



## Article

# Influence of *L. thermotolerans* and *S. cerevisiae* Commercial Yeast Sequential Inoculation on Aroma Composition of Red Wines (Cv Trnjak, Babić, Blatina and Frankovka)

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**Abstract:** Even though *Saccharomyces cerevisiae* starter cultures are still largely used nowadays, the non-*Saccharomyces* contribution is re-evaluated, showing positive enological characteristics. Among them, *Lachancea thermotolerans* is one of the key yeast species that are desired for their contribution to wine sensory characteristics. The main goal of this work was to explore the impact of *L. thermotolerans* commercial yeast strain used in sequential inoculation with *S. cerevisiae* commercial yeast on the main enological parameters and volatile aroma profile of Trnjak, Babić, Blatina, and Frankovka red wines and compare it with wines produced by the use of *S. cerevisiae* commercial yeast strain. In all sequential fermented wines, lactic acid concentrations were significantly higher, ranging from 0.20 mg/L in Trnjak up to 0.92 mg/L in Frankovka wines, while reducing alcohol levels from 0.1% *v/v* in Trnjak up to 0.9% *v/v* in Frankovka wines. Among volatile compounds, a significant increase of ethyl lactate and isobutyl acetate, geraniol, and geranyl acetate was detected in all wines made by use of *L. thermotolerans*. In Babić wines, the strongest influence of sequential fermentation was connected with higher total terpenes and total ester concentrations, while Trnjak sequentially fermented wines stood up with higher total aldehyde, volatile phenol, and total lactone concentrations. Control wines, regardless of variety, stood up with higher concentrations of total higher alcohols, especially isoamyl alcohol. The present work contributed to a better understanding of the fermentation possibilities of selected non-*Saccharomyces* strains in the overall red wine quality modeling.

**Keywords:** *L. thermotolerans*; volatile aroma compounds; red grape varieties



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## 1. Introduction

Wine quality is influenced by many factors starting from the geographical origin of the grapes, varietal grape must composition, vinification process, and microbial activity of yeast species used. Wine is a complex mixture of chemical compounds that contribute differently to overall quality. Among them, volatile aroma compounds that can be divided according to their origin into varietal (grape) aromas, fermentative aromas, and aging aromas are some of the most important contributors to flavor perception. Between grape varieties, there is a notable sensory difference in aroma composition that is usually not really perceptible at pre-fermentative stages but strongly influenced by microbial activity during wine production [1]. Nowadays in winemaking, *Saccharomyces cerevisiae* commercial starter cultures are still largely used with the main goal being the assurance

of more predictable and desired final wine quality results. However, some evidence suggests that the continuous use of commercial yeast can significantly reduce the variability of autochthonous yeasts as well as aromatic complexity and uniqueness of the wine [2,3]. In the last decade, the contribution and important role of non-*Saccharomyces* wine yeasts were re-evaluated in many works [2,4–7] showing positive enological characteristics that are more or less absent in *S. cerevisiae*. Among them, *Lachancea thermotolerans* is one of the key yeast species that is desired for their positive contribution to wine sensory characteristics [8]. According to Gobbi et al. [9], the association of *L. thermotolerans* and *S. cerevisiae* significantly reduced ethanol levels from 0.7 to 0.9% *v/v*, especially when fermentation was carried out at lower temperatures. In the work by Binati et al. [7] the highest potential to reduce ethanol content was achieved by the use of *L. thermotolerans* strains. The possibility to increase lactic acid concentrations and at the same time reduce volatile acidity was confirmed by [4,10], while increased production of 2-phenylethyl alcohol was described as a characteristic of *L. thermotolerans* by Beckner et al. [11]. The same authors noted significantly higher production of terpenes nerol and terpine-4-ol as well as 3-methylthio-1-propanol. A study evaluating the impact of several non-*Saccharomyces* yeasts in sequential inoculation with *S. cerevisiae* showed that an *L. thermotolerans*–*S. cerevisiae* combination had the most potential for increased chemical complexity of the Shiraz volatile profile [12]. In the work by Whitener et al. [13], *L. thermotolerans* fermentation showed a higher amount of acetate esters and certain terpenes but also the lowest amount of both total acidity and malic acid, which is in agreement with previous data that had indicated *S. cerevisiae* as a poor L-malate metabolizer compared to non-*Saccharomyces* yeasts. Nowadays, based on previously published results, commercial non-*Saccharomyces* starter cultures have been developed for use in wine production, but compared to *S. cerevisiae*, little work has been done with commercial starter cultures that can point out what specific chemical profile to expect based on grape variety and overall fermentation conditions. The varieties Trnjak, Babić, and Blatina are native red grapevine varieties grown in the Dalmatia wine region (Croatia) used for the production of high-quality red wines. Typically, they have lower levels of total acidity and higher pH values in grape juice and wine, and this is especially expressed in years with extremely high temperatures. Frankovka (syn. Blaufraenkisch) is a variety mostly distributed in the continental part of Croatia and Istria but also in neighboring regions of Slovenia, Hungary, and Austria. Usually, it is used for the production of fresh and fruity red wines, which are also hard to obtain in years with elevated temperatures, which have become more and more frequent in the last decades. The aims of the present study were to explore the impact of the *L. thermotolerans* commercial yeast strain (Laktia, Lallemand Inc., Montreal, QC, Canada), used in sequential inoculation with *S. cerevisiae* commercial yeast (Uvaferm BDX, Lallemand Inc. Montreal, QC, Canada), on the main enological parameters and volatile aroma profiles of Trnjak, Babić, Blatina, and Frankovka red wines and to compare it with control wines produced by use of an *S. cerevisiae* commercial strain. The present work contributes to a better understanding of the fermentation possibilities of selected commercial non-*Saccharomyces* strains in overall red wine quality modeling.

## 2. Materials and Methods

### 2.1. Yeast Strains

The commercial *S. cerevisiae* and *L. thermotolerans* strains were provided from Lallemand Inc., Montreal, QC, Canada as active dry yeasts. Both yeast strains were precultured in the same grape must at 25 °C for 72 h. Each yeast strain was added at approximately  $1 \times 10^7$  cells/mL, and fermentations were carried out at 20 °C according to the manufacturer's instructions. The cell concentrations were determined by counting under a light microscope (Zeiss Axioscope2-Plus microscope (Carl Zeiss Ltd., Oberkochen, Germany).

## 2.2. Fermentation Trials

Grape varieties Trnjak and Blatina were grown in the Mostar vineyard, Bosnia and Herzegovina, while the other two grape varieties were grown in Croatia, namely Babic in the Jadrtovac vineyard (located near Šibenik) and Frankovka in the experimental Jazbina vineyard (located in Zagreb). For each grape variety (Blatina, Trnjak, Babić, Frankovka), 150 kg of grapes harvested in 2019 was destemmed, crushed, and distributed evenly into three 50 L stainless steel fermenters. Basic chemical composition of the grapes was as follows: for Blatina, initial sugar 220 g/L, total acidity 6.05 g/L as tartaric acid, yeast assimilable nitrogen 240 mg/L, and pH 3.39; for Trnjak, 205 g/L, total acidity 7.03 g/L as tartaric acid, yeast assimilable nitrogen 270 mg/L, and pH 3.52; for Babić, initial sugar 235 g/L, total acidity 7.60 g/L as tartaric acid, yeast assimilable nitrogen 220 mg/L, and pH 3.30; for Frankovka, initial sugar 230 g/L, total acidity 7.75 g/L as tartaric acid, yeast assimilable nitrogen 245 mg/L, and pH 3.32. In all variants, sulfur dioxide (SO<sub>2</sub>), in a concentration of 50 mg/L, was added to prevent oxidation and inhibit indigenous bacterial or fungal growth. The control variants were inoculated by *S. cerevisiae* Uvaferm BDX (control culture), while the sequential variants were inoculated with *L. thermotolerans* LAKTIA strain with the addition of the *S. cerevisiae* Uvaferm BDX after 2 days of fermentation. The maceration process, at 20 °C, lasted for 7 days, and during that period, mash aeration and cap management were carried out by mechanical mixing. Alcoholic fermentation finished by the end of the maceration process, and at that moment wines were separated from the pomace, and the solid pulp left behind was pressed by use of a hydropress (Lancman VS-A 80, Gomark d.o.o., Vranksko, Slovenia). Free run wines and pressed wines were mixed. The course of fermentation was monitored by sugar consumption, and it was considered complete when the residual sugar concentrations were under 1.5 g/L. In all variants, fermentation started 24 h after inoculation and lasted between 10 and 12 days. In that period, fermentation kinetics was monitored by the decomposition of sugars showing no marked difference. The final wines were bottled in 750 mL glass bottles with screw caps and transported to the laboratory of the Department of Viticulture and Enology, Faculty of the Agriculture University of Zagreb, for chemical analysis.

## 2.3. Physicochemical Analysis

Basis wine parameters including alcohol content (% *v/v*), pH values, and total and volatile acidity were quantified applying methods recommended by the International Organization of Vine and Wine (OIV, 2016) [14].

## 2.4. Organic Acids Analysis

Analysis of individual acids (malic and lactic acid) was done by an Agilent Series 1100 HPLC system equipped with a diode array detector (Agilent, Palo Alto, CA, USA). In brief, the determination was performed isocratically with the flow rate set to 0.6 mL/min with 0.065% phosphoric acid (p.a. Merck, Darmstadt, Germany) as a mobile phase. An Aminex HPX-87H column, 300 × 7.8 mm i.d. (Bio-Rad Laboratories, Hercules, CA, USA), was heated at 65 °C, while the detector was set to 210 nm [15].

## 2.5. Volatile Compounds Determination

Volatile compound analysis of wine samples was performed according to the described method [15]. Isolation of analytes was performed by solid-phase extraction (SPE) on LiChrolut EN cartridges (200 mg/3 mL, Merck, Darmstadt, Germany). First, 50 mL of sample was loaded to the column that was previously conditioned by successive washing with 3 mL dichloromethane (UHPLC gradient grade J.T. Baker, Deventer, The Netherlands), methanol (UHPLC gradient grade J.T. Baker, Deventer, The Netherlands), and 13% aqueous ethanol (LiChrosolv, Merck, Darmstadt, Germany) solution. After the passage of the sample through the column, residual sugars and other polar compounds were washed out with 3 mL of water. The column was dried by the passing of air. The evaluation of analytes was done by 1 mL of dichloromethane. As a quality control, 50 mL of water was loaded to the SPE

column instead of the sample. Quantitative and qualitative analyses were performed on a Thermo Scientific Trace 1300 system coupled with ISQ 7000 mass spectrometer with a ZB-WAX column (60 m × 0.32 mm i.d., with 0.5 µm film thickness, Phenomenex, Torrance, CA, USA). The temperature program was as follows: 40 °C for 15 min, from 40 to 250 °C with increments of 2 °C per minute, and 250 °C for 15 min. The transfer line was set to 250 °C, and the flow rate of helium was 1 mL/min. The MS was operated in electron ionization (EI) mode at 70 eV with total ion current (TIC) monitoring. Identification was done by comparing retention times and mass spectra with those of standards. A list of used standards, linear retention indices, and other parameters for identification and quantification are presented in Supplementary Materials Table S1. Quantification was done by calibration curves. The curves (based on quantification ions) were constructed with Chromeleon™ Chromatography Data System (CDS) software. For all available standards (Table S1), six different concentrations were prepared. For two compounds (Terpendiol I and II) semi-quantitative analysis was performed. Their concentrations were expressed in equivalents of similar compounds, with the assumption that a response factor was equal to one.

