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Impact of Grape Variety, Yeast and Malolactic Fermentation on Volatile Compounds and Fourier Transform Infrared Spectra in Red Wines

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Volatile compounds are very important to the flavour and quality of the wine. The study aimed to determine the effect of grape variety (Rondo and Zweigelt), yeast, malolactic fermentation (MLF) and yeast×MLF interaction on the content of volatile compounds in red wines. The wines were produced by sequential inoculation with five commercial yeast strains and a commercial lactic acid bacteria (LAB) strain (induced malolactic fermentation) as well as by inoculation with five commercial yeast strains and without LAB inoculation (spontaneous malolactic fermentation). The volatile compounds were determined by headspace solid-phase microextraction/gas chromatography-mass spectrometry (HS-SPME-GC/MS). Forty-six volatile compounds belonging to alcohols, esters, acids, aldehydes, ketones, furan compounds, sulfur compounds and volatile phenols were identified in the wines. The grape variety was the factor with a significant impact on the highest number of volatile compounds, 32 out of 46. Furthermore, 7 compounds were affected by yeast, 10 by MLF and only 3 by yeast×MLF interaction. Characteristic bands in Fourier transform infrared (FTIR) spectra were assigned to the vibrations of functional groups of volatile compounds. The whole FTIR spectra were analysed in detail; three characteristic spectral ranges such as 3650–2700, 1750–1500, and below 1500 cm^{-1} were shown for different classes of volatile compounds. The most remarkable spectral changes were observed for the last two areas.

INTRODUCTION

Volatile compounds (VOCs) largely determine the aroma of the wine. The concentration of VOCs in wines ranges from several mg/L to a few ng/L [Welke *et al.,* 2012; Zhu *et al.,* 2016]. These volatile compounds originate from grapes (varietal aromas) and are secondary products of fermentation processes (fermentation aromas) and aging (post-fermentation aromas) [Callejon *et al*., 2010]. Alcoholic fermentation (AF) and malolactic fermentation (MLF) processes play a fundamental role in creating the taste and aroma of the product [Gammacurta *et al*., 2014, 2017].

Yeasts, responsible for AF, transform sugar into ethanol, carbon dioxide, and minor secondary metabolites, including higher alcohols, esters, fatty acids, aldehydes, ketones, and others [Englezos *et al.*, 2018]. Yeasts also release varietal aromatic compounds from grapes. The ability to synthesize secondary metabolites and to release varietal compounds depends on the yeast species and strain [Blanco *et al*., 2014; Callejon *et al*., 2010; Gammacurta *et al*., 2017]. The primary yeast species responsible for AF is *Saccharomyces cerevisiae* [Azzolini *et al.*, 2012]. A wide range of selected yeast strains are commercially available that guarantee fermentation control and wine quality. However, some wineries are interested in selecting autochthonous (indigenous) yeast starters because of their ability to ensure the sensory characteristic of wines originating from a specific *terroir* [Blanco *et al*., 2014; Tufariello *et al*., 2014].

During MLF, L-malic acid is an enzymatically decarboxylated into L-lactic acid and carbon dioxide by lactic acid bacteria (LAB), mainly *Oenococcus oeni* [Tristezza *et al.*, 2016]. This process is an important step in the production of most red and some white and sparkling wines [Abrahamse & Bartowsky, 2012; Costello *et al*., 2012; Lasik-Kurdyś *et al*., 2018]. MLF reduces the total acidity (by slightly increasing the pH), causes microbiological stability, and modifies wine aroma and taste [Costello *et al*., 2012]. Aroma modification is associated with citric acid catabolism by LAB, resulting in the production of diacetyl, acetic acid, acetoin, and 2,3-butanediol [Malherbe *et al*., 2012]. MLF typically takes place after AF and can occur spontaneously, carried out by indigenous LAB populations, or may be induced by starter culture inoculation to control this step of winemaking. Two types of LAB

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inoculation are commonly used: traditional inoculation after AF (sequential) or simultaneous inoculation with the yeast and LAB (co-inoculation) [Abrahamse & Bartowsky, 2012; Antalick *et al*., 2013]. The undoubted advantage of simultaneous inoculation is the reduction of the vinification time. However, this inoculation may slow down the growth and deteriorate the viability of yeast cells, leading to a delay or inhibition of AF, and increase volatile acidity caused by higher acetic acid production [Stój, 2020a].

In recent years, several authors have conducted studies on the influence of grape variety and microorganisms on the content of volatile compounds in red wines [Cañas *et al.*, 2012; Cioch-Skoneczny *et al.*, 2021; Englezos *et al.*, 2018; Tristezza *et al.*, 2016]. The grape variety affects not only varietal aromas (C13-norisoprenoids, lactones, and terpenes) but also other volatile compounds, such as higher alcohols, esters, and fatty acids [Vilanova *et al*., 2007]. Both grape cultivar and yeast species significantly impacted content of ethyl esters in wines. The effect of grape cultivar on the content of higher alcohols seems more significant than the effect of yeast species. Fatty acid (acetic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid and octanoic acid) contents showed significant differences as an effect of yeast species [Liu *et al*., 2017]. A specific volatile profile corresponded to each yeast/LAB combination, wherein the yeast strain had a predominant effect on aromatic compounds [Gammacurta *et al.*, 2017]. The wine matrix, particularly pre-MLF pH, ethanol content, and grape source affected the ability of LAB strains to modulate volatile compounds in wines [Costello *et al*., 2012].

