82 ^{a} (±0.24)	156.4 ^{b} (±6.6)
Čačanska Lepotica	151.0 ^{c} (±1.0)	119.4 ^{ab} (±2.6)	32.1 ^{b} (±2.0)	44.5 ^{b} (±1.6)	41.4 ^{a} (±2.5)	11.90 ^{a} (±0.53)	1.04 ^{b} (±0.12)	6.56 ^{ab} (±0.33)	0.98 ^{a} (±0.05)	212.1 ^{a} (±5.1)
Significance	**	***	***	***	***	***	***	***	***	**

Values with different superscript roman letters (a–c) in the same column indicate statistically significant differences at $p < 0.05$; $n = 5$; 0.001 ***; 0.01 **.

Total extract of analyzed samples decreased after fermentation, which was caused by intense consumption of sugars during that process and high fermentation efficiency ranging from 66.6 to 99.3% (Table 2). The highest fermentation efficiency and concentration of ethanol (more than 7% vol.) was determined in musts fermented with Ethanol RED

(*S. cerevisiae*) or with *S. cerevisiae* S1, while lowest efficiency was shown for musts fermented spontaneously. According to the manufacturer's specification, Ethanol RED is more resistant to ethanol concentration exceeding 18% vol. and provides higher fermentation efficiency than many other yeast strains [16]. This would explain the results described in the current paper.

Table 2. Chemical composition of plum jerkums fermented spontaneously or with different yeast starter cultures.

Plum Cultivars	Type of Fermentation	Total Extract	Titratable Acidity	Ethanol	Free Amino Nitrogen	Fermentation Efficiency
		[g/L]		[% vol.]	[mg/L]	[%]
Węgierka Zwykła	*S. cerevisiae* S1	42.0 ^{ab} (±2.2)	3.72 ^{e} (±0.12)	6.9 ^{b} (±0.2)	46.29 ^{d} (±1.98)	93.8 ^{ab} (±1.2)
	H. uvarum H2	44.7 ^{ab} (±1.2)	3.63 ^{e} (±0.21)	6.8 ^{b} (±0.1)	51.67 ^{cd} (±2.18)	92.3 ^{ab} (±2.1)
	Spontaneous fermentation	39.0 ^{ab} (±0.8)	3.73 ^{e} (±0.23)	5.7 ^{c} (±0.1)	52.46 ^{c} (±1.02)	78.4 ^{bc} (±0.9)
	Ethanol RED (*S. cerevisiae*)	45.3 ^{ab} (±0.5)	4.43 ^{d} (±0.07)	7.2 ^{ab} (±0.2)	47.46 ^{d} (±1.06)	97.8 ^{a} (±1.4)
Węgierka Dąbrowicka	*S. cerevisiae* S1	36.3 ^{b} (±2.1)	9.14 ^{a} (±0.03)	7.9 ^{a} (±0.1)	57.61 ^{c} (±2.61)	98.8 ^{a} (±1.3)
	H. uvarum H2	33.0 ^{bc} (±3.7)	6.53 ^{b} (±1.73)	7.8 ^{a} (±0.2)	45.32 ^{d} (±1.51)	98.6 ^{a} (±0.8)
	Spontaneous fermentation	48.7 ^{a} (±5.8)	8.55 ^{a} (±0.72)	5.4 ^{c} (±0.3)	44.91 ^{d} (±6.02)	67.6 ^{c} (±2.1)
	Ethanol RED (*S. cerevisiae*)	46.0 ^{a} (±0.8)	8.33 ^{a} (±0.40)	8.0 ^{a} (±0.3)	54.41 ^{c} (±3.31)	99.3 ^{a} (±0.3)
Stanley	*S. cerevisiae* S1	44.7 ^{ab} (±0.5)	5.81 ^{c} (±0.13)	7.6 ^{a} (±0.3)	66.33 ^{b} (±3.03)	96.4 ^{a} (±0.8)
	H. uvarum H2	43.0 ^{ab} (±0.8)	6.10 ^{b} (±0.38)	7.4 ^{a} (±0.1)	59.11 ^{c} (±0.61)	93.6 ^{ab} (±1.0)
	Spontaneous fermentation	42.7 ^{ab} (±1.2)	5.38 ^{c} (±0.32)	6.7 ^{b} (±1.1)	57.72 ^{c} (±0.83)	84.8 ^{b} (±0.6)
	Ethanol RED (*S. cerevisiae*)	39.7 ^{ab} (±1.6)	7.19 ^{ab} (±0.41)	7.8 ^{a} (±0.9)	63.32 ^{bc} (±0.54)	98.9 ^{a} (±1.2)
Čačanska Lepotica	*S. cerevisiae* S1	28.0 ^{c} (±1.6)	5.47 ^{c} (±0.41)	7.4 ^{a} (±0.3)	70.65 ^{a} (±1.44)	97.6 ^{a} (±0.6)
	H. uvarum H2	37.3 ^{b} (±2.1)	5.65 ^{c} (±0.36)	7.0 ^{ab} (±0.2)	69.93 ^{ab} (±2.40)	91.1 ^{ab} (±0.8)
	Spontaneous fermentation	53.0 ^{a} (±7.4)	4.93 ^{cd} (±0.18)	5.1 ^{c} (±0.7)	72.49 ^{a} (±1.43)	66.6 ^{c} (±4.3)
	Ethanol RED (*S. cerevisiae)*	46.0 ^{a} (±0.8)	5.64 ^{c} (±0.12)	7.5 ^{a} (±0.2)	73.65 ^{a} (±3.89)	97.7 ^{a} (±1.1)
Significance		**	*	***	**	**

Values with different superscript roman letters (a–e) in the same column indicate statistically significant differences at $p < 0.05$;; $n = 5$; 0.001 ***; 0.01 **; 0.05 *.

In the current study, it has also been demonstrated that microorganisms used for fermentation and plum cultivar affected the rate of fermentation. Generally, the highest fermentation rate was noted in Węgierka Dąbrowicka and Čačanska Lepotica (except spontaneous fermentation) (Figures 1–4), however, in the case of the first cultivar, weight loss occurred successively throughout the whole fermentation process. It could be associated

to the fact that this cultivar demonstrated the highest titratable acidity (12.22 g of malic acid/L) and period of adaptation of yeast was the longest. The final weight losses were highest during fermentation carried out with both strains of S. cerevisiae S1 (about 8% for Čačanska Lepotica and 6% for Węgierka Dąbrowicka—*S. cerevisiae* S1, and about 6% for both cultivars fermented with Ethanol RED *S. cerevisiae*). However, musts obtained from those cultivars fermented spontaneously demonstrated the lowest fermentation efficiency. A possible explanation of that phenomenon is that microorganisms involved in spontaneous fermentation required more time for the adaptation to the conditions provided for the fermentation process. We noted that the turbulent stage of fermentation was delayed and started after the fourth day. This resulted in lower weight losses, especially in variants obtained from Čačanska Lepotica. On the other hand, decreasing the rate of fermentation could minimize heat release and improve the formation of different volatiles such as terpenes [17].

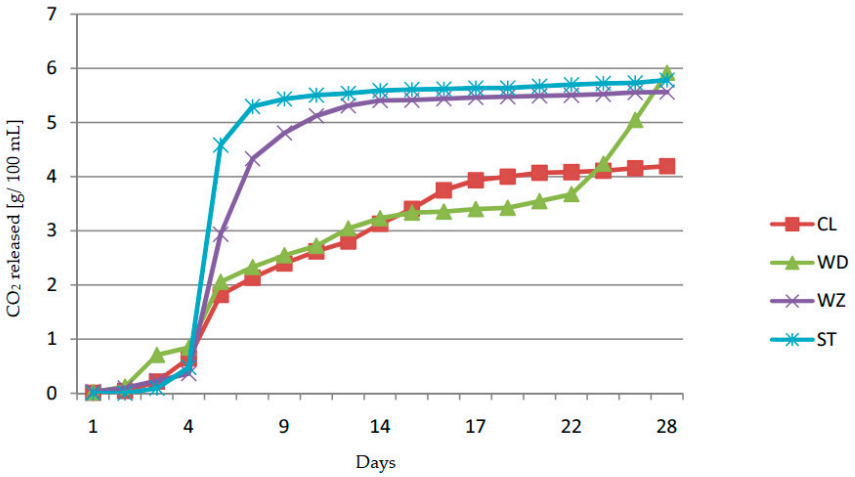


Figure 1. Fermentation dynamics of plum must fermented spontaneously, n = 5, STD < 5%. Abbreviations: CL—Čačanska Lepotica, WD—Węgierka Dąbrowicka, WZ—Węgierka Zwykła, ST—Stanley.