### 2.6. Determination of Odor Activity Values and Relative Odor Contributions

Each chemical substance can have a specific influence on the wine aroma. It can be presented by the odor activity value (OAV) and relative odor contributions (ROCs). Thus, they can be used as markers in determining the role of a specific compound in the sample aroma composition. OAV is calculated as the quotient of its concentration (c) and corresponding odor detection threshold (t) reported in the literature [16]. Volatile aroma substances with an  $OAV \geq 1$  can have a direct impact on aroma, and they are usually marked as one of the most significant volatile substances or the most active odors [17]. Volatiles with  $OAVs < 1$  can also positively influence the wine aroma complexity and aromatic intensity of other compounds through synergistic effects [18]. The ROC of each aroma compound is calculated as the ratio of the OAV of the respective compound to the total OAVs of each wine [19].

### 2.7. Statistical Analysis

Means and standard deviations were calculated for all parameters related to physicochemical properties of wines as well as for all the volatile organic compounds obtained after analyses. One-way ANOVA was performed for all parameters separately due to the significant differences among the four cultivars studied; to define common effects of *L. thermotolerans* yeast in sequential fermentation with *S. cerevisiae* against control wine, data for volatile organic compounds were standardized within cultivars using z-score normalization. One-way ANOVA and two-sided Dunnett test were performed using standardized data to compare the treatment (*L. thermotolerans*) with control for data from all four cultivars. The analysis was carried out with XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA).

## 3. Results and Discussion

### 3.1. Physicochemical Composition

The results of basic physicochemical analysis of wines are presented in Table 1 showing that the use of *L. thermotolerans* yeast in sequential fermentation with *S. cerevisiae* can be used as one useful tool for alcohol content reduction in wines by the production of lactic acid, thus leading to biological acidification. Previous studies [7,20] have already pointed out that the use of non-*Saccharomyces* yeasts can reduce the alcohol content of wine, which is in accordance with our data. Reducing alcohol levels ranged from 0.1% v/v in Trnjak wines up to 0.9% v/v in Frankovka wines. In the work by Sgouros et al. [21], the alcohol reduction by use of the high lactate-producing *L. thermotolerans* strain (P-HO1) in sequential inoculation with *S. cerevisiae*, produced the highest levels of lactic acid ever recorded in mixed fermentations (10.4 g/L), increasing thereby the acidity and reducing ethanol by 1.6% vol. In our work, lactic acid concentrations were also significantly higher in all

sequential fermented wines, not depending on variety, ranging from 0.20 mg/L in Trnjak wines up to 0.92 mg/L in Frankovka wines. Natural *S. cerevisiae* strains produce only traces of D-lactic acid during alcoholic fermentation, and levels between 100 and 500 mg/L have been reported in final wines [22]. Higher lactic acid concentrations had a positive effect on total acidity and pH values of sequential fermented wines, ensuring better wine stability as well as aging potential and overall quality. This is especially important nowadays with global climate change influencing grape composition and resulting in lower acidity and increasing sugar concentrations [23]. Volatile acidity is one of the important parameters influencing wine quality, and it is also strongly dependent on the type of yeast conducting alcoholic fermentation. In the past, non-*Saccharomyces* yeasts were considered undesired and one of the reasons was higher acetic acid production. Nowadays, published studies have generated highly variable results, showing that some of them can have desirable enological properties connected with low production of volatile acidity [4]. Among non-*Saccharomyces* yeasts, *L. thermotolerans* stood out as a low acetic acid producer, which has been shown in our work, with volatile acidity not differing compared to values achieved in fermentation conducted by *S. cerevisiae* commercial yeast. Differences observed in malic acid concentrations could be connected with the esterification process, resulting in diethyl malate presence (Table 1) or the weak but possible ability of *S. cerevisiae* to metabolize L-malic acid during wine fermentation [22].

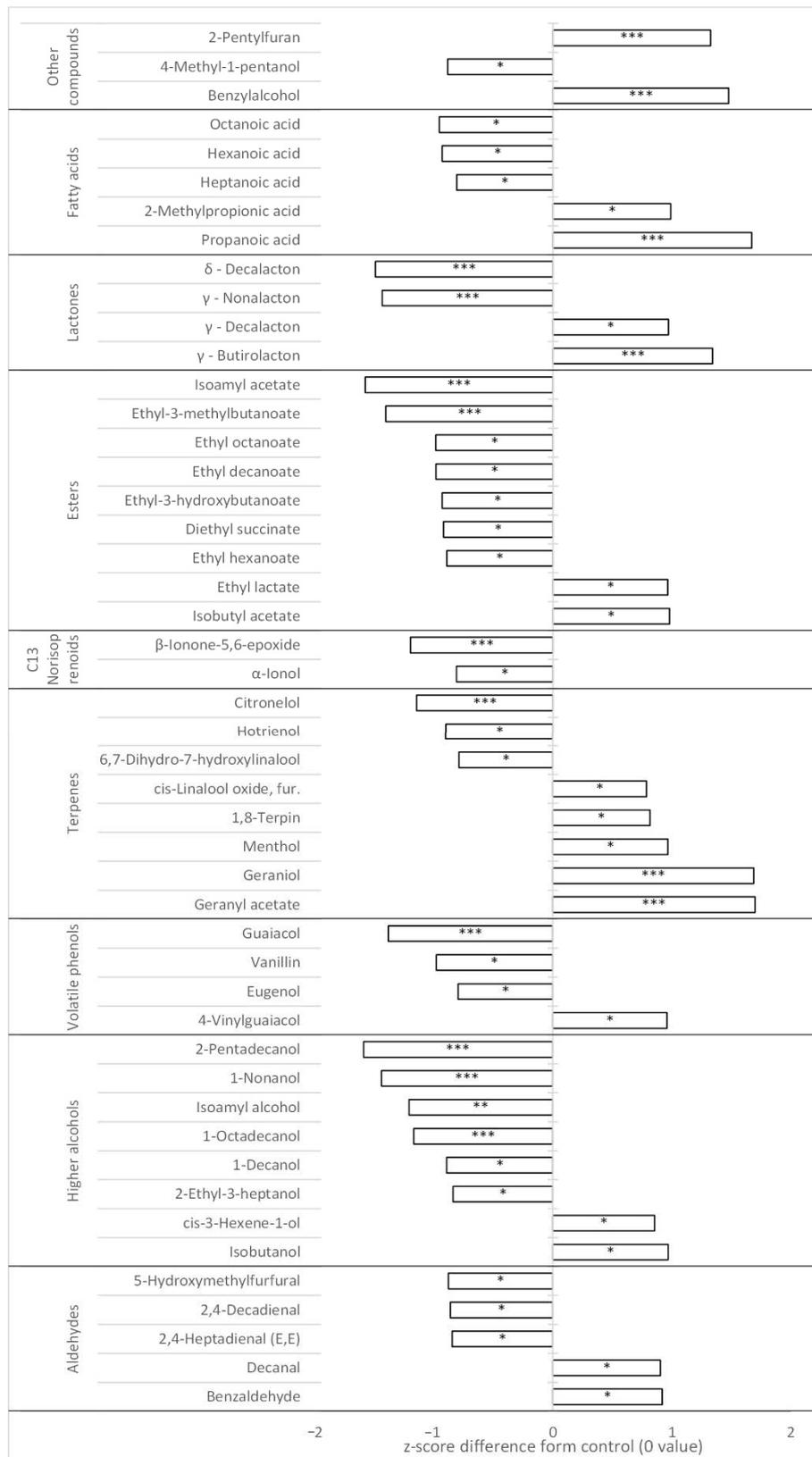
**Table 1.** Physicochemical properties of Babić, Blatina, Frankovka, and Trnjak wines.

	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Compounds								
Alcohol (% v/v)	13.7 ± 0.1 <sup>a</sup>	13.0 ± 0.0 <sup>b</sup>	12.9 ± 0.0 <sup>a</sup>	12.5 ± 0.0 <sup>b</sup>	13.5 ± 0.0 <sup>a</sup>	12.6 ± 0.1 <sup>b</sup>	11.8 ± 0.0 <sup>a</sup>	11.7 ± 0.0 <sup>a</sup>
Total acidity* (g/L)	6.60 ± 0.04 <sup>b</sup>	7.75 ± 0.02 <sup>a</sup>	5.33 ± 0.05 <sup>b</sup>	5.85 ± 0.07 <sup>a</sup>	7.55 ± 0.04 <sup>b</sup>	10.10 ± 0.02 <sup>a</sup>	4.82 ± 0.03 <sup>b</sup>	6.90 ± 0.04 <sup>a</sup>
Volatile acidity** (g/L)	0.47 ± 0.01 <sup>a</sup>	0.50 ± 0.00 <sup>a</sup>	0.44 ± 0.00 <sup>a</sup>	0.54 ± 0.01 <sup>b</sup>	0.64 ± 0.00 <sup>a</sup>	0.67 ± 0.00 <sup>a</sup>	0.37 ± 0.00 <sup>a</sup>	0.35 ± 0.01 <sup>a</sup>
pH	3.40 ± 0.01 <sup>a</sup>	3.33 ± 0.00 <sup>b</sup>	3.46 ± 0.01 <sup>a</sup>	3.35 ± 0.00 <sup>b</sup>	3.38 ± 0.00 <sup>a</sup>	3.30 ± 0.00 <sup>b</sup>	3.86 ± 0.01 <sup>a</sup>	3.76 ± 0.01 <sup>b</sup>
Malic acid (g/L)	0.79 ± 0.05 <sup>a</sup>	0.62 ± 0.03 <sup>b</sup>	1.08 ± 0.04 <sup>a</sup>	0.76 ± 0.05 <sup>b</sup>	0.75 ± 0.01 <sup>a</sup>	0.50 ± 0.03 <sup>b</sup>	1.14 ± 0.02 <sup>a</sup>	1.10 ± 0.01 <sup>a</sup>
Lactic acid (g/L)	0.16 ± 0.02 <sup>b</sup>	0.99 ± 0.04 <sup>a</sup>	0.09 ± 0.05 <sup>b</sup>	0.81 ± 0.02 <sup>a</sup>	0.11 ± 0.04 <sup>b</sup>	1.09 ± 0.01 <sup>a</sup>	0.12 ± 0.02 <sup>b</sup>	0.32 ± 0.04 <sup>a</sup>

\* Tartaric acid and \*\* acetic acid equivalents. Concentrations expressed as mean ± standard deviation ( $n = 3$ ). Means with different superscript letters, for each variety separately, in the same row differ significantly ( $p \leq 0.05$ ).