The composition of grapes depends on the varietal and clonal genotype of the grapevine as well as the interaction of the genotype and phenotype with many environmental factors (*terroir*) [Styger *et al*., 2011]. One of the most popular red grape hybrids in Poland is Rondo (non-*Vitis vinifera*). It derives from a cross between two species of *Vitis, Zarya severa* × *Saint Laurent* [Wojdyło *et al*., 2018]. However, noble grape varieties, such as Zweigelt (*Vitis vinifera*), cover a small area of Poland [Stój *et al*., 2020b]. To the best of our knowledge, there have been no studies on the varieties of grapes cultivated in Poland and the wine factors influencing the concentration of different classes of volatile compounds in red wines. The study aimed to determine the effects of Rondo and Zweigelt varieties, yeast, MLF, and yeast×MLF interaction on the content of volatile compounds in wines. The wines were produced by sequential inoculation of grape pulp with five commercial yeast strains (*S. cerevisiae* or *S. cerevisiae* × *S. bayanus*) and a commercial LAB (*O. oeni*) as well as by inoculation with five commercial yeast strains and without LAB inoculation. Moreover, the analysis of Fourier transform infrared (FTIR) spectra used in conjunction with the multivariate analysis allowed for assigning vibration bands to specific functional groups of volatile compounds.

MATERIALS AND METHODS

Winemaking

Details of winemaking are presented in our previous article [Stój *et al*., 2020b]. The grapes of Zweigelt and Rondo varieties were obtained from 'Małe Dobre' and 'Dom Bliskowice' vineyards, respectively. The vineyards are located in the Lublin Province, Poland. The grapes were harvested manually in 2017. The grape pulp was subjected to alcoholic fermentation by using five commercial yeast strains, four *S. cerevisiae*: SafOEno™ SC 22, Essentiale Grand Cru (Lesaffre, Marcq-en-Barœul, France), Siha Active Yeast 8, Siha Rubino Cru (Eaton, Tinton Falls, NJ, USA); and one *S. cerevisiae* × *S. bayanus* – SafOEno™ HD S62 (Lesaffre), the same for the Zweigelt and Rondo varieties. Spontaneous MLF occurred in one part of the wines, *i.e*. that without LAB inoculation, and induced MLF – in the other part of the wines, *i.e.* that with *O. oeni* (Viniflora Oenos, Eaton) inoculation. *O. oeni* starter culture was added after the completion of AF (sequential inoculation) to the wines in which induced malolactic fermentation was carried out. We did not obtain complete reduction of malic acid in any of the wines. The concentrations of malic and lactic acids in the final wines are presented in the supplementary material to the previous article [Stój *et al.*, 2020b]. The experiments were performed in duplicate. The following abbreviations are used for the wines: Z1-Z5 – Zweigelt wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria; R1-R5 – Rondo wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

Reagents

Sodium chloride and hydrochloric acid were obtained from POCh (Gliwice, Poland). Sodium chloride was oven-dried at 200°C overnight. Hydrochloric acid was dissolved in water at a concentration of 78 g/L. 4-Hydroxy-4-methyl-2-pentanone (the internal standard of volatile compound analysis) was purchased from Sigma-Aldrich (Saint Louis, MO, USA) and prepared in water at a concentration of 7 mg/L. A mixture of *n*-alkanes (C_7-C_{30}) for the calculations of linear retention indices (RI) was supplied by Supelco (Bellefonte, PA, USA). All chemicals were of analytical grade.

Determination of volatile compounds

The volatile compounds were determined by headspace solid-phase microextraction and gas chromatography-mass spectrometry analysis (HS-SPME-GC/MS) according to our previous method [Stój *et al*., 2017a,b] with slight modification.

A fiber holder and an 85 μm CAR/PDMS fiber were used (Supelco). Before each analysis, the fiber was conditioned by inserting it into the auxiliary GC injection port at 280°C for 5 min. Then, 0.9 g of NaCl, 3 mL of wine, 50 μL of HCl, 100 μL of 4-hydroxy-4-methyl-2-pentanone, and a magnetic stirring bar were placed in a glass vial of 7 mL. The vial was capped with a PTFE-silicone septum (Supelco) on which a screw cap with a hole was placed. The vial was placed in a block on an MS7-H550-S magnetic stirrer with a hotplate (DLAB Scientific Co., Beijing, China). The sample was incubated at 40°C

for 15 min, and then the fiber was exposed to the headspace (HS) at 40°C for 30 min. The incubation and microextraction were carried out with continuous stirring at a minimum speed. The fiber was thermally desorbed in the GC injection port for 2 min at 220°C in the split-less mode.

The samples were analyzed using a gas chromatograph combined with a quadrupole mass spectrometer (GCMS--QP2010, Shimadzu, Kyoto, Japan). All analyses were made in triplicate. Chromatographic separations were carried out using a VF-WAXms capillary column (60 m, 0.25 mm ID, 0.25 μm film thickness, 100% polyethylene glycol, Agilent, Santa Clara, CA, USA). Helium was used as the carrier gas at a flow rate of 1.8 mL/min. The column oven temperature was held at 34°C for 5 min, then raised to 100°C at a rate of 3°C/min and held for 6 min, and finally raised to 220°C at a rate of 5°C/min and held for 15 min. The total run time was 72 min. The specification of the mass spectrometer was as follows: electron ionization source with a temperature of 200°C, 70 eV ionization energy and