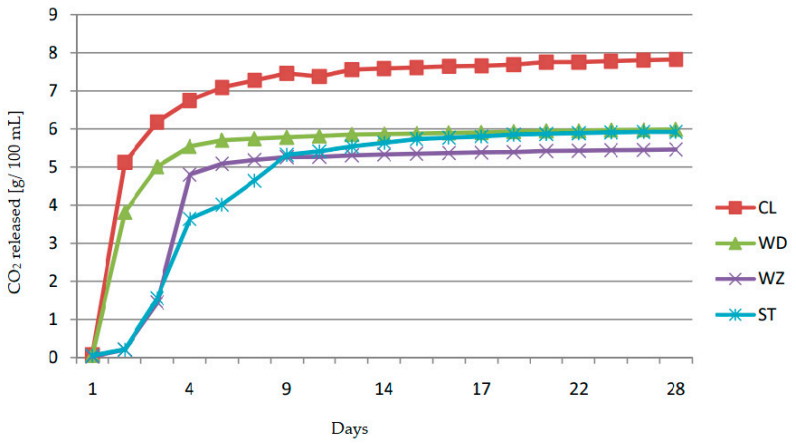


Figure 2. Fermentation dynamics of plum must fermented with *S. cerevisiae* S1, n = 5, STD < 5%. Abbreviations: CL—Čačanska Lepotica, WD—Węgierka Dąbrowicka, WZ—Węgierka Zwykła, ST—Stanley.

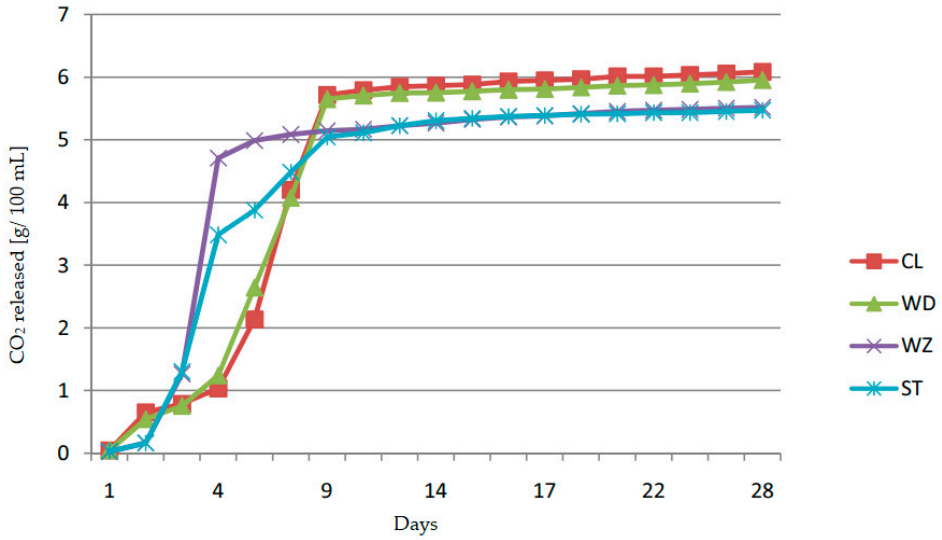


Figure 3. Fermentation dynamics of plum must fermented with *H. uvarum* H2, $n = 5$, STD < 5%. Abbreviations: CL—Čačanska Lepotica, WD—Węgierka Dąbrowicka, WZ—Węgierka Zwykła, ST—Stanley.

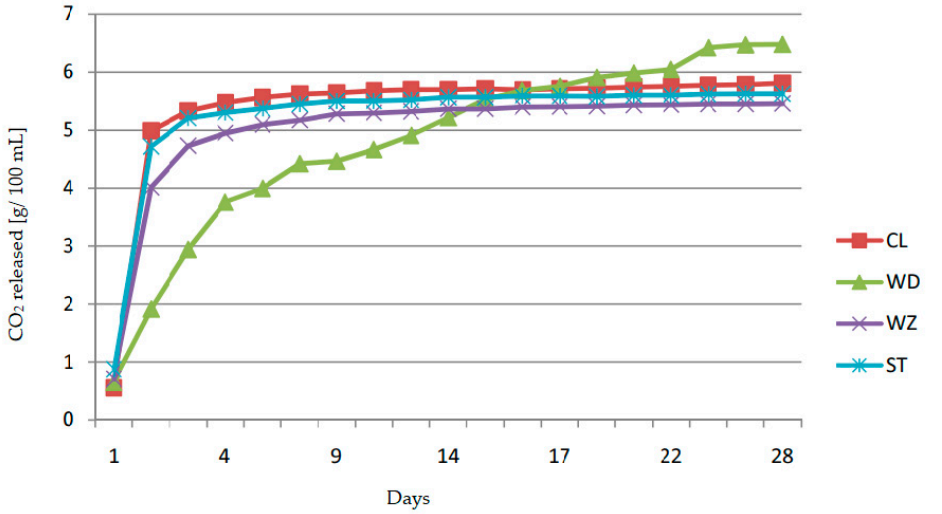


Figure 4. Fermentation dynamics of plum must fermented with Ethanol RED (*S. cerevisiae)*, $n = 5$, STD < 5%. Abbreviations: CL—Čačanska Lepotica, WD—Węgierka Dąbrowicka, WZ—Węgierka Zwykła, ST—Stanley.

Analyzed plum musts had high content of sugar-free extract (26.7–41.4 g/L) which is affected by, e.g., polyols, nitrogenous compounds, non-volatile organic acids, tannins, vitamins, pigments, and mineral salts. The quantity of these compounds determines the proper growth of microorganisms during fermentation [4]. Such high level of that parameter could be connected to the high level of titratable acidity of analyzed musts, which ranged from 9.06 (Węgierka Zwykła) to 12.22 g of malic acid/L (Węgierka Dąbrowicka). Similar or slightly lower titratable acidity (9.76 g of malic acid/L—Węgierka Zwykła and 7.55 g of malic acid/L—Węgierka Dąbrowicka) was demonstrated by Satora et al., 2017 [4]. On the other hand, Pashova et al., 2006 [14] showed that Red Damson and Blackthorn cultivars demonstrated higher acidity, which were 18.1 and 27.3 g of malic acid/L, respectively. The prevailing organic acid in analyzed plum musts in the current study was malic acid, which was also confirmed in other published studies [14,18]. Quantities of citric and succinic acids were lower and in most cases did not exceed 1 g/L (Table 3).

Table 3. Profile of organic acids, sugars, and glycerol concentrations in plum jerkums.