### 3.2. Volatile Compound Composition

In Table 2 one hundred and twenty-one individual volatile compounds are presented, quantified, and classified into several chemical classes (aldehydes, higher alcohols, volatile phenols, terpenes, C13-norisoprenoids, lactones, esters, fatty acids, sulfur compounds, other compounds, other alcohols), showing significant difference among red wines produced by the use of pure *S. cerevisiae* commercial yeast and the combination of *L. thermotolerans* and *S. cerevisiae* commercial yeast within four cultivars. Significant varietal effects were obtained for the majority of volatile compounds except for *trans*-3-hexene-1-ol, tyrosol, 1,8-terpin, 8-hydroxylinalool, neralidol, menthol,  $\beta$ -ionone-5,6-epoxide, nonanoic acid, 1,4-butanediol, and acetoin. For this reason, standardized data were used to define the common effects of *L. thermotolerans* and *S. cerevisiae* on volatile compounds. In Figure 1, results of the two-sided Dunnett test using standardized data (z-scores) are presented only for volatile compounds with significant differences against the control for all cultivars.



**Figure 1.** Common significant effects of *L. thermotolerans* yeast in sequential fermentation with *S. cerevisiae* against control wines expressed as the difference of z-score from control (presented as 0 value) for all four cultivars using z-score standardization within cultivars for volatile aroma compounds with significant effect only; significance level: \*  $p < 0,05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  with two-sided Dunnett test.

### 3.2.1. Aldehydes

Aldehyde concentration is connected with the degree of ripeness, treatments before fermentation, enzymatic oxidation, and breakdown of grape lipids, as well as variety. Comparing Babić, Blatina, Frankovka, and Trnjak total aldehydes concentrations, the highest ones were detected in Trnjak wines, while there were no marked differences between the others. Trnjak wines were also the only ones with a positive influence of sequential fermentation on total aldehyde concentration as well as 5-hydroxymethylfurfural and furfural concentrations. In order to protect themselves, yeasts reduce both furfural and HMF to their furyl acid or alcohol derivatives through NAD(P)H-dependent reductive pathways that utilize a range of aldehyde dehydrogenases involved in glycolysis and ethanol fermentation. Under aerobic conditions, *S. cerevisiae* transforms furfural to furoic acid, while under anaerobic fermentation, the primary product is furfuryl alcohol [24]. These detoxification processes lead to a lack of NADH, suggesting that furfural reduction competes for NADH and results in a decrease in cell growth and ethanol formation [25,26]. Accordingly, *L. thermotolerans* may have a stronger ability to reduce these aldehydes, even though there was no significant difference in furfuryl alcohol production between control and sequential fermentation wines. Decanal, as the only individual aldehyde with OAV > 1 in Blatina, Frankovka, and Trnjak wines produced with *L. thermotolerans*, was significantly higher compared to control wines with a notable odor contribution.

### 3.2.2. C13-Norisoprenoids and Terpenes

These two groups of chemical compounds primarily generate the varietal odor profile of wines that are characterized by floral and fruity aromas and are mainly translocated from the grape to the must during the crushing, pressing, and settling process in free volatile form or bound to sugars. Thus, higher enzymatic activity by the action of endogenous or exogenous glycosidase enzymes during the winemaking process can influence their release. Previous works [27–29] have shown those non-*Saccharomyces* yeasts and among them also certain strains of *L. thermotolerans* can have high  $\beta$ -glucosidase activity. Only in Babić wines was total terpene concentration significantly higher in sequentially fermented wines due to the higher concentrations of linalool, 8-hydroxylinalool, tetrahydrolinalool, farnesol, neral, geraniol, and geranyl acetate. Significantly higher concentrations of geraniol and geranyl acetate were present in all sequentially fermented variants, not depending on variety, which is in accordance with data published by Beckner Whitener et al. [13]. Farnesol has also been positively connected with *L. thermotolerans* activity [13], while in the work by Whitener et al. [12], linalool was indicated as a key compound in Shiraz wines with higher amounts in *L. thermotolerans*-*S. cerevisiae* sequential fermentation. In Blatina and Trnjak wines, no significant difference was detected in total terpene concentrations among variants, but among detected individual terpenes, the concentrations of 1,8-terpin stood up showing significantly higher concentrations in Blatina, Trnjak, and also Frankovka wine samples produced by sequential fermentation. Neral concentrations were higher in Babić and Blatina wines, while nerol was presented in higher concentrations in Frankovka and Trnjak sequential variants. Terpene-4-ol was also among compounds pointed out as one whose concentration can be influenced by *L. thermotolerans* activity [11]. Our data showed a significant increase in Blatina and Trnjak wines. Only in Frankovka control wines was total terpene concentration significantly higher compared to sequentially fermented wines, mainly due to the presence of linalool and citronellol, which showed higher ROCs (Table 2). In addition, as shown in Figure 1, significantly lower concentrations of citronellol were presented in all sequential fermentation wines compared to the control. Comparing total C13-norisoprenoids concentrations, no significant influence of *L. thermotolerans* yeast, not depending on variety, was noted, while only in Blatina control wines were higher concentrations of  $\beta$ -damascenone and TDN noted.

### 3.2.3. Higher Alcohols and Esters

Among fermentation aroma compounds, higher alcohols and esters can be strongly influenced by the type of yeasts used and fermentation conditions [4]. The concentrations of higher alcohols not exceeding the amount of 300 mg/L can positively influence the formation of wine complexity [30], which was not the case in our samples. Slightly higher concentrations were present in Frankovka and Trnjak wines, mainly due to 2-methyl-1-butanol content, but as can be seen from Table 2, with values under the odor detection threshold. In the analyzed red wines, total higher alcohol concentrations were significantly higher in control variants, except in Trnjak wines, where no marked differences were noted. There was a 13% lower total concentration of higher alcohols, with the greatest difference observed for isoamyl alcohol when *L. thermotolerans* was used, which was also reported by [20]. Gobbi et al. [9] also reported that in sequential inoculation, *L. thermotolerans* reduced isoamyl alcohol and isobutanol concentrations. In our work, isoamyl alcohol reduction was also noted in all sequential variants, not depending on variety, while isobutanol concentrations differed according to variety, with higher concentrations in Babić, Frankovka, and Trnjak sequentially fermented wines and lower in Blatina wines. Escribano et al. [31] pointed out *L. thermotolerans* as a top 1-propanol and 1-hexanol producing species when a pure culture was used, while in our study, results were different between varieties. In Babić wines, sequential fermentation positively influenced 1-hexanol concentrations, in Trnjak there were no differences, while in Blatina and Frankovka control wines, higher concentrations were present. Among all higher alcohols detected, only 1-hexanol was above the OAV. Higher phenylethyl alcohol was detected in Babić and Trnjak sequentially produced wines, while in Blatina and Trnjak wines, higher concentrations were presented in control wines. Similar results were presented in the work by Comitini et al. [27], where just one of the *L. thermotolerans* strains tested showed a statistically significant difference in phenylethyl alcohol concentration, while Benito et al. [20] noted that between non-*Saccharomyces* yeasts tested, *L. thermotolerans* was the best producer of phenylethyl alcohol but with lower concentrations compared to fermentation by *S. cerevisiae* yeast. Chen et al. [32] observed in *L. thermotolerans* conducted fermentation a decrease of approximately 15 mg/L of phenylethyl alcohol compared to the wines produced with *S. cerevisiae* yeast, while no differences were detected for 2-phenylethyl acetate. In our study, total esters concentrations were significantly higher in Babić and Frankovka sequentially fermented wines, while in Blatina and Trnjak wines, higher concentrations were in control variants. Babić and Trnjak sequentially produced wines that had higher concentrations of 2-phenylethyl acetate, even though in the work by Chen et al. [32], no differences were noted. Isoamyl acetate stood out with significantly lower concentrations in all sequentially fermented wines, which is in accordance with data published by [9]. Among ethyl esters, the most abundant was ethyl lactate, whose concentrations were significantly higher in all wines produced by sequential fermentation as a result of the greater lactic acid production involved with *L. thermotolerans*, which is also in accordance with previously published data [32,33]. From the data presented in Figure 1, it can be noted that in sequentially fermented wines, esters and higher alcohols were mainly presented in lower concentrations compared to control wines.

### 3.2.4. Fatty Acids

Initial must composition, as well as agricultural conditions and variety, can have a strong influence on fatty acids present in wine [34,35], which was confirmed by our data. In our work, total fatty acid concentrations were significantly higher in Babić and Frankovka sequentially fermented wines, mainly due to higher 2-methylpropionic acid concentrations, while in the other two, there was no difference. Fatty acid concentrations can also be significantly influenced by *L. thermotolerans* in combined fermentations, where lower production of hexanoic and octanoic acid was noted [27], which was also the case in Blatina, Frankovka, and Trnjak sequentially fermented wines. Babić wines produced with *L. thermotolerans* were the only ones with a higher concentration of isovaleric acid, which

has been pointed out by previously published work [31] as one whose concentrations can also be influenced by the action of *L. thermotolerans*.

### 3.2.5. Lactones

Lactones mostly arise from the cyclization of the corresponding  $\gamma$ -hydroxycarboxylic acids, which are unstable molecules that can be formed by glutamic acid deamination and the decarboxylation process, pantolactone being an example [36–38]. Lactones may also come from grapes, as is the case in Riesling, where they contribute to the varietal aroma [39,40]. Our results show that  $\gamma$ -butyrolactone was the most abundant lactone in all analyzed wines, with significantly higher concentrations in Babić, Frankovka, and Trnjak sequentially produced wines in which also total lactone concentrations were significantly higher compared to control wines. In the work by Escribano et al. [31] *L. thermotolerans* was a higher  $\gamma$ -butyrolactone producer when compared with some non-*Saccharomyces* yeasts, but with no significant difference when compared to *S. cerevisiae*. Nakamura et al. [41] analyzed  $\gamma$ -nonalactone in 38 Californian and French wines, in which  $\gamma$ -nonalactone concentrations ranged from 0 to 16  $\mu\text{g/L}$  in white samples and 12 to 43  $\mu\text{g/L}$  in red ones. Concentrations of  $\gamma$ -nonalactone in our wines were in agreement with the results of Nakamura et al. [41] but significantly higher in Babić, Frankovka, and Trnjak control wines.

### 3.2.6. Volatile Phenols

Volatile phenols, such as guaiacol, eugenol, vanillin, 4-vinylguaiacol, and 4-vinylphenol, are relevant components of the hydrolysates obtained from fractions of precursors extracted from grapes or wines [42,43]. Among them, vinylguaiacol and vinylphenol can be formed by yeast phenolic acid decarboxylases or by enzymatic or acid hydrolyses of their glycosides, having a strong influence on wine quality if present at high levels [44]. Significantly higher concentrations of 4-vinylguaiacol and 4-vinylphenol were noted in Babić and Trnjak sequentially fermented wines but with no impact on wine sensory profile, as OAVs were  $<1$ . According to our data only eugenol concentrations were above the odor detection threshold, but with no significant differences between variants except in Babić wines, where control wines were more abundant. Even though the levels of vanillin derived from the grape cannot rival levels released by some types of oak wood, they can be released from a large number of grape precursors, for instance during enzymatic hydrolysates from grape berry skin or by oxidation of 4-vinylguaiacol [43]. Diversity in vanillin concentrations between *V. vinifera* aromatic varieties was also noted in work by D'Onofrio et al. [45]. In our work, higher concentrations of vanillin in Babić and Frankovka control wines may be connected with lower concentrations of 4-vinylguaiacol in the same ones.

**Table 2.** Individual volatile compound concentrations ( $\mu\text{g/L}$ ) of Blatina, Babić, Frankovka, and Trnjak red wines. Concentrations expressed as mean  $\pm$  standard deviation ( $n = 3$ ). Means with different superscript letters, for each variety separately, in the same row differ significantly ( $p \leq 0.05$ ).

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
<b>Aldehydes</b>										
2,4-Decadienal	270 [46]	Floral [47]	$0.17 \pm 0.01^a$	$0.04 \pm 0.02^b$	$0.34 \pm 0.01^a$	$0.12 \pm 0.01^b$	$0.31 \pm 0.04^a$	$0.18 \pm 0.01^b$	$0.11 \pm 0.01^a$	$0.14 \pm 0.02^a$
2,4-Heptadienal (E,E)			$3.53 \pm 0.06^a$	$2.66 \pm 0.05^b$	$4.47 \pm 0.03^a$	$3.85 \pm 0.01^b$	$2.68 \pm 0.44^a$	$1.60 \pm 0.21^a$	$0.10 \pm 0.02^a$	$0.13 \pm 0.01^a$
2,4-Hexadienal			$1.88 \pm 0.11^a$	$1.55 \pm 0.01^a$	$1.46 \pm 0.05^a$	$1.14 \pm 0.03^b$	$1.31 \pm 0.14^a$	$1.36 \pm 0.11^a$	$1.31 \pm 0.19^a$	$1.54 \pm 0.04^a$
2,4-Nonadienal	0.09 [48]	Cucumber [26]	$0.09 \pm 0.01^a$	$0.02 \pm 0.01^b$	$0.05 \pm 0.01^a$	$0.08 \pm 0.01^a$	$0.56 \pm 0.01^b$	$0.77 \pm 0.03^a$	$0.37 \pm 0.31^a$	$0.44 \pm 0.39^a$
2-Octenal			$1.29 \pm 0.49^b$	$2.15 \pm 0.09^a$	$4.21 \pm 1.01^a$	$3.72 \pm 0.66^a$	$6.35 \pm 0.74^a$	$6.64 \pm 0.78^a$	$1.27 \pm 0.48^b$	$4.11 \pm 0.47^a$
5-Hydroxymethyl-furfural	100,000 [49]	Almond [50]	$2.75 \pm 0.05^a$	$0.38 \pm 0.01^b$	$0.61 \pm 0.06^a$	$0.18 \pm 0.02^b$	$1.23 \pm 0.01^a$	$0.04 \pm 0.01^b$	$0.15 \pm 0.19^a$	$0.20 \pm 0.02^a$
Benzaldehyde	4 [51]		$14.90 \pm 0.28^a$	$15.71 \pm 0.67^a$	$11.73 \pm 0.59^a$	$9.84 \pm 0.03^b$	$34.14 \pm 1.58^a$	$27.52 \pm 2.33^a$	$34.60 \pm 1.24^b$	$50.00 \pm 8.60^a$
Benzaldehyde	350 [43]	Bitter, almond [52]	$7.54 \pm 0.06^b$	$7.81 \pm 0.06^a$	$3.50 \pm 0.28^b$	$4.38 \pm 0.02^a$	$8.51 \pm 0.21^a$	$7.30 \pm 0.16^b$	$122.19 \pm 6.80^b$	$155.29 \pm 10.11^a$
Decanal	0.1–2 [53]		$2.67 \pm 0.01^a$	$2.19 \pm 0.10^b$	$1.74 \pm 0.06^b$	$2.12 \pm 0.01^a$	$3.48 \pm 0.10^b$	$3.89 \pm 0.01^a$	$1.60 \pm 0.16^b$	$2.18 \pm 0.23^a$
Furfural	770 [54]	Almond, yeast [55]	$5.31 \pm 0.69^a$	$2.07 \pm 0.01^b$	$1.93 \pm 0.09^a$	$1.74 \pm 0.02^a$	$0.65 \pm 0.01^a$	$0.13 \pm 0.01^b$	$3.23 \pm 0.23^b$	$6.79 \pm 0.48^a$
$\Sigma$			$40.11^a$	$34.56^b$	$30.01^a$	$27.17^b$	$59.20^a$	$49.42^b$	$164.86^b$	$220.74^a$
<b>Higher alcohols</b>										
1-Butanol	150,000 [56]	Medicinal [43]	$185.27 \pm 1.41^a$	$153.08 \pm 3.13^b$	$168.70 \pm 1.63^a$	$155.30 \pm 1.54^b$	$156.25 \pm 2.85^b$	$243.53 \pm 1.00^a$	$108.29 \pm 9.09^b$	$192.29 \pm 13.62^a$
1-Decanol	5000 [57]	Pear, waxy, violet [57]	$5.23 \pm 0.01^a$	$4.80 \pm 0.03^b$	$1.15^b \pm 0.01^b$	$6.05 \pm 0.01^a$	$2.20 \pm 0.01^a$	$0.64 \pm 0.04^b$	$2.73 \pm 0.33^a$	$1.89 \pm 0.35^b$
1-Heptanol	425 [51]	Oily [47]	$29.01 \pm 0.92^a$	$32.15 \pm 1.58^a$	$19.22 \pm 0.62^a$	$17.68 \pm 0.71^a$	$19.34 \pm 0.03^a$	$7.78 \pm 0.16^b$	$32.72 \pm 0.96^a$	$31.72 \pm 4.75^a$
1-Hexanol	2500 [55]	Grass just cut [43]	$1464.07 \pm 1.56^b$	$1484.66 \pm 1.46^a$	$1872.75 \pm 4.77^a$	$1764.33 \pm 1.21^b$	$868.37 \pm 1.45^a$	$729.93 \pm 0.52^b$	$2581.10 \pm 41.61^a$	$2642.37 \pm 225.20^a$
1-Nonanol			$4.18 \pm 0.10^a$	$3.78 \pm 0.01^b$	$5.64 \pm 0.08^a$	$4.29 \pm 0.01^b$	$4.98 \pm 0.13^a$	$1.50 \pm 0.06^b$	$5.40 \pm 0.27^a$	$4.58 \pm 1.20^a$
1-Octadecanol			$22.97 \pm 0.69^a$	$0.22 \pm 0.01^b$	$0.34 \pm 0.01^a$	$0.33 \pm 0.04^a$	$25.16 \pm 0.56^a$	$0.07 \pm 0.03^b$	$1.74 \pm 0.16^a$	$0.29 \pm 2.59^a$
1-Octanol	110–130 [53]	Chemical [43]	$34.66 \pm 0.66^b$	$39.62 \pm 0.70^a$	$9.45 \pm 0.04^b$	$11.79 \pm 0.49^a$	$15.14 \pm 1.10^a$	$11.12 \pm 0.30^b$	$22.99 \pm 1.85^a$	$10.46 \pm 2.69^b$
1-Pentanol	64,000 [37]	Bitter, almond, balsamic [37]	$0.48 \pm 0.01^b$	$34.97 \pm 0.97^a$	$32.37 \pm 1.06^a$	$30.04 \pm 0.73^a$	$30.42 \pm 1.20^a$	$26.80 \pm 0.13^a$	$0.10 \pm 0.06^a$	$0.13 \pm 0.02^a$

Table 2. Cont.

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
2-Pentadecanol			$0.95 \pm 0.01^a$	$0.24 \pm 0.02^b$	$0.34 \pm 0.04^a$	$0.17 \pm 0.03^b$	$1.01 \pm 0.02^a$	$0.33 \pm 0.01^b$	$3.07 \pm 0.32^a$	$2.28 \pm 0.51^a$
2-Pentene-1-ol			$0.14 \pm 0.04^b$	$0.38 \pm 0.01^a$	$3.07 \pm 0.03^a$	$2.65 \pm 0.01^b$	$0.24 \pm 0.07^a$	$0.15 \pm 0.07^a$	$2.77 \pm 0.20^b$	$3.85 \pm 0.30^a$
2-Methyl-1-butanol	30,000 [58]	Whiskey, burnt, nail polish [59]	$13,752.80 \pm 69.76^b$	$14,600.50 \pm 1.87^a$	$12,760.75 \pm 3.58^b$	$13,794.74 \pm 3.50^a$	$19,820.03 \pm 396.27^a$	$16,713.06 \pm 437.03^b$	$25,883.52 \pm 353.92^a$	$26,051.04 \pm 77.75^a$
2-Ethyl-1-hexanol			$3.93 \pm 0.08^b$	$5.50 \pm 0.07^a$	$0.11 \pm 0.01^a$	$0.10 \pm 0.01^a$	$1.11 \pm 0.01^a$	$0.77 \pm 0.01^b$	$2.14 \pm 0.17^a$	$2.68 \pm 0.58^a$
2-Ethyl-3-heptanol			$1.66 \pm 0.01^a$	$0.96 \pm 0.04^b$	$1.91 \pm 0.11^a$	$1.63 \pm 0.03^a$	$0.15 \pm 0.07^a$	$0.10 \pm 0.05^a$	$0.26 \pm 0.29^a$	$0.35 \pm 0.22^a$
<i>trans</i> -2-Hexene-1-ol	100 [60]	Herbaceous, green [47]	$11.73 \pm 0.78^a$	$11.75 \pm 0.73^a$	$5.08 \pm 0.02^a$	$4.89 \pm 0.13^a$	$5.63 \pm 0.42^b$	$7.90 \pm 0.01^a$	$7.87 \pm 0.19^a$	$7.48 \pm 0.79^a$
<i>cis</i> -3-Hexene-1-ol	400 [43]	Grass, green [43]	$16.57 \pm 0.07^b$	$21.59 \pm 0.76^a$	$76.66 \pm 0.69^a$	$72.26 \pm 0.47^b$	$18.95 \pm 0.69^a$	$19.95 \pm 0.37^a$	$141.29 \pm 5.90^b$	$190.06 \pm 18.90^a$
<i>trans</i> -3-Hexene-1-ol	1000 [43]	Grass, resinous, cream [43]	$47.73 \pm 0.74^a$	$32.66 \pm 19.75^a$	$30.22 \pm 1.05^a$	$29.88 \pm 0.61^a$	$25.74 \pm 0.88^a$	$28.33 \pm 0.78^a$	$31.50 \pm 0.78^a$	$31.29 \pm 3.71^a$
Phenylethyl alcohol	14,000 [61]	Floral, rose, honey [57]	$5176.93 \pm 1.85^b$	$6465.90 \pm 4.74^a$	$6974.79 \pm 0.50^a$	$6695.82 \pm 0.47^b$	$9971.16 \pm 0.51^a$	$6307.75 \pm 0.52^b$	$7311.89 \pm 188.33^a$	$7536.44 \pm 708.53^a$
Isoamyl alcohol	30,000 [58]	Alcohol, nail polish [57]	$12,130.23 \pm 7.52^a$	$5566.17 \pm 0.70^b$	$10,960.85 \pm 6.62^a$	$10,430.94 \pm 0.34^b$	$17,083.44 \pm 23.84^a$	$14,469.53 \pm 23.50^b$	$9621.08 \pm 4725.72^a$	$9022.92 \pm 2075.75^b$
Isobutanol	40,000 [37]	Alcohol, nail polish [57]	$4548.47 \pm 1.29^b$	$6089.32 \pm 6.86^a$	$3414.33 \pm 5.55^a$	$3055.67 \pm 3.63^b$	$6135.18 \pm 0.98^b$	$6702.63 \pm 3.66^a$	$3370.62 \pm 160.10^b$	$5077.13 \pm 394.26^a$
$\Sigma$			$37,436.96^a$	$34,548.24^b$	$36,337.69^a$	$36,078.53^b$	$54,184.46^a$	$45,271.83^b$	$49,131.05^a$	$50,809.20^a$
<b>Volatile phenols</b>										
4-Vinylguaiacol	40 [62]	Clove, curry [43]	$0.93 \pm 0.08^b$	$1.57 \pm 0.02^a$	$19.62 \pm 0.71^a$	$15.01 \pm 0.06^b$	$0.90 \pm 0.01^b$	$3.19 \pm 0.08^a$	$10.51 \pm 0.56^b$	$26.23 \pm 4.79^a$
4-Vinylphenol	180 [61]	Phenolic, medicinal [43]	$0.30 \pm 0.02^b$	$1.04 \pm 0.01^a$	$28.49 \pm 1.48^a$	$26.75 \pm 0.99^a$	$3.54 \pm 0.01^a$	$1.07 \pm 0.03^b$	$6.96 \pm 1.00^b$	$50.32 \pm 4.95^a$
Eugenol	6 [54]	Cinnamon, clove [43]	$2.38 \pm 0.04^a$	$1.87 \pm 0.03^b$	$9.39 \pm 0.22^a$	$9.12 \pm 0.09^a$	$4.08 \pm 0.04^a$	$4.28 \pm 0.23^a$	$33.98 \pm 0.87^a$	$30.16 \pm 4.02^a$
Guaiacol	9.5 [55]	Smoky, hospital [55]	$5.70 \pm 0.29^a$	$2.25 \pm 0.05^b$	$1.48 \pm 0.01^a$	$1.07 \pm 0.04^b$	$1.95 \pm 0.06^a$	$1.87 \pm 0.04^a$	$3.40 \pm 0.09^a$	$2.86 \pm 0.74^a$
Homovanillyl alcohol			$85.19 \pm 0.57^b$	$90.39 \pm 1.12^a$	$17.21 \pm 0.08^a$	$16.78 \pm 0.95^a$	$124.39 \pm 1.66^a$	$118.71 \pm 0.74^b$	$41.77 \pm 2.30^b$	$72.86 \pm 10.72^a$