Plum Cultivar	Type of Fermentation	Citric Acid	Malic Acid	Succinic Acid	Lactic Acid	Acetic Acid	Glycerol	Fructose	Sucrose	Glucose
Węgierka Zwykła	*S. cerevisiae* S1	0.29 ^{a}	0.99 ^{c}	2.01 ^{a}	9.38 ^{a}	0.28 ^{c}	3.10 ^{d}	0.47 ^{d}	1.44 ^{b}	2.79 ^{bc}
	H. uvarum H2	0.00 ^{d}	0.00 ^{d}	1.39 ^{b}	0.48 ^{d}	0.59 ^{bc}	2.54 ^{d}	1.69 ^{c}	0.42 ^{d}	0.00 ^{d}
	Spontaneous fermentation	0.00 ^{d}	0.00 ^{d}	0.12 ^{d}	0.75 ^{d}	0.47 ^{c}	5.42 ^{c}	1.01 ^{c}	0.16 ^{d}	0.00 ^{d}
	Ethanol RED	0.00 ^{d}	0.00 ^{d}	0.17 ^{d}	0.89 ^{d}	0.69 ^{b}	5.14 ^{c}	0.22 ^{d}	0.59 ^{d}	2.16 ^{b}
Węgierka Dąbrowicka	*S. cerevisiae* S1	0.19 ^{b}	3.50 ^{a}	2.15 ^{a}	6.94 ^{b}	0.84 ^{b}	6.92 ^{bc}	0.61 ^{d}	0.32 ^{d}	2.63 ^{bc}
	H. uvarum H2	0.24 ^{b}	3.21 ^{a}	2.26 ^{a}	5.39 ^{bc}	0.93 ^{ab}	6.25 ^{bc}	0.00 ^{e}	2.46 ^{a}	4.14 ^{b}
	Spontaneous fermentation	0.34 ^{a}	1.07 ^{c}	1.29 ^{b}	4.62 ^{c}	0.34 ^{c}	5.01 ^{c}	6.58 ^{b}	1.16 ^{c}	1.45 ^{c}
	Ethanol RED	0.29 ^{a}	3.32 ^{a}	1.91 ^{ab}	3.19 ^{d}	0.57 ^{bc}	8.88 ^{ab}	2.35 ^{c}	0.00 ^{d}	9.85 ^{a}
Stanley	*S. cerevisiae* S1	0.14 ^{c}	1.23 ^{c}	1.57 ^{b}	6.38 ^{b}	0.25 ^{c}	7.93 ^{b}	11.28 ^{a}	0.85 ^{cd}	0.65 ^{cd}
	H. uvarum H2	0.07 ^{d}	0.00 ^{d}	1.16 ^{b}	2.99 ^{c}	1.07 ^{a}	6.65 ^{bc}	12.66 ^{a}	1.53 ^{b}	0.84 ^{c}
	Spontaneous fermentation	0.15 ^{c}	0.10 ^{d}	0.73 ^{c}	3.32 ^{c}	0.67 ^{b}	6.67 ^{bc}	10.72 ^{a}	2.08 ^{ab}	0.91 ^{c}
	Ethanol RED	0.23 ^{b}	2.97 ^{a}	2.39 ^{a}	6.39 ^{b}	1.29 ^{a}	10.02 ^{a}	14.05 ^{a}	1.52 ^{b}	0.00 ^{d}
Čačanska Lepotica	*S. cerevisiae* S1	0.20 ^{b}	2.00 ^{b}	2.38 ^{a}	8.45 ^{a}	0.25 ^{c}	5.50 ^{c}	2.50 ^{c}	1.33 ^{c}	0.00 ^{d}
	H. uvarum H2	0.16 ^{c}	2.36 ^{ab}	2.58 ^{a}	6.51 ^{b}	0.18 ^{c}	5.73 ^{c}	3.32 ^{c}	0.00 ^{d}	0.01 ^{d}
	Spontaneous fermentation	0.28 ^{a}	0.35 ^{d}	1.43 ^{b}	5.98 ^{b}	1.33 ^{a}	5.70 ^{c}	0.00 ^{e}	0.21 ^{d}	0.84 ^{c}
	Ethanol RED	0.25 ^{ab}	3.56 ^{a}	2.33 ^{a}	5.50 ^{bc}	0.36 ^{c}	6.41 ^{bc}	0.33 ^{d}	0.00 ^{d}	5.91 ^{b}
SD pooled		0.06	0.92	0.57	1.35	0.27	1.94	1.37	0.53	0.35
Significance		***	**	***	**	***	**	**	***	**

Values with different superscript roman letters (a–e) in the same column indicate statistically significant differences at $p < 0.05$; $n = 5$; 0.001 ***; 0.01 **.

The level of titratable acidity significantly decreased after fermentation (Table 2), which was probably caused by the assimilation of some organic acids, mainly malic and citric acids, in which concentration also decreased the most significantly among detected acids. However, after fermentation, concentration of succinic acid increased, especially in samples fermented with *Hanseniaspora uvarum* H2 and *Saccharomyces cerevisiae* S1 isolated from plum fruits (Table 3). Succinic acid is a common by-product of alcoholic fermentation and it is confirmed that it is a dominant non-volatile carboxylic acid synthetized by yeasts. It must be highlighted that succinic acid plays a major role in shaping the taste of wine [19]. Succinic acid could originate from either sugar or amino acid catabolism of yeast; however, it is connected to growth conditions and available nitrogen sources. Therefore, higher concentration of that organic acid in fermented musts produced from Węgierka Dąbrowicka and Čačanska Lepotica could be related to a higher concentration of free amino nitrogen in those plum cultivars. Moreover, reactions of tricarboxylic acid cycle determine its direct formation [19].

Volatile acidity of wines is mostly built up by the acetic acid. That substance could be formed in biochemical reaction during fermentation or could be directly produced by acetic or lactic acid bacteria (LAB). When acetic acid is present above a certain concentration (1.2 g/L volatile acidity expressed as acetic acid), it decreases the quality of wine and it also inhibits the performance of yeasts [20,21]. A slightly higher amount of this compound was present in samples obtained from Stanley cultivar fermented with Ethanol RED and from Čačanska Lepotica fermented spontaneously. We did not notice any correlation between concentrations of acetic and lactic acids—low concentrations of acetic acid was determined in samples that contained significant quantities of lactic acid and the other way around.

Lactic acid was detected in all analyzed fermented plum musts and its quantities ranged from 0.48 to 9.38 g/L. Highest concentration of lactic acid was present in samples fermented with *S. cerevisiae* S1. It is possible that during the fermentation of those variant lactic acid bacteria (LAB) produced that acid. Based on the profile of organic acids (Table 3) it could be presumed that malolactic fermentation did not occur before the time the samples were collected because, in the majority of cases, the concentration of malic acid was higher in all variants fermented with *S. cerevisiae* S1.

Glycerol enhances taste characteristics, defines texture of a wine, and as such its bouquet, taste, and smell. On the contrary to the compounds described above, it is mostly produced by yeast [22]. Glycerol contributes to the sugar-free extract so it is not surprising that values of that parameter demonstrated in the current study were relatively high (2.54–10.02 g/L).

The concentration of free amino nitrogenous compounds in plum musts contributes to the aroma of obtained jerkums. It ranged from 119.6 mg/L to 212.1 mg/L and it decreased after fermentation, which could be possibly due to a high demand for nitrogenous substances by microorganisms present in those musts. This group of nutrients affects the rate of fermentation by enhancing growth of microorganisms. Moreover, it could prevent the formation of stuck or the occurrence of sluggish fermentation. Those compounds could be also utilized for the formation of higher alcohols. It seems that branched chain aromatic amino acids are the most important in those processes [23].

3.2. Volatile Compounds of Fresh Plum Musts

Qualitative and quantitative composition of volatiles of plums is very diverse and statistically significantly varies among cultivars. More than 50 volatiles were identified in plum musts of which esters were the dominant group. Among them, ethyl acetate, ethyl butanoate, butyl butanoate, and ethyl decanoate were the most abundant (Table 4). Those esters (except butyl butanoate) were also present in fermented plum musts. Esters contribute to floral and fruity aroma notes in fruit and those esters have sweet, pineapple, banana, and nut-like aroma, respectively [24,25]. Some of those compounds were characteristic for particular cultivars, e.g., butyl butanoate for Węgierka Zwykła, hexyl hexanoate for Węgierka Zwykła and Stanley, ethyl tridecanoate for Węgierka Dąbrowicka and Čačanska Lepotica. Due to numerous transformations of volatile compounds during fermentation, we could not assign specific esters to plum jerkums obtained from particular cultivars. However, we observed that samples fermented with *S. cerevisiae* S1 or *H. uvarum* H2 demonstrated the highest concentration of esters, followed by spontaneous fermentation and musts fermented with Ethanol RED (four times lower concentration, e.g., methyl hexanoate, isoamyl lactate). Differences in the concentration of esters are associated with the fact that esters are produced during fermentation by yeast cells in an enzyme-catalyzed intracellular reaction [26]. Generally, *H. uvarum* is known as a good ester producer and it increases formation of some acetate esters. According to the literature, *H. uvarum* were able to produce ethyl acetate, geranyl acetate, and isoamyl acetate in model solution (GPYM—glucose, peptone, yeast extract, malt extract medium) [27]. Combined cultures of *Hanseniaspora* spp. and *S. cerevisiae* increased the formation of esters and improved sensory properties of wines [28]. It is worth mentioning that in the current study, the samples fermented with *S. cerevisiae* S1 demonstrated the highest concentration of isoamyl lactate. This could be related to the higher concentration of lactic acid in samples fermented with this type of yeast, which probably resulted from spontaneous malolactic fermentation.