Table 2. Cont.

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Vanillin	200 [58]	Vanilla [43]	$17.72 \pm 1.40^a$	$5.57 \pm 0.22^b$	$8.15 \pm 0.01^a$	$7.18 \pm 0.02^b$	$7.76 \pm 0.31^a$	$4.19 \pm 0.11^b$	$16.46 \pm 2.05^a$	$17.44 \pm 2.00^a$
$\Sigma$			$112.22^a$	$102.69^a$	$84.32^a$	$75.89^a$	$142.60^a$	$133.30^a$	$113.11^b$	$199.87^a$
<b>Terpenes</b>										
1,8-Terpin			$1.77 \pm 0.04^a$	$1.01 \pm 0.02^b$	$0.36 \pm 0.01^b$	$1.43 \pm 0.06^a$	$0.95 \pm 0.06^b$	$1.26 \pm 0.01^a$	$0.99 \pm 0.22^b$	$2.08 \pm 0.88^a$
6,7-Dihydro-7-hydroxylinalool			$33.04 \pm 0.93^a$	$9.23 \pm 0.08^b$	$6.56 \pm 0.15^a$	$5.46 \pm 0.13^b$	$12.60 \pm 0.59^a$	$12.05 \pm 1.07^a$	$5.50 \pm 0.96^a$	$6.18 \pm 2.41^a$
8-Hydroxylinalool			$1.74 \pm 0.02^b$	$3.61 \pm 0.02^a$	$2.01 \pm 0.06^a$	$26.33 \pm 34.33^a$	$27.60 \pm 0.89^a$	$24.17 \pm 0.28^b$	$9.35 \pm 1.84^a$	$13.72 \pm 3.09^a$
$\alpha$ -Pinene			$0.12 \pm 0.01^a$	$0.10 \pm 0.01^a$	$0.04 \pm 0.01^b$	$0.54 \pm 0.02^a$	$0.22 \pm 0.01^a$	$0.15 \pm 0.06^a$	$1.10 \pm 0.26^a$	$1.99 \pm 0.90^a$
$\alpha$ -Terpineol	330 [63]	Lilac, floral, sweet [43]	$1.95 \pm 0.06^a$	$1.18 \pm 0.02^b$	$2.37 \pm 0.04^a$	$2.75 \pm 0.21^a$	$9.90 \pm 0.21^a$	$8.42 \pm 0.42^b$	$6.87 \pm 0.19^a$	$7.28 \pm 1.07^a$
2,6-Dimethyl-3,7-octadiene-2,6-diol			$0.87 \pm 0.03^a$	$0.08 \pm 0.01^b$	$0.17 \pm 0.04^a$	$0.17 \pm 0.03^a$	$55.68 \pm 0.63^a$	$52.18 \pm 1.41^a$	$0.87 \pm 0.23^a$	$1.10 \pm 0.41^a$
2,6-Dimethyl-7-octene-2,6-diol			$32.62 \pm 0.70^a$	$7.98 \pm 0.01^b$	$5.93 \pm 0.01^a$	$1.74 \pm 0.02^b$	$1.89 \pm 0.09^b$	$2.48 \pm 0.01^a$	$4.98 \pm 0.48^a$	$7.37 \pm 2.82^a$
$\beta$ -Ocimene			$0.69 \pm 0.06^a$	$0.38 \pm 0.02^b$	$0.56 \pm 0.04^a$	$0.82 \pm 0.09^a$	$0.43 \pm 0.04^a$	$0.28 \pm 0.02^b$	$0.38 \pm 0.17^a$	$0.27 \pm 0.05^a$
$\alpha$ -Farnesen			$1.83 \pm 0.03^a$	$1.18 \pm 0.01^b$	$0.64 \pm 0.07^a$	$0.73 \pm 0.03^a$	$0.73 \pm 0.06^a$	$0.78 \pm 0.01^a$	$1.21 \pm 0.03^a$	$0.93 \pm 0.55^a$
<i>cis,trans</i> - $\alpha$ -Farnesene			$1.74 \pm 0.02^a$	$1.75 \pm 0.04^a$	$1.18 \pm 0.02^a$	$1.13 \pm 0.04^a$	$1.95 \pm 0.06^a$	$1.65 \pm 0.01^b$	$1.82 \pm 0.27^b$	$2.44 \pm 0.16^a$
<i>trans</i> - $\beta$ -Farnesene	87 [64]		$0.83 \pm 0.08^a$	$0.73 \pm 0.02^a$	$0.95 \pm 0.04^a$	$0.72 \pm 0.01^b$	$0.85 \pm 0.06^a$	$0.66 \pm 0.05^a$	$0.59 \pm 0.10^a$	$0.68 \pm 0.05^a$
<i>cis</i> - $\beta$ -Farnesene			$2.27 \pm 0.03^a$	$1.18 \pm 0.01^b$	$0.87 \pm 0.02^a$	$0.65 \pm 0.01^b$	$1.18 \pm 0.01^a$	$0.90 \pm 0.08^b$	$0.90 \pm 0.08^b$	$1.43 \pm 0.13^a$
<i>cis</i> -Linalool oxide, furan	6000 [55]	Flower [55]	$0.57 \pm 0.03^a$	$0.65 \pm 0.04^a$	$0.16 \pm 0.01^a$	$0.01 \pm 0.00^b$	$0.91 \pm 0.02^b$	$1.04 \pm 0.01^a$	$0.44 \pm 0.22^a$	$0.81 \pm 0.17^a$
Citronellol	40 [63]	Rose [65]	$39.05 \pm 0.28^a$	$35.06 \pm 0.89^b$	$23.84 \pm 0.45^a$	$22.99 \pm 1.00^a$	$59.13 \pm 0.33^a$	$42.88 \pm 0.54^b$	$45.75 \pm 2.55^a$	$43.35 \pm 7.64^a$
Farnesol	20 [66]	Floral, clove [47]	$2.39 \pm 0.14^b$	$14.13 \pm 0.21^a$	$8.40 \pm 0.23^a$	$7.33 \pm 0.08^b$	$43.92 \pm 0.29^a$	$39.77 \pm 0.65^b$	$9.44 \pm 0.35^a$	$9.12 \pm 2.82^a$
Geraniol	20 [61]	Citrus [43]	$6.65 \pm 2.11^b$	$12.05 \pm 1.24^a$	$0.96 \pm 0.04^b$	$4.42 \pm 0.10^a$	$0.56 \pm 0.04^b$	$14.10 \pm 0.18^a$	$12.03 \pm 0.42^b$	$17.39 \pm 1.73^a$
Geranyl acetate	9 [67]	Flowery [67]	$4.41 \pm 0.05^b$	$8.83 \pm 0.03^a$	$3.54 \pm 0.01^b$	$3.67 \pm 0.04^a$	$5.47 \pm 0.01^b$	$7.04 \pm 0.01^a$	$3.11 \pm 0.43^b$	$6.82 \pm 1.27^a$
Hotrienol	110 [52]	Fresh, floral, sweet [52]	$12.31 \pm 1.34^a$	$8.84 \pm 0.01^a$	$1.74 \pm 0.01^b$	$2.62 \pm 0.04^a$	$155.03 \pm 0.13^a$	$100.95 \pm 0.28^b$	$2.83 \pm 0.33^a$	$0.82 \pm 0.82^b$
Linalool	25 [61]	Citrus, floral, sweet [43]	$22.31 \pm 1.25^b$	$30.66 \pm 0.85^a$	$22.93 \pm 0.32^a$	$21.56 \pm 0.69^a$	$76.33 \pm 0.08^a$	$40.06 \pm 0.09^b$	$18.82 \pm 0.36^a$	$18.57 \pm 3.05^a$

Table 2. Cont.