Table 4. The composition of volatile compounds in musts obtained from various plum cultivars [µg/L].

Compounds [µg/L]	LRI [2]	Węgierka Zwykła	Węgierka Dąbrowicka	Stanley	Čačanska Lepotica	OT [4]	Sig.	Characteristic Aroma
Esters								
Ethyl acetate	868	6667 ^{b}	5966 ^{b}	6540 ^{b}	10118 ^{a}	5000	*	sweet, solvent
Isobutyl acetate	1011	5.5 ^{b}	10.6 ^{ab}	5.4 ^{b}	16.4 ^{a}	66	**	fig-like, banana
Ethyl butanoate [3]	1033	112 ^{b}	112 ^{b}	119 ^{b}	229 ^{a}	1	***	pineapple
Ethyl 2-methylbutyrate	1048	7.6 ^{a}	8.0 ^{a}	10.9 ^{a}	12.3 ^{a}	0.3	ns	berry, tropical
Butyl butanoate	1215	151 ^{a}	0 ^{b}	0 ^{b}	0 ^{b}	100	***	fruity banana pineapple sweet
Ethyl hexanoate	1230	23 ^{a}	15 ^{a}	21 ^{a}	24 ^{a}	1	ns	apple peel, pineapple
Hexyl butanoate	1416	10.5 ^{a}	0 ^{b}	10.7 ^{a}	2.2 ^{b}	250	***	green, fruity, estry vegetative
Ethyl octanoate	1438	7.8 ^{ab}	6.7 ^{b}	9.2 ^{ab}	12.4 ^{a}	15	*	Fruity, winey, sweet
3-hexenyl butanoate	1464	0.5 ^{bc}	0 ^{c}	1.8 ^{b}	10.1 ^{a}	20,000	***	Fresh, green apple, fruity
Hexyl hexanoate	1585	0.6 ^{a}	0 ^{b}	0.6 ^{a}	0 ^{b}	6.4	**	Herbaceous
Ethyl decanoate	1612	311 ^{b}	382 ^{ab}	311 ^{b}	474 ^{a}	510	*	Sweet, fatty, nut-like, winey
1-methylethyl dodecanoate	1800	2.4 ^{d}	24.6 ^{a}	9.3 ^{b}	5.3 ^{c}	-	***	-
Ethyl dodecanoate	1812	210 ^{a}	261 ^{a}	242 ^{a}	208 ^{a}	2000	ns	Oily, fatty, floral
Ethyl tridecanoate	1994	0 ^{b}	34 ^{a}	0 ^{b}	60 ^{a}	-	***	-
Ethyl tetradecanoate	2093	37 ^{b}	44 ^{b}	35 ^{b}	85 ^{a}	4000	***	Mild, waxy, soapy
Alcohols								
1-butanol	1144	18.6 ^{b}	0 ^{b}	83.7 ^{a}	0 ^{b}	500	***	banana harsh alcoholic sweet
1-hexanol	1348	2088 ^{bc}	3001 ^{b}	8691 ^{a}	1112 ^{c}	2500	***	herbal ethereal alcoholic green
3-hexen-1-ol	1385	6.0 ^{b}	8.9 ^{b}	4.5 ^{b}	23.5 ^{a}	70	***	grassy-green freshly cut grass
2-hexen-1-ol	1409	10.6 ^{a}	4.9 ^{b}	4.6 ^{b}	2.2 ^{b}	400	***	Sharp green leafy
2-ethyl-1-hexanol	1487	2.8 ^{a}	0 ^{c}	2.2 ^{ab}	1.5 ^{b}	138	***	citrus fresh floral oily sweet
1-nonanol	1642	10.2 ^{a}	18.4 ^{a}	12.0 ^{a}	10.3 ^{a}	50	ns	citrus
Benzyl alcohol	1858	4.7 ^{a}	2.5 ^{a}	3.7 ^{a}	4.0 ^{a}	10,000	ns	floral rose phenolic balsamic
Phenol	1972	0.1 ^{b}	0.5 ^{ab}	0.5 ^{ab}	0.9 ^{a}	5900	*	phenolic plastic rubber
2-phenoxyethanol	2114	0.5 ^{a}	0 ^{b}	0.6 ^{a}	0.4 ^{ab}	-	*	mild rose balsam cinnamyl

Table 4. *Cont.*

Compounds [µg/L]	LRI [2]	Węgierka Zwykła	Węgierka Dąbrowicka	Stanley	Čačanska Lepotica	OT [4]	Sig.	Characteristic Aroma
Carbonyl compounds								
5-methyl-3-hexanone	1069	2205 ^{b}	3140 ^{a}	1753 ^{b}	3365 ^{a}	-	***	pleasant fruity
Hexanal	1076	1122 ^{b}	7783 ^{a}	7118 ^{a}	8562 ^{a}	4.5–5	***	grassy
4-methyl-2-hexanone	1113	0 ^{b}	1.5 ^{a}	2.8 ^{a}	2.4 ^{a}	-	***	pleasant fruity
2-hexenal	1199	291 ^{bc}	467 ^{ab}	549 ^{a}	134 ^{c}	17	**	green
Nonanal [3]	1392	4.0 ^{ab}	2.5 ^{b}	5.9 ^{a}	2.3 ^{b}	1	**	aldehydic rose orange peel
2,4-hexadienal	1406	0.3 ^{a}	0.6 ^{a}	0.8 ^{a}	0.6 ^{a}	60	ns	Green, fruity, aldehydic, citrus
Decanal	1491	7.7 ^{a}	0 ^{b}	2.0 ^{b}	0 ^{b}	2	**	aldehydic orange peel citrus
Benzaldehyde	1513	2.1 ^{b}	0.7 ^{b}	2.2 ^{b}	6.1 ^{a}	350	**	spicy bitter-almond
Acetophenone	1640	10.8 ^{a}	4.0 ^{bc}	0 ^{c}	7.7 ^{ab}	65	***	pungent hawthorn almond
Terpenoids								
p-Cymene	1259	1.2 ^{bc}	0.9 ^{c}	1.6 ^{b}	2.6 ^{a}	-	***	solvent, gasoline, citrus
Bornylene	1506	0.7 ^{a}	0 ^{b}	0 ^{b}	0.3 ^{ab}	-	**	-
Linalool	1536	13.7 ^{a}	1.4 ^{c}	4.3 ^{b}	5.0 ^{b}	6	***	Floral, woody, lavender
Calamenene	1567	0.5 ^{b}	3.2 ^{a}	1.3 ^{ab}	0.5 ^{b}	-	***	Herb spice
ß-Cyclocitral	1595	0.5 ^{b}	0 ^{c}	0 ^{c}	0.9 ^{a}	5	***	Minty, citrus
Menthol	1617	0.8 ^{a}	0.2 ^{b}	0 ^{b}	0.2 ^{ab}	-	*	Cooling, fresh, sweet, minty
Damascenone	1804	3.6 ^{b}	17.9 ^{a}	6.8 ^{b}	4.0 ^{b}	0.002	**	Apple, rose honeys
Geraniol	1816	0.3 ^{a}	1.4 ^{a}	1.4 ^{a}	1.4 ^{a}	40	ns	floral fruity rose waxy citrus
Geranyl acetone	1828	3.3 ^{a}	1.3 ^{a}	1.4 ^{a}	3.1 ^{a}	60	ns	rose leaf magnolia aldehydic
ß-ionone	1918	0.6 ^{b}	0.9 ^{ab}	2.2 ^{a}	2.1 ^{a}	7	*	violet raspberry woody fruity
p-cresol	1967	0.3 ^{a}	0.2 ^{a}	0.2 ^{a}	0.1 ^{a}	55	ns	phenolic narcissus
Eugenol	2136	0.4 ^{a}	1.6 ^{a}	1.0 ^{a}	1.4 ^{a}	6	ns	sweet spicy clove woody
Lactones								
Γ-nonanolactone	2128	1.1 ^{ab}	2.0 ^{a}	1.2 ^{ab}	0.5 ^{b}	65	*	coconut creamy waxy buttery waxy peach coconut buttery
Γ-decanolactone	2328	3.2 ^{a}	2.9 ^{a}	2.6 ^{a}	0.2 ^{b}	11	**	