	ODT (µg/L)	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Neral			1.16 ± 0.01 <sup>b</sup>	4.09 ± 0.03 <sup>a</sup>	1.04 ± 0.02 <sup>b</sup>	1.15 ± 0.01 <sup>a</sup>	4.98 ± 0.07 <sup>a</sup>	1.64 ± 0.02 <sup>b</sup>	2.34 ± 0.15 <sup>a</sup>	0.85 ± 0.25 <sup>b</sup>
Nerolidol	250 [51]	Rose, apple, green, waxy [47]	1.16 ± 0.04 <sup>a</sup>	0.95 ± 0.06 <sup>a</sup>	0.64 ± 0.01 <sup>b</sup>	1.25 ± 0.06 <sup>a</sup>	0.85 ± 0.04 <sup>a</sup>	0.76 ± 0.04 <sup>a</sup>	1.04 ± 0.20 <sup>a</sup>	0.85 ± 0.23 <sup>a</sup>
Neric acid			3.26 ± 0.04 <sup>b</sup>	3.56 ± 0.05 <sup>a</sup>	3.09 ± 0.01 <sup>a</sup>	2.74 ± 0.06 <sup>b</sup>	3.89 ± 0.10 <sup>a</sup>	3.85 ± 0.06 <sup>a</sup>	3.07 ± 0.47 <sup>a</sup>	3.01 ± 0.24 <sup>a</sup>
Nerol	300 [63]	Rose, fruity, floral [43]	3.45 ± 0.01 <sup>a</sup>	3.51 ± 0.52 <sup>a</sup>	1.95 ± 0.01 <sup>a</sup>	1.59 ± 0.01 <sup>b</sup>	1.15 ± 0.01 <sup>b</sup>	3.68 ± 0.04 <sup>a</sup>	5.01 ± 0.18 <sup>b</sup>	6.07 ± 0.58 <sup>a</sup>
Menthol			0.63 ± 0.05 <sup>b</sup>	1.14 ± 0.02 <sup>a</sup>	0.43 ± 0.06 <sup>b</sup>	0.91 ± 0.06 <sup>a</sup>	0.67 ± 0.03 <sup>b</sup>	0.85 ± 0.01 <sup>a</sup>	1.07 ± 0.34 <sup>a</sup>	0.92 ± 0.26 <sup>a</sup>
Ocimenol			0.90 ± 0.01 <sup>a</sup>	0.64 ± 0.07 <sup>b</sup>	2.66 ± 0.05 <sup>a</sup>	0.02 ± 0.01 <sup>b</sup>	1.37 ± 0.19 <sup>a</sup>	0.06 ± 0.04 <sup>b</sup>	2.56 ± 0.64 <sup>b</sup>	4.46 ± 0.98 <sup>a</sup>
Terpendiol I			3.21 ± 0.12 <sup>a</sup>	1.77 ± 0.03 <sup>b</sup>	6.03 ± 0.03 <sup>a</sup>	5.38 ± 0.02 <sup>b</sup>	0.21 ± 0.02 <sup>a</sup>	0.30 ± 0.02 <sup>a</sup>	2.06 ± 0.12 <sup>a</sup>	0.18 ± 3.30 <sup>b</sup>
Terpendiol II			0.65 ± 0.01 <sup>b</sup>	1.10 ± 0.01 <sup>a</sup>	0.40 ± 0.08 <sup>a</sup>	0.45 ± 0.06 <sup>a</sup>	74.29 ± 0.95 <sup>b</sup>	94.80 ± 0.59 <sup>a</sup>	1.99 ± 1.04 <sup>a</sup>	0.80 ± 0.41 <sup>a</sup>
Terpinene-4-ol			4.38 ± 0.13 <sup>a</sup>	2.16 ± 0.04 <sup>b</sup>	1.97 ± 0.03 <sup>b</sup>	2.57 ± 0.04 <sup>a</sup>	0.97 ± 0.03 <sup>a</sup>	0.54 ± 0.02 <sup>b</sup>	4.84 ± 4.15 <sup>b</sup>	6.52 ± 0.68 <sup>a</sup>
Tetrahydrolinalool			21.68 ± 0.78 <sup>b</sup>	89.23 ± 0.65 <sup>a</sup>	33.82 ± 0.47 <sup>a</sup>	31.93 ± 0.69 <sup>a</sup>	28.00 ± 0.93 <sup>a</sup>	19.43 ± 0.43 <sup>b</sup>	11.60 ± 0.28 <sup>a</sup>	10.50 ± 1.68 <sup>a</sup>
Linalyl formate			2.33 ± 0.03 <sup>a</sup>	0.28 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	2.44 ± 0.04 <sup>a</sup>	0.33 ± 0.03 <sup>b</sup>	0.74 ± 0.01 <sup>a</sup>	0.25 ± 0.14 <sup>a</sup>	0.27 ± 0.23 <sup>a</sup>
<b>Σ</b>			209.89 <sup>b</sup>	247.06 <sup>a</sup>	135.19 <sup>a</sup>	155.41 <sup>a</sup>	571.97 <sup>a</sup>	477.40 <sup>b</sup>	162.80 <sup>a</sup>	176.80 <sup>a</sup>
<b>C13 Norisoprenoids</b>										
α-Ionol			0.32 ± 0.01 <sup>a</sup>	0.26 ± 0.05 <sup>a</sup>	0.29 ± 0.06 <sup>a</sup>	0.24 ± 0.02 <sup>a</sup>	0.30 ± 0.01 <sup>a</sup>	0.21 ± 0.04 <sup>a</sup>	0.41 ± 0.03 <sup>a</sup>	0.44 ± 0.16 <sup>a</sup>
β-Ionon	3.4 [68]	Flora l [68]	0.13 ± 0.03 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.17 ± 0.04 <sup>a</sup>	0.17 ± 0.03 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.02 <sup>a</sup>	0.53 ± 0.02 <sup>a</sup>	0.60 ± 0.07 <sup>a</sup>
β-Ionone-5,6-epoxide			0.28 ± 0.02 <sup>a</sup>	0.16 ± 0.01 <sup>b</sup>	0.06 ± 0.02 <sup>a</sup>	0.04 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>	0.28 ± 0.20 <sup>a</sup>	0.17 ± 0.11 <sup>a</sup>
β-Damascenone	0.05 [58]	Sweet, fruity, floral, honey [61]	9.24 ± 0.42 <sup>a</sup>	8.51 ± 0.02 <sup>a</sup>	3.76 ± 0.01 <sup>a</sup>	2.45 ± 0.05 <sup>b</sup>	5.54 ± 0.02 <sup>a</sup>	5.61 ± 0.25 <sup>a</sup>	4.79 ± 0.25 <sup>a</sup>	6.52 ± 1.17 <sup>a</sup>
TDN	2 [69]	Petrol, kerosene [55]	1.10 ± 0.01 <sup>a</sup>	1.24 ± 0.07 <sup>a</sup>	0.40 ± 0.01 <sup>a</sup>	0.32 ± 0.01 <sup>b</sup>	0.71 ± 0.02 <sup>a</sup>	0.62 ± 0.06 <sup>a</sup>	0.54 ± 0.02 <sup>a</sup>	0.87 ± 0.46 <sup>a</sup>
<b>Σ</b>			11.06 <sup>a</sup>	10.26 <sup>a</sup>	4.67 <sup>a</sup>	3.20 <sup>b</sup>	6.83 <sup>a</sup>	6.69 <sup>a</sup>	6.55 <sup>a</sup>	8.59 <sup>a</sup>
<b>Esters</b>										
Ethyl butanoate	20 [54]	Pineapple, apple, peach [57]	174.39 ± 1.16 <sup>b</sup>	190.00 ± 1.34 <sup>a</sup>	65.44 ± 1.63 <sup>b</sup>	77.66 ± 0.05 <sup>a</sup>	166.28 ± 4.18 <sup>a</sup>	106.34 ± 0.49 <sup>b</sup>	142.49 ± 4.12 <sup>a</sup>	127.40 ± 14.59 <sup>a</sup>

Table 2. Cont.

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Ethyl decanoate	200 [62]	Floral, grape, fruity [59]	$12.97 \pm 0.45^b$	$38.47 \pm 1.69^a$	$18.07 \pm 0.81^a$	$10.90 \pm 0.35^b$	$28.38 \pm 0.71^a$	$13.85 \pm 0.73^b$	$28.47 \pm 0.14^a$	$9.20 \pm 2.30^b$
Ethyl furoate	16,000 [54]		$0.23 \pm 0.04^b$	$2.13 \pm 0.03^a$	$3.24 \pm 0.08^a$	$0.06 \pm 0.01^b$	$3.29 \pm 0.01^a$	$2.35 \pm 0.02^b$	$1.94 \pm 1.25^a$	$1.54 \pm 0.16^a$
Ethyl hexanoate	14 [61]	Fruity, green apple, banana [59]	$346.14 \pm 1.05^b$	$403.95 \pm 1.88^a$	$76.55 \pm 1.51^a$	$69.89 \pm 0.28^b$	$207.57 \pm 2.20^a$	$204.53 \pm 0.83^a$	$381.84 \pm 4.62^a$	$187.62 \pm 28.00^b$
Ethyl hydrogen succinate			$0.15 \pm 0.00^b$	$20.03 \pm 0.62^a$	$2707.20 \pm 2.91^a$	$2395.19 \pm 0.53^b$	$0.20 \pm 0.00^b$	$5.93 \pm 0.06^a$	$6361.92 \pm 117.27^a$	$2854.00 \pm 906.31^b$
Ethyl lactate	154,000 [54]	Butter [57]	$1754.17 \pm 1.44^b$	$2889.01 \pm 0.16^a$	$3307.43 \pm 2.89^a$	$3108.26 \pm 2.14^a$	$1528.19 \pm 2.88^b$	$3189.99 \pm 15.93^a$	$1844.03 \pm 58.14^b$	$2419.02 \pm 145.90^a$
Ethyl linalyl acetal			$0.48 \pm 0.01^a$	$0.26 \pm 0.01^b$	$0.25 \pm 0.01^b$	$0.44 \pm 0.02^a$	$0.65 \pm 0.06^a$	$0.54 \pm 0.01^a$	$0.37 \pm 0.09^a$	$0.36 \pm 0.15^a$
Ethyl linoleate			$0.01 \pm 0.04^b$	$0.45 \pm 0.01^a$	$0.44 \pm 0.01^b$	$1.61 \pm 0.02^a$	$1.56 \pm 0.01^a$	$0.65 \pm 0.06^b$	$0.29 \pm 0.55^a$	$0.72 \pm 0.20^a$
Ethyl octanoate	580 [62]	Sweet, floral, fruity, pear [57]	$217.15 \pm 2.98^b$	$367.23 \pm 2.76^a$	$95.21 \pm 1.86^a$	$76.83 \pm 0.45^b$	$187.34 \pm 0.03^a$	$110.71 \pm 0.72^b$	$249.47 \pm 2.11^a$	$80.23 \pm 16.60^b$
Ethyl vanillate	3000 [62]	Creamy, vanilla [59]	$0.01 \pm 0.03^a$	$0.01 \pm 0.08^a$	$0.28 \pm 0.02^a$	$0.06 \pm 0.04^b$	$0.04 \pm 0.01^a$	$0.08 \pm 0.01^a$	$0.04 \pm 0.02^a$	$0.10 \pm 0.06^a$
Ethyl-2-hydroxy-3-methyl butanoate			$7.04 \pm 0.11^b$	$31.11 \pm 0.24^a$	$11.75 \pm 0.56^a$	$10.91 \pm 0.39^a$	$9.10 \pm 0.08^a$	$6.57 \pm 0.02^b$	$3.63 \pm 0.11^a$	$3.16 \pm 0.60^a$
Ethyl-2-methylbutanoate	18 [54]	Apple, strawberry [59]	$6.17 \pm 0.16^a$	$1.19 \pm 0.09^b$	$3.90 \pm 0.12^a$	$3.94 \pm 0.03^a$	$6.63 \pm 0.05^a$	$3.67 \pm 0.34^b$	$1.80 \pm 0.47^a$	$1.90 \pm 0.45^a$
Ethyl-3-hydroxybutanoate	20,000 [65]	Grape, fruity, caramel [70]	$30.00 \pm 0.27^b$	$43.80 \pm 0.97^a$	$17.78 \pm 0.95^a$	$15.19 \pm 1.00^a$	$29.79 \pm 0.63^a$	$12.95 \pm 0.46^b$	$31.34 \pm 0.38^a$	$12.86 \pm 3.63^b$
Ethyl-3-methylbutanoate	3 [54]	Fruity, pineapple [47]	$9.31 \pm 0.04^a$	$5.52 \pm 0.04^b$	$6.55 \pm 0.06^a$	$6.33 \pm 0.01^b$	$21.31 \pm 0.67^a$	$7.45 \pm 0.28^b$	$4.40 \pm 0.31^a$	$3.99 \pm 0.66^a$
Isoamyl acetate	30 [61]	Banana [57]	$1017.78 \pm 2.05^a$	$886.04 \pm 0.78^b$	$527.92 \pm 1.85^a$	$478.02 \pm 0.70^b$	$2276.20 \pm 0.98^a$	$1456.07 \pm 1.24^b$	$661.26 \pm 19.59^a$	$597.79 \pm 52.38^b$
Isobutyl acetate	6140 [57]	Apple, banana [59]	$42.57 \pm 1.00^a$	$48.36 \pm 1.62^a$	$57.91 \pm 0.33^a$	$53.54 \pm 1.01^b$	$67.15 \pm 0.59^b$	$76.71 \pm 0.73^a$	$35.41 \pm 3.49^b$	$81.15 \pm 2.91^a$
Hexyl acetate	670 [56]	Fruity, green, sweet [59]	$35.55 \pm 1.37^a$	$12.21 \pm 1.35^b$	$3.30 \pm 0.05^a$	$3.02 \pm 0.04^b$	$9.59 \pm 0.71^a$	$10.70 \pm 0.50^a$	$15.94 \pm 1.16^b$	$28.79 \pm 1.94^a$
Methyl vanillate			$9.46 \pm 0.05^a$	$8.65 \pm 0.05^b$	$0.01 \pm 0.00^a$	$0.02 \pm 0.01^a$	$77.75 \pm 0.71^b$	$80.97 \pm 0.37^a$	$21.71 \pm 0.34^a$	$21.51 \pm 3.64^a$