Table 4. *Cont.*

Compounds [µg/L]	LRI [2]	Węgierka Zwykła	Węgierka Dąbrowicka	Stanley	Čačanska Lepotica	OT [4]	Sig.	Characteristic Aroma
Hydrocarbons								
Acenaphthene	2154	1.5 [a]	0.8 [a]	1.5 [a]	1.4 [a]	80	*	Pungent
Other compounds								
Dimethyl sulfoxide	1204	2.0 [a]	2.3 [a]	4.3 [a]	2.6 [a]	-	ns	Garlic-like
Benzothiazole [3]	1952	8.8 [a]	7.8 [a]	11.0 [a]	9.3 [a]	80	ns	Sulphurous, rubbery, burnt

Values with different superscript roman letters (a–e) in the same row indicate statistically significant differences at $p < 0.05$; $n = 5$; ns—not significant; 0.001 ***; 0.01 **; 0.05 *. [2] LRI—linear retention index. [3] Determined semi-quantitatively by measuring the relative peak area of each identified compound, according to the NIST database, in relation to that of the internal standard. [4] OT—Odor thresholds in spirits or wines [25].

The next largest groups of volatile compounds were alcohols and carbonyl compounds. Hexanol (with herbal ethereal alcoholic green aroma) was the most abundant of the nine alcohols detected in fresh musts and constituted of over 90% of alcohols. This is in agreement with the results presented in other studies which indicated hexanol as a characteristic compound in plums [24,25,29]. The origin of C6 alcohols, e.g., 1-hexanol, is related to the lipoxygenase activity, which occurs in plants mainly in fruits. It brakes unsaturated fatty acids and the products of such reaction are precursors of short chain alcohols. After fermentation, in analyzed samples some of other alcohols have occurred, e.g., propanol and isobutanol. Those compounds could be produced by yeast from amino acids and they naturally occur in wines [30,31]. As well as in the case of esters, the concentration of most of these compounds were higher in juices fermented with *H. uvarum* H2 or *S. cerevisiae* S1, especially in Węgierka Zwykła and Stanley cultivars in comparison to samples fermented with Ethanol RED (Table 5). Musts fermented with *S. cerevisiae* S1 demonstrated higher concentrations of propanol, isobutanol, butanol, and 2-phenylethanol. Concentration of the last of these compounds was also high in samples fermented with Ethanol RED (*S. cerevisiae*). It is claimed, that *Saccharomyces* genus is able to produce significant amounts of 2-phenylethanol and it is currently receiving attention as yeast having a great prospect in development of biotechnological production of this substance [32].

Increased production of higher alcohols by *H. uvarum* was claimed, but there is no information on the increased synthesis of methanol by this strain. However, according to our studies, concentration of methanol in samples fermented with *H. uvarum* H2 was the highest and exceeded 950 mg/L. According to results presented by other authors, concentration of methanol in plum wines varies from 175 mg/L to almost 1000 mg/L [33,34]. Methanol is produced by the hydrolysis of methyl ester groups in pectins from fruits, so it is present in wines and spirits. The enzyme which catalyzes that reaction (pectin methyl esterase) occurs not only in plants, but it may also be produced by various microorganisms [9]. The main reason standing behind high methanol concentrations in our samples was the application of pectinolytic preparation and using whole plum fruits, including skins and stones.

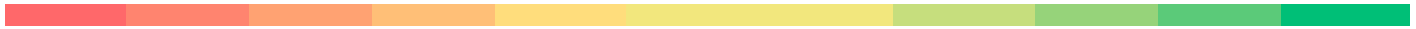
Table 5. Aroma composition of plum jerkums [μg/L].

	LRI	Węgierka Zwykła				Węgierka Dąbrowicka				Stanley				Čačanska Lepotica				Methods	Significance
		S. cerevisiae S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED		
Esters																			
Ethyl acetate	599	76,600 ^{e}	78,513 ^{e}	104,256 ^{cd}	74,717 ^{e}	104,351 ^{cd}	193,967 ^{a}	202,212 ^{a}	75,380 ^{e}	45,933 ^{f}	51,830 ^{f}	94,536 ^{d}	117,636 ^{c}	24,323 ^{g}	25,456 ^{g}	154,846 ^{b}	16,920 ^{g}	FID	***
Isobutyl acetate	771	8.1 ^{a}	0.8 ^{f}	4.9 ^{bc}	2.5 ^{cdef}	1.5 ^{ef}	3.9 ^{bcde}	5.6 ^{ab}	1.7 ^{def}	1.4 ^{ef}	0.8 ^{f}	4.6 ^{bc}	1.3 ^{ef}	1.3 ^{ef}	0.6 ^{f}	4.4 ^{bcd}	2.98 $^{b–f}$	MS	***
Ethyl butanoate	789	37.6 ^{a}	8.1 ^{bcd}	9.1 ^{bcd}	1.9 ^{d}	1.9 ^{d}	14.1 ^{bc}	1.6 ^{d}	2.0 ^{d}	8.3 ^{bcd}	10.1 ^{bcd}	7.8 ^{bcd}	3.7 ^{cd}	16.4 ^{b}	16.3 ^{b}	8.7 ^{bcd}	9.7 ^{bcd}	MS	***
Ethyl 2-hydroxypropanoate	798	1118.5 ^{bc}	946.9 ^{bc}	762.3 ^{cd}	170.3 ^{d}	3187.2 ^{a}	1061.5 ^{bc}	1186.6 ^{bc}	140.9 ^{d}	1637.7 ^{b}	966.0 ^{bc}	1066.3 ^{bc}	122.3 ^{d}	736.1 ^{cd}	1050.4 ^{bc}	619.4 ^{cd}	67.9 ^{d}	MS	***
3-Methylbutyl acetate	872	4.7 ^{def}	3.5 ^{ef}	9.3 ^{bcd}	10.3 ^{bc}	3.6 ^{ef}	6.3 ^{cdef}	8.9 ^{bcd}	1.2 ^{f}	5.2 ^{cdef}	7.7 ^{bcde}	8.7 ^{bcde}	1.6 ^{f}	7.1 ^{bcde}	12.4 ^{b}	9.5 ^{bcd}	18.6 ^{a}	MS	***
Methyl hexanoate	915	4.4 ^{c}	0.7 ^{efg}	1.4 ^{defg}	0.2 ^{g}	2.1 ^{de}	11.0 ^{a}	0.9 ^{efg}	0.2 ^{g}	4.6 ^{c}	8.0 ^{b}	0.9 ^{efg}	0.5 ^{fg}	1.8 ^{def}	2.6 ^{d}	1.9 ^{def}	0.5 ^{fg}	MS	***
(s)-i-butyl lactate	960	4.8 ^{bcde}	5.8 ^{bcd}	2.9 ^{defg}	0.5 ^{g}	13.5 ^{a}	3.6 ^{cdef}	2.6 ^{efg}	0.2 ^{g}	7.6 ^{b}	5.7 ^{bcd}	3.5 ^{cdef}	0.2 ^{g}	1.9 ^{efg}	1.2 ^{fg}	3.7 ^{cdef}	0.0 ^{g}	MS	***
Ethyl hexanoate	986	77.7 ^{bcd}	11.6 ^{h}	38.7 ^{efgh}	40.9 ^{defg}	25.9 ^{fgh}	179.1 ^{a}	18.9 ^{gh}	13.7 ^{h}	80.9 ^{bc}	95.2 ^{b}	54.0 $^{c–g}$	33.7 $^{e–h}$	63.5 $^{b–f}$	65.4 ^{bcde}	41.1 $^{d–h}$	69.4 ^{bcde}	MS	***
Isoamyl lactate	1047	18.4 ^{ab}	21.6 ^{a}	2.6 ^{cd}	0.1 ^{d}	24.6 ^{a}	8.9 ^{cd}	3.6 ^{cd}	0.1 ^{d}	24.1 ^{a}	18.2 ^{ab}	3.8 ^{cd}	0.1 ^{d}	11.2 ^{bc}	10.0 ^{bc}	2.2 ^{cd}	0.1 ^{d}	MS	***
Ethyl methyl succinate	1070	18.4 ^{ab}	25.1 ^{a}	7.5 ^{cdef}	2.7 ^{ef}	10.7 ^{bcde}	11.5 ^{bcd}	0.4 ^{f}	0.4 ^{f}	14.6 ^{bc}	18.6 ^{ab}	5.7 ^{def}	1.8 ^{f}	4.1 ^{def}	5.9 ^{def}	6.2 ^{def}	2.1 ^{f}	MS	***
Methyl octanoate	1107	4.4 ^{bc}	0.4 ^{e}	1.7 ^{de}	1.6 ^{de}	2.2 ^{cde}	10.5 ^{a}	1.3 ^{e}	1.7 ^{de}	3.8 ^{cd}	6.3 ^{b}	1.4 ^{de}	1.6 ^{de}	1.3 ^{e}	1.8 ^{de}	1.8 ^{de}	1.9 ^{de}	MS	***
Ethyl benzoate	1144	281.0 ^{a}	166.1 $^{b–f}$	247.0 ^{ab}	156.0 $^{b–f}$	85.3 ^{fg}	91.4 ^{efg}	105.1 ^{defg}	30.7 ^{g}	213.7 ^{abc}	207.7 ^{abcd}	174.0 $^{b–f}$	152.3 $^{b–f}$	126.2 $^{c–g}$	144.5 $^{b–f}$	191.5 $^{a–e}$	119.7 $^{c–g}$	MS	***
Diethyl succinate	1149	1955.0 ^{b}	3202.7 ^{a}	1593.7 ^{bcde}	1620.7 ^{bcd}	1390.8 ^{bcde}	1709.0 ^{bcd}	64.9 ^{g}	338.3 ^{fg}	1558.0 ^{bcde}	1730.3 ^{bc}	1209.7 ^{bcde}	1398.7 ^{bcde}	865.9 ^{defg}	1070.2 ^{cdef}	743.2 ^{efg}	1213.9 ^{bcde}	MS	***
Ethyl octanoate	1180	74.0 ^{b}	8.7 ^{d}	68.7 ^{bc}	31.3 ^{bcd}	57.2 ^{bcd}	330.4 ^{a}	48.8 ^{bcd}	16.8 ^{cd}	75.0 ^{b}	78.6 ^{b}	70.8 ^{b}	52.6 ^{bcd}	84.3 ^{b}	81.9 ^{b}	70.2 ^{b}	74.0 ^{b}	MS	***
Ethyl decanoate	1397	41.1 ^{bcd}	16.9 ^{cd}	52.6 ^{bc}	20.8 ^{cd}	52.5 ^{bc}	117.1 ^{a}	38.5 ^{bcd}	14.5 ^{d}	47.0 ^{bcd}	65.5 ^{b}	67.9 ^{b}	17.1 ^{cd}	52.4 ^{bcd}	62.7 ^{b}	52.6 ^{bc}	72.6 ^{b}	MS	***
Ethyl isopentyl succinate	1421	4.8 ^{a}	5.2 ^{a}	2.3 ^{c}	2.2 ^{c}	5.0 ^{a}	5.1 ^{a}	0.3 ^{d}	1.0 ^{cd}	5.9 ^{a}	5.6 ^{a}	2.1 ^{cd}	2.3 ^{c}	2.6 ^{bc}	2.6 ^{bc}	2.1 ^{cd}	4.4 ^{ab}	MS	***
Ethyl dodecanoate	1581	34.9 ^{bcd}	46.5 ^{bc}	34.0 ^{bcd}	15.9 ^{cd}	84.5 ^{a}	42.6 ^{bcd}	31.0 ^{bcd}	13.5 ^{d}	45.4 ^{bc}	43.9 ^{bcd}	49.9 ^{b}	18.1 ^{cd}	44.4 ^{bcd}	33.5 ^{bcd}	61.5 ^{ab}	61.2 ^{ab}	MS	***
Ethyl tetradecanoate	1790	7.9 ^{bcd}	13.1 ^{b}	8.2 ^{bcd}	8.1 ^{bcd}	35.0 ^{a}	12.1 ^{bc}	7.6 ^{bcd}	2.8 ^{d}	14.0 ^{b}	12.5 ^{b}	7.8 ^{bcd}	5.3 ^{cd}	8.1 ^{bcd}	5.4 ^{cd}	8.6 ^{bcd}	12.2 ^{bc}	MS	***
Ethyl hexadecanoate	1990	41.5 $^{c–g}$	58.3 ^{bcde}	34.9 ^{defg}	55.2 $^{b–f}$	146.6 ^{a}	85.9 ^{b}	22.4 ^{g}	45.9 $^{c–g}$	70.5 ^{bc}	65.7 ^{bcd}	30.5 ^{efg}	62.6 ^{bcd}	15.5 ^{g}	24.7 ^{fg}	33.9 ^{defg}	65.2 ^{bcd}	MS	***
Alcohols																			
Methanol	382	1,763,141 ^{a}	1,604,822 ^{a}	908,463 ^{bc}	892,061 ^{bc}	289,671 ^{ef}	981,453 ^{b}	1,031,741 ^{b}	435,692 ^{de}	653,663 ^{cd}	981,323 ^{b}	770,277 ^{bc}	663,322 ^{cd}	245,341 ^{ef}	964,122 ^{b}	112,285 ^{f}	101,331 ^{f}	FID	***
Propanol	543	331,832 ^{c}	283,571 ^{e}	130,221 ^{g}	125,921 ^{gh}	509,675 ^{a}	240,771 ^{f}	250,788 ^{g}	292,321 ^{de}	381,141 ^{b}	382,263 ^{b}	321,697 ^{cd}	406,823 ^{b}	234,256 ^{f}	316,467 ^{cd}	96,651 ^{h}	119,851 ^{gh}	FID	***
Isobutanol	629	187,873 ^{c}	157,952 ^{d}	97,726 ^{fg}	198,263 ^{bc}	244,759 ^{a}	204,076 ^{bc}	212,523 ^{b}	130,967 ^{e}	100,969 ^{fg}	99,567 ^{fg}	832,967 ^{g}	112,063 ^{ef}	46,673 ^{h}	99,713 ^{fg}	25,600 ^{h}	33,013 ^{h}	FID	***
Butanol	653	11,171 ^{cd}	22,277 ^{a}	1834 ^{ef}	3356 ^{ef}	2043 ^{ef}	1687 ^{ef}	0 ^{f}	1810 ^{ef}	15,913 ^{b}	14,843 ^{bc}	5233 ^{e}	9960 ^{d}	213 ^{f}	0 ^{f}	647 ^{f}	0 ^{f}	FID	***
Amyl alcohols	740	83,167 ^{g}	455,136 ^{b}	217,710 ^{d}	621,077 ^{a}	71,897 ^{gh}	45,643 ^{hi}	272,343 ^{c}	46,077 ^{hi}	167,463 ^{ef}	145,333 ^{f}	38,873 ^{i}	266,590 ^{c}	80,873 ^{g}	185,700 ^{e}	24,574 ^{i}	86,897 ^{g}	FID	***
Pentanol	757	241 ^{c}	92 ^{c}	2161 ^{a}	220 ^{c}	0 ^{c}	0 ^{c}	0 ^{c}	0 ^{c}	0 ^{c}	321 ^{c}	1632 ^{b}	0 ^{c}	0 ^{c}	0 ^{c}	121 ^{c}	0 ^{c}	FID	***
2,3-Butanediol	768	119.9 ^{de}	778.3 ^{b}	284.3 ^{de}	138.3 ^{de}	737.1 ^{bc}	1156.4 ^{a}	383.6 ^{cd}	80.6 ^{de}	192.7 ^{de}	973.0 ^{ab}	362.7 ^{d}	146.7 ^{de}	131.3 ^{de}	0.00 ^{e}	230.0 ^{de}	166.5 ^{de}	MS	***
(Z)-3-Hexen-1-ol	858	1.1 ^{e}	1.1 ^{e}	5.8 $^{ab\ c}$	1.9 ^{de}	7.8 ^{a}	2.6 ^{de}	5.7 $^{ab\ c}$	1.2 ^{de}	2.6 ^{de}	1.2 ^{e}	6.1 ^{ab}	2.1 ^{de}	3.1 ^{bcde}	0.8 ^{e}	4.6 ^{bcd}	2.8 ^{cde}	MS	***
Hexanol	862	18,096 ^{bcd}	19,480 ^{bcd}	18,076 ^{bcd}	5761 ^{ef}	22,333 ^{bc}	23,310 ^{b}	23,697 ^{b}	7557 ^{e}	15,581 ^{d}	17,526 ^{cd}	29,541 ^{a}	65,901 ^{ef}	19,787 ^{bcd}	20,200 ^{bcd}	3757 ^{ef}	1747 ^{f}	FID	***
Benzyl alcohol	1006	28.8 ^{a}	28.2 ^{a}	17.6 $^{a–e}$	18.1 ^{abcd}	13.3 ^{cde}	8.3 ^{de}	5.4 ^{e}	13.9 ^{cde}	24.7 ^{abc}	20.6 ^{abcd}	17.1 $^{a–e}$	27.3 ^{ab}	13.3 ^{cde}	15.6 ^{bcde}	23.8 ^{abc}	23.4 ^{abc}	MS	***
Phenylethanol	1114	65,111 ^{a}	39,700 ^{fgh}	42,221 $^{e–h}$	44,515 $^{c–h}$	59,463 ^{ab}	33,640 ^{hi}	38,444 ^{gh}	55,211 ^{abcd}	56,100 ^{abc}	57,063 ^{ab}	43,931 $^{d–h}$	52,081 $^{b–e}$	48,973 $^{b–g}$	50,587 $^{b–f}$	23,541 ^{i}	37,526 ^{gh}	FID	***
1-Nonanol	1166	3.2 ^{de}	2.8 ^{de}	3.3 ^{de}	2.7 ^{de}	4.1 ^{bcde}	5.7 ^{bc}	4.8 ^{bcd}	1.9 ^{e}	3.3 ^{de}	3.8 ^{bcde}	2.8 ^{de}	3.4 ^{cde}	5.9 ^{b}	3.9 ^{bcde}	8.9 ^{a}	4.8 ^{bcd}	MS	***
Terpenoids																			
cis-Linalol oxide	1066	2.4 ^{a}	2.3 ^{a}	2.0 ^{ab}	1.5 ^{b}	0.3 ^{c}	0.4 ^{c}	0.3 ^{c}	0.1 ^{c}	0.5 ^{c}	0.6 ^{c}	0.6 ^{c}	0.5 ^{c}	0.1 ^{c}	0.2 ^{c}	0.4 ^{c}	0.3 ^{c}	MS	***
à-Terpineol	1176	3.9 ^{bc}	5.4 ^{a}	2.5 $^{c–g}$	1.4 ^{fg}	2.9 ^{bcde}	3.3 ^{bcd}	2.9 $^{b–f}$	1.8 ^{defg}	3.3 ^{bcd}	4.2 ^{ab}	2.6 $^{c–g}$	1.8 ^{efg}	1.3 ^{g}	1.3 ^{g}	2.4 ^{defg}	1.5 ^{efg}	MS	***
Eugenol	1339	2.2 ^{bc}	3.5 ^{a}	2.6 ^{b}	2.2 ^{bc}	0.5 ^{fg}	0.5 ^{fg}	0.1 ^{fg}	0.1 ^{fg}	0.7 ^{fg}	0.6 ^{fg}	0.1 ^{g}	0.1 ^{g}	1.7 ^{cd}	1.5 ^{cde}	0.8 ^{efg}	0.9 ^{def}	MS	***
(E)-β-Damascenone	1384	1.2 $^{a–e}$	1.2 $^{a–e}$	1.6 ^{ab}	0.3 ^{de}	0.4 ^{cde}	1.2 $^{a–e}$	1.7 ^{a}	0.2 ^{e}	1.2 $^{a–e}$	1.3 ^{abcd}	1.6 ^{ab}	0.3 ^{de}	1.1 $^{a–e}$	0.8 $^{a–e}$	1.5 ^{abc}	0.5 $^{b–e}$	MS	***
Geranyl acetone	1434	2.7 ^{ab}	2.3 ^{bc}	2.9 ^{ab}	0.7 ^{d}	2.8 ^{ab}	3.6 ^{a}	3.6 ^{a}	0.5 ^{d}	2.7 ^{ab}	3.1 ^{ab}	3.1 ^{ab}	0.9 ^{ab}	1.0 ^{d}	1.2 ^{cd}	2.9 ^{ab}	0.9 ^{d}	MS	***