Table 2. Cont.

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Geranium acid methyl ester			$4.65 \pm 0.08^a$	$3.47 \pm 0.01^b$	$0.01 \pm 0.00^a$	$0.01 \pm 0.01^a$	$6.42 \pm 0.30^a$	$4.45 \pm 0.40^b$	$3.30 \pm 0.13^a$	$3.04 \pm 1.09^a$
Diethyl glutarate			$0.28 \pm 0.02^a$	$0.06 \pm 0.02^b$	$0.23 \pm 0.03^a$	$0.16 \pm 0.03^a$	$0.30 \pm 0.05^a$	$0.25 \pm 0.01^a$	$0.32 \pm 0.01^b$	$1.04 \pm 0.11^a$
Diethyl malate			$40.82 \pm 0.89^a$	$20.63 \pm 0.85^b$	$67.72 \pm 0.81^a$	$62.87 \pm 0.68^b$	$19.69 \pm 0.85^a$	$15.75 \pm 0.69^b$	$45.87 \pm 2.59^b$	$80.58 \pm 8.91^a$
Diethyl succinate	200,000 [37]	Overripe, aged [55]	$438.85 \pm 0.83^b$	$865.90 \pm 5.18^a$	$434.01 \pm 1.80^a$	$408.95 \pm 0.84^b$	$473.02 \pm 0.77^a$	$171.29 \pm 18.46^b$	$428.85 \pm 9.81^a$	$340.64 \pm 38.95^b$
2-Phenylethyl acetate	250 [71]	Rose, honey, tobacco [57]	$0.50 \pm 0.07^b$	$1.08 \pm 0.02^a$	$13.04 \pm 0.33^a$	$12.11 \pm 0.18^a$	$0.49 \pm 0.03^a$	$0.47 \pm 0.04^a$	$15.51 \pm 2.19^b$	$95.62 \pm 2.04^a$
$\Sigma$			$4148.45^b$	$5839.52^a$	$7418.19^a$	$6795.92^b$	$5120.67^b$	$5482.20^a$	$10,280.21^a$	$6952.26^b$
<b>Lactones</b>										
$\gamma$ -Butirolactone	10,000 [57]	Coconut, caramel [43]	$477.88 \pm 2.38^b$	$544.56 \pm 1.42^a$	$463.59 \pm 9.15^a$	$463.10 \pm 0.22^a$	$525.68 \pm 0.76^b$	$721.52 \pm 0.86^a$	$235.97 \pm 23.02^b$	$415.74 \pm 28.48^a$
$\gamma$ -Decalactone	1000 [57]	Peach, fruity [43]	$2.30 \pm 0.01^a$	$1.78 \pm 0.02^b$	$1.42 \pm 0.38^b$	$2.62 \pm 0.10^a$	$2.60 \pm 0.01^b$	$10.73 \pm 0.79^a$	$1.88 \pm 0.52^b$	$13.99 \pm 0.39^a$
$\gamma$ -Hexalactone	1600 [72]	Sweet, cake, peach [43]	$5.36 \pm 0.22^a$	$5.12 \pm 0.04^a$	$3.20 \pm 0.07^a$	$2.95 \pm 0.01^b$	$3.88 \pm 0.02^a$	$4.22 \pm 0.35^a$	$3.90 \pm 0.16^b$	$6.44 \pm 0.41^a$
$\gamma$ -Nonalactone	25 [49]	Coconut, peach [43]	$24.00 \pm 0.27^a$	$19.31 \pm 0.02^b$	$27.96 \pm 0.64^a$	$27.25 \pm 1.73^a$	$22.19 \pm 0.71^a$	$14.92 \pm 0.93^b$	$53.18 \pm 1.31^a$	$39.68 \pm 6.15^b$
$\gamma$ -Octalactone	7 [49]		$2.30 \pm 0.01^a$	$0.74 \pm 0.02^b$	$2.26 \pm 0.01^a$	$1.72 \pm 0.01^b$	$2.39 \pm 0.38^a$	$0.98 \pm 0.01^b$	$1.11 \pm 2.01^a$	$3.16 \pm 0.65^a$
$\gamma$ -Undecalactone	60 [49]	Apricot, peach [47]	$0.56 \pm 0.04^a$	$0.36 \pm 0.04^b$	$0.56 \pm 0.01^a$	$0.34 \pm 0.01^b$	$0.53 \pm 0.05^a$	$0.66 \pm 0.01^a$	$0.44 \pm 0.03^b$	$0.81 \pm 0.09^a$
$\delta$ -Decalactone			$3.54 \pm 0.01^a$	$3.37 \pm 0.26^a$	$2.90 \pm 0.01^a$	$2.77 \pm 0.01^b$	$3.39 \pm 0.23^a$	$1.99 \pm 0.16^b$	$4.11 \pm 0.11^a$	$2.76 \pm 0.68^b$
$\Sigma$			$515.93^b$	$575.24^a$	$501.88^a$	$500.74^a$	$560.64^b$	$755.00^a$	$300.57^b$	$482.57^a$
<b>Fatty acids</b>										
Butanoic acid	400 [55]	Rancid, cheesy [43]	$56.80 \pm 0.12^a$	$69.13 \pm 0.61^a$	$220.62 \pm 0.75^a$	$223.53 \pm 3.06^a$	$50.25 \pm 0.76^a$	$33.28 \pm 0.52^b$	$526.76 \pm 16.87^a$	$628.68 \pm 105.07^a$
Heptanoic acid	3000 [53]	Rancid, cheesy [43]	$13.05 \pm 0.42^a$	$11.69 \pm 0.68^a$	$9.61 \pm 0.18^a$	$8.76 \pm 0.29^a$	$5.32 \pm 0.45^b$	$13.64 \pm 0.68^a$	$17.56 \pm 0.35^a$	$14.38 \pm 1.82^b$
Hexanoic acid	420 [53]	Cheesy, oily [57]	$1261.73 \pm 41.87^b$	$1420.06 \pm 1.02^a$	$546.11 \pm 1.05^a$	$513.36 \pm 1.12^b$	$707.79 \pm 0.63^a$	$570.43 \pm 0.95^b$	$1929.66 \pm 23.44^a$	$1045.24 \pm 407.61^b$
Isovaleric acid	33 [61]	Sweet, rancid [43]	$4.50 \pm 0.23^b$	$6.13 \pm 0.01^a$	$3.06 \pm 0.02^a$	$3.19 \pm 0.01^a$	$5.53 \pm 0.68^a$	$3.76 \pm 0.10^a$	$3.33 \pm 0.36^a$	$3.14 \pm 0.40^a$
Nonanoic acid			$10.04 \pm 0.16^a$	$10.75 \pm 0.62^a$	$15.69 \pm 0.64^a$	$9.28 \pm 0.10^b$	$9.91 \pm 0.10^b$	$12.55 \pm 0.75^a$	$9.94 \pm 1.42^a$	$11.74 \pm 1.91^a$

Table 2. Cont.