Table 5. *Cont.*

	LRI	Węgierka Zwykła				Węgierka Dąbrowicka				Stanley				Čačanska Lepotica				Methods	Significance
		S. cerevisiae S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED	*S. cerevisiae* S1	*H. uvarum* H2	Spontaneous Fermentation	Ethanol RED		
Carbonyl compounds																			
Acetaldehyde	360	6490 ^{de}	24,700 ^{b}	5310 ^{ef}	6440 ^{de}	770 ^{g}	4530 ^{efg}	4440 ^{efg}	10,340 ^{cd}	6710 ^{de}	12,990 ^{c}	6690 ^{de}	43,000 ^{a}	5460 ^{e}	13,580 ^{c}	13,860 ^{c}	1150 ^{fg}	FID	***
Acetone	469	2886 ^{b}	2800 ^{b}	2133 ^{b}	2233 ^{b}	2367 ^{bc}	1267 ^{c}	2113 ^{bc}	2656 ^{b}	1244 ^{c}	1233 ^{c}	9172 ^{a}	952 ^{c}	2213 ^{bc}	3444 ^{b}	2786 ^{b}	3133 ^{b}	FID	***
Hexanal	780	4.22 ^{d}	4.00 ^{d}	7.94 ^{bc}	1.08 ^{fg}	3.41 ^{def}	1.40 ^{efg}	12.74 ^{a}	0.00 ^{g}	4.28 ^{d}	3.54 ^{de}	9.94 ^{b}	0.79 ^{g}	3.26 ^{def}	0.00 ^{g}	7.04 ^{c}	1.53 ^{efg}	MS	***
Benzaldehyde	930	2.7 ^{fg}	3.5 ^{ef}	6.6 ^{cd}	4.9 ^{de}	3.3 ^{ef}	2.3 ^{fg}	6.5 ^{cd}	9.6 ^{a}	3.5 ^{ef}	3.4 ^{ef}	7.4 ^{bc}	8.8 ^{ab}	1.5 ^{g}	1.3 ^{g}	7.4 ^{bc}	3.7 ^{ef}	MS	***
2-Heptenal	936	0.6 ^{bc}	0.8 ^{a}	0.7 ^{ab}	0.2 ^{d}	0.2 ^{d}	0.0 ^{e}	0.5 ^{c}	0.1 ^{de}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	0.0 ^{e}	MS	***
Lactones																			
Butyrolactone	908	2.6 ^{b}	4.6 ^{b}	6.3 ^{b}	5.8 ^{b}	24.7 ^{ab}	197.9 ^{a}	177.6 ^{ab}	5.9 ^{b}	17.5 ^{ab}	6.7 ^{b}	8.8 ^{b}	6.4 ^{b}	10.9 ^{b}	2.9 ^{b}	3.7 ^{b}	7.3 ^{b}	MS	**
(R)-γ-decalactone	1428	1.7 ^{c}	1.7 ^{c}	1.7 ^{c}	1.2 ^{c}	34.0 ^{ab}	24.9 ^{b}	43.9 ^{a}	6.9 ^{c}	1.6 ^{c}	1.4 ^{c}	1.5 ^{c}	1.2 ^{c}	0.5 ^{c}	1.2 ^{c}	1.0 ^{c}	0.6 ^{c}	MS	***
ç-Dodecalactone	1655	1.6 ^{d}	1.8 ^{d}	1.9 ^{d}	1.6 ^{d}	13.4 ^{a}	5.4 ^{c}	9.4 ^{b}	2.5 ^{cd}	2.5 ^{cd}	2.1 ^{cd}	2.4 ^{cd}	2.4 ^{cd}	0.2 ^{d}	0.1 ^{d}	0.2 ^{d}	0.2 ^{d}	MS	***