	ODT ( $\mu\text{g/L}$ )	Odor Descriptor	Babić Control	Babić Lachancea	Blatina Control	Blatina Lachancea	Frankovka Control	Frankovka Lachancea	Trnjak Control	Trnjak Lachancea
Octanoic acid	500 [54]	Rancid, oily [59]	1185.08 $\pm$ 0.49 <sup>b</sup>	1394.94 $\pm$ 3.65 <sup>a</sup>	430.56 $\pm$ 0.98 <sup>a</sup>	402.15 $\pm$ 1.41 <sup>b</sup>	724.85 $\pm$ 2.14 <sup>a</sup>	506.16 $\pm$ 1.13 <sup>b</sup>	1655.69 $\pm$ 16.56 <sup>a</sup>	511.98 $\pm$ 354.13 <sup>b</sup>
Propanoic acid	8100 [56]	Rancid, oily [47]	6.61 $\pm$ 0.07 <sup>b</sup>	25.82 $\pm$ 1.10 <sup>a</sup>	3.31 $\pm$ 0.09 <sup>a</sup>	3.60 $\pm$ 0.11 <sup>a</sup>	7.52 $\pm$ 0.01 <sup>b</sup>	12.04 $\pm$ 0.49 <sup>a</sup>	49.25 $\pm$ 2.48 <sup>b</sup>	63.41 $\pm$ 4.59 <sup>a</sup>
Decanoic acid	1000 [71]	Rancid, waxy [43]	8.37 $\pm$ 0.03 <sup>b</sup>	149.26 $\pm$ 1.29 <sup>a</sup>	5.89 $\pm$ 0.09 <sup>b</sup>	137.57 $\pm$ 2.79 <sup>a</sup>	7.23 $\pm$ 0.07 <sup>a</sup>	6.90 $\pm$ 0.13 <sup>a</sup>	361.78 $\pm$ 1.75 <sup>a</sup>	96.62 $\pm$ 82.42 <sup>b</sup>
2-Methylpropionic acid			1168.90 $\pm$ 0.54 <sup>b</sup>	1281.00 $\pm$ 0.64 <sup>a</sup>	975.71 $\pm$ 0.74 <sup>a</sup>	929.75 $\pm$ 1.13 <sup>b</sup>	2050.21 $\pm$ 1.27 <sup>b</sup>	5175.83 $\pm$ 52.74 <sup>a</sup>	704.78 $\pm$ 72.86 <sup>b</sup>	2457.96 $\pm$ 145.03 <sup>a</sup>
$\Sigma$			4226,30 <sup>b</sup>	4368.78 <sup>a</sup>	2210.54 <sup>a</sup>	2231.17 <sup>a</sup>	3568.59 <sup>b</sup>	6344.59 <sup>a</sup>	4833.16 <sup>a</sup>	5258.74 <sup>a</sup>
<b>Other alcohols</b>										
1,4-Butanediol			1.03 $\pm$ 0.03 <sup>a</sup>	3.33 $\pm$ 0.03 <sup>a</sup>	0.52 $\pm$ 0.05 <sup>a</sup>	0.55 $\pm$ 0.01 <sup>a</sup>	0.36 $\pm$ 0.03 <sup>b</sup>	29.22 $\pm$ 14.21 <sup>a</sup>	1.31 $\pm$ 0.13 <sup>b</sup>	2.91 $\pm$ 0.33 <sup>a</sup>
4-Ethylcyclohexanol			3.13 $\pm$ 0.18 <sup>a</sup>	1.98 $\pm$ 0.01 <sup>b</sup>	4.38 $\pm$ 0.01 <sup>a</sup>	3.67 $\pm$ 0.01 <sup>b</sup>	1.60 $\pm$ 0.11 <sup>a</sup>	0.97 $\pm$ 0.04 <sup>b</sup>	7.30 $\pm$ 0.26 <sup>a</sup>	5.19 $\pm$ 0.83 <sup>b</sup>
4-Methyl-1-pentanol	50,000 [57]	Almond, toasted [47]	32.27 $\pm$ 1.38 <sup>a</sup>	38.05 $\pm$ 1.42 <sup>a</sup>	32.67 $\pm$ 0.74 <sup>a</sup>	30.85 $\pm$ 0.47 <sup>a</sup>	82.99 $\pm$ 0.48 <sup>a</sup>	35.19 $\pm$ 0.40 <sup>b</sup>	23.06 $\pm$ 0.67 <sup>b</sup>	32.45 $\pm$ 4.95 <sup>a</sup>
Furfuryl alcohol	15,000 [57]	Sweet, nutty [59]	1.52 $\pm$ 0.37 <sup>a</sup>	1.94 $\pm$ 0.02 <sup>b</sup>	0.99 $\pm$ 0.08 <sup>a</sup>	0.92 $\pm$ 0.04 <sup>a</sup>	1.32 $\pm$ 0.01 <sup>a</sup>	0.61 $\pm$ 0.04 <sup>b</sup>	5.45 $\pm$ 0.43 <sup>a</sup>	1.91 $\pm$ 0.48 <sup>b</sup>
Benzylalcohol	10,000 [63]	Roasted, toasted, sweet, fruity [43]	17.25 $\pm$ 0.63 <sup>b</sup>	20.65 $\pm$ 0.61 <sup>a</sup>	8.73 $\pm$ 0.01 <sup>b</sup>	9.22 $\pm$ 0.09 <sup>a</sup>	48.97 $\pm$ 0.12 <sup>a</sup>	50.62 $\pm$ 0.56 <sup>a</sup>	16.61 $\pm$ 1.34 <sup>a</sup>	14.52 $\pm$ 2.03 <sup>a</sup>
$\Sigma$			55.20 <sup>b</sup>	65.95 <sup>a</sup>	47.29 <sup>a</sup>	45.21 <sup>a</sup>	135.24 <sup>a</sup>	116.31 <sup>a</sup>	53.73 <sup>a</sup>	56.98 <sup>a</sup>
<b>Other compounds</b>										
Acetoin	150,000 [54]	Buttery, creamy [57]	17.26 $\pm$ 0.82 <sup>b</sup>	71.91 $\pm$ 0.33 <sup>a</sup>	63.05 $\pm$ 0.19 <sup>a</sup>	65.95 $\pm$ 0.95 <sup>a</sup>	13.57 $\pm$ 1.66 <sup>b</sup>	21.02 $\pm$ 1.17 <sup>a</sup>	41.39 $\pm$ 1.36 <sup>a</sup>	30.82 $\pm$ 4.74 <sup>a</sup>
Acetyl furane			0.56 $\pm$ 0.01 <sup>b</sup>	0.85 $\pm$ 0.05 <sup>a</sup>	1.82 $\pm$ 0.05 <sup>a</sup>	1.55 $\pm$ 0.21 <sup>a</sup>	1.28 $\pm$ 0.07 <sup>a</sup>	1.57 $\pm$ 0.03 <sup>a</sup>	0.77 $\pm$ 0.67 <sup>a</sup>	0.85 $\pm$ 0.40 <sup>a</sup>
2H-Pyran-2,6(3H)-dione			59.45 $\pm$ 0.77 <sup>b</sup>	71.65 $\pm$ 0.91 <sup>a</sup>	46.21 $\pm$ 0.52 <sup>a</sup>	37.07 $\pm$ 1.00 <sup>b</sup>	70.63 $\pm$ 0.59 <sup>a</sup>	62.16 $\pm$ 1.32 <sup>b</sup>	58.86 $\pm$ 1.95 <sup>a</sup>	38.03 $\pm$ 6.98 <sup>b</sup>
2-Pentylfuran	2000 [64]		260.59 $\pm$ 1.61 <sup>b</sup>	329.93 $\pm$ 0.34 <sup>a</sup>	243.75 $\pm$ 2.16 <sup>a</sup>	247.47 $\pm$ 1.10 <sup>a</sup>	355.53 $\pm$ 1.60 <sup>a</sup>	358.77 $\pm$ 0.48 <sup>a</sup>	285.39 $\pm$ 3.23 <sup>a</sup>	251.00 $\pm$ 5.41 <sup>a</sup>
$\Sigma$			337.86 <sup>b</sup>	474.34 <sup>a</sup>	354.83 <sup>a</sup>	352.04 <sup>a</sup>	441.01 <sup>a</sup>	443.52 <sup>a</sup>	386.41 <sup>a</sup>	320.70 <sup>b</sup>
ODT—odor detection threshold										

### 3.3. Odor Active Values (OAVs) and Relative Odor Contributions (ROCs)

To evaluate the influence of individual volatile compounds on the overall aroma of each red variety of wine, OAVs and ROC indexes were calculated and are presented in Table 2. From a total of 122 compounds, only 17 exceeded the threshold values ( $OAV > 1$ ). Between them, the most abundant were esters, with four individual compounds, followed by terpenes, aldehydes, and fatty acids, with three compounds each, and higher alcohols, volatile phenols, C13-norisoprenoides, and lactones, with only one compound each. In Babić wines, the highest OAV was  $\beta$ -damascenone, with no marked ROC differences between control and sequential fermentation wines. The use of *L. thermotolerans* in Babić wines positively influenced total ester, terpene, and fatty acid ROCs with higher ethyl hexanoate, linalool, hexanoic, and octanoic acid OAVs. The ROC of isoamyl acetate was noted in all wines, but especially in Frankovka and Babić control wines. Comparing OAVs in Blatina wines, the highest one was connected with  $\beta$ -damascenone, with higher values in control wine, while the strong influence of sequential fermentation was noted with the presence of aldehydes, especially decanal, which resulted in an almost 10% higher total ROC. Blatina wines produced with the use of *L. thermotolerans* also stood up with higher total fatty acid and total ester ROCs as well as  $\gamma$ -nonalactone and eugenol values. On the contrary, in Frankovka and Trnjak wines, the total ROC of esters, fatty acids, terpenes,  $\gamma$ -nonalactone, and eugenol was stronger in control variants, while *L. thermotolerans* positively influenced total aldehydes and  $\beta$ -damascenone OAV, especially in Trnjak wines.

## 4. Conclusions

In conclusion, the data from the presented work pointed out positive effects of *L. thermotolerans* yeast on overall wine composition, although they were different between the varieties used. For the first time, the influence of an equal sequential fermentation strategy was applied in the production of four different grape varieties of wines. The resulting production of L-lactic acid regardless of primary grape must composition pointed out the use of *L. thermotolerans* as an effective acidification tool of the fermenting grape must as well as a possible path for reduction of wine alcohol content. In Babić wines, the strongest influence of sequential fermentation was connected with higher total terpene and total ester concentrations, mainly due to the higher farnesol, linalool, neral, geraniol, and geranyl acetate presence, and due to the concentrations of mostly all ethyl esters being above the odor detection threshold, such as ethyl decanoate, ethyl octanoate, and ethyl hexanoate. Blatina sequentially fermented wines can be singled out by higher concentrations of some individual terpenes, such as geraniol, geranyl acetate, neral, and 1,8 terpin but a lower concentration of total esters and ethyl lactate, whose presence was significantly higher in Babić, Frankovka, and Trnjak wines. Significantly higher concentrations of ethyl lactate, together with some already mentioned individual terpenes, were present in Frankovka sequentially fermented wines, while Trnjak sequentially fermented wines stood up with higher total aldehyde volatile phenols and total lactone concentrations. Control wines, regardless of variety, stood up with higher concentrations of total higher alcohols, among them especially isoamyl alcohol. In addition, higher concentrations of citronellol, isoamyl acetate, and vanillin were defined in all control wines, as were total esters in Blatina and Frankovka wines. Thus, the most significantly different profiles between *S. cerevisiae* yeast fermentation and sequential fermentations were observed in total aldehyde, higher alcohol, ester, and terpene concentrations. Our data also showed that multivariate analysis differences in the volatile aroma compounds can be a useful tool leading to an optimal selection of yeasts with the main purpose of producing high-quality varietal wines.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2311-5637/7/1/4/s1>, Table S1: Identification and quantification parameters for GC-MS analysis.

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**Data Availability Statement:** Data available on request due to restrictions eg privacy or ethical. The data presented in this study are available on request from the corresponding author. The data are not publicly available there are part of PhD thesis.

**Conflicts of Interest:** The authors declare no conflict of interest.

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