Values with different superscript roman letters (a–e) in the same row indicate statistically significant differences at $p < 0.05$; $n = 5$; 0.001 ***; 0.01 **; 0.05 *. LRI—linear retention index; the amounts of components were determined semi-quantitatively by measuring the relative peak area of each identified compound, according to the National Institute of Standards and Technology (NIST) database, in relation to that of the internal standard. MS—SPME–GC–MS (Solid Phase Microextraction–Gas Chromatography–Mass Spectrometry); FID—GC–FID (Gas Chromatography–Flame Ionization Detector. Color determination from lowest (0%) to highest (100%) concentration of volatile compounds [µg/L]. The highest concentration of a specific compound in a row is in the darkest green and the lowest content is in the darkest red.



In the current study, the most abundant aldehydes in fresh musts were 5-methyl-3-hexanone (fruity aroma) and hexanal (grassy scents). Moreover, except for hexanal, we identified other aldehydes and alcohols of six-carbon atoms in all cultivars. 2-hexenal, hexanal, 2-hexenol, 3-hexenol, and hexanol are characteristic for plums and contribute to the green note of the fruit. Those compounds might be formed when the fruit tissues are crushed or blended and the compounds of cytosol and cell walls are exposed to lipoxygenases. The presence of these compounds is probably due to lipoxygenase activity, which is initialized by the disruption of the fruit tissues when it is crushed or blended [35]. Other carbonyl compound identified in plum musts was nonanal (woody-like aroma) which could be found in waxes that cover the skin of plum fruit [35]. Some carbonyl compounds were characteristic for particular cultivars (regardless of the type of fermentation carried out), e.g., 2-heptenal in fermented musts obtained from Węgierka Dąbrowicka and Węgierka Zwykład and 2-buten-1-one, from Węgierka Dąbrowicka and Čačanska Lepotica.

Some of the carbonyl compounds were also identified in jerkums and their concentration was usually higher in samples fermented spontaneously than in other types of fermentation. These compounds included, e.g., hexanal, benzaldehyde, and 2-heptenal.

The acetaldehyde is one of the most important carbonyl compounds produced during fermentation, which at low levels contributes to fruity flavors, while high concentrations (200 mg/L) cause flatness in wines [36]. It was present in all analyzed jerkums. Its concentration did not exceed this value and was not dependent on the type of fermentation used.

Among terpenoids, linalool, p-cymene, geraniol, and geranyl acetone were dominant compounds in analyzed plum musts. Those compounds (with the exception of geraniol) were also detected in plums *Prunus domestica* L. cv. Horvin as described by Pino et al. [25]. The terpenoids, namely the monoterpenols, were reported as volatile components of fruits responsible for a wide spectrum of aromas, mostly perceived as very pleasant. The compounds are responsible for the varietal aroma of fruits, and at least some of them are present in their glycosidic compounds. The concentration of linalool, which is also present in fresh plum, may increase during technological processing, mostly, by its release from glycosidic forms. That process may occur due to the heat release [37,38].

Bornylene is an interesting compound which was not detected in plum fruit, so far, however its occurrence was confirmed in kiwi fruit and wines obtained from that fruit [39]. However, this compound was present in Węgierka Zwykła and Čačanska Lepotica cultivars in the current study. After fermentation, the profile of terpenoids was changed and the highest concentration of those compounds was determined in samples fermented spontaneously or with *H. uvarum* H2. Despite the fact that analyzed samples were immediately frozen after fermentation and stored at −18 °C, some volatile compounds could be oxidized or transformed into each other. Probably, linalool oxidized to cis-linalool oxide (which was present after fermentation) and the aroma of the latter volatile is less intense. In addition to the oxidation processes, other enzymatic and non-enzymatic reactions occur during storage. Terpenoids are reduced by 50–60% after 3 months of storage of wines [40]. It was also noted that during that period those compounds undergo various transformations which result in the formation of cyclic compounds, ketones, lactones (α-(alpha), β-ionone (beta), and vitispirane [40]. Transformation of terpenoids requires appropriate conditions, e.g., in acidic solutions, geraniol is converted to the cyclic terpene α-terpineol [41], which also occurred in jerkums (Table 5).

The last group of volatile compounds in the analyzed plums was lactones. Among them, γ-nonanolactone and γ-decanolactone were detected. Lactones are formed from the corresponding hydroxy acids. These compounds, particularly γ-lactones, are important compounds in terms of their contribution to the aroma and, in general, pleasant fruity aroma descriptors [37]. Some studies confirmed γ-dodecalactone as the major lactone in plums, especially in Japanese plum (*P. salicina*) and candied plum (*P. domestica*) [25,37]. Completely different lactones were found in the fermented samples—γ-butyrolactone and ç-dodecalactone. The γ-butyrolactone, the most common and important lactone in

fermented foods, has a creamy, oily, fatty, or caramel aroma and a milky, creamy taste, with fruity peach-like after-scents [42].

4. Conclusions

Our research proved that fermentation type had a significant impact on chemical composition and volatile profile of plum jerkums. Among four analyzed cultivars of plums, Węgierka Zwykła and Stanley demonstrated most diverse profile of volatile compounds. Musts fermented with *H. uvarum* H2 presented higher concentration of terpenoids, e.g., α-terpineol, eugenol, and esters, e.g., ethyl propanoate, methyl hexanoate, isoamyl lactate, than samples fermented with Ethanol RED. As well as in the case of esters, the concentration of some higher alcohols was higher in musts fermented with *H. uvarum* H2 or *S. cerevisiae* S1, e.g., propanol, isobutanol, butanol, and 2-phenylethanol. Concentration of the last of those compounds was also high in samples fermented with Ethanol RED (*S. cerevisiae*), which confirms that the *Sacharomyces* genus is able to produce significant amounts of 2-phenylethanol. Despite the fact that the majority of non-*Saccharomyces* yeasts produce high levels of volatile compounds, their application could cause technological disadvantages. However, in the current paper, the application of indigenous strains of yeast demonstrated desired results, e.g., highest fermentation efficiency and concentration of ethanol was determined in musts fermented with Ethanol RED (*S. cerevisiae)* and also with *S. cerevisiae* S1, while lowest efficiency was shown for musts fermented spontaneously. Based on our results it could be concluded that indigenous strains of yeast present in plums demonstrate great potential for the production of plum jerkums of high quality. In the future we are planning to involve olfactometer detector for the determination of sensory active aroma compounds.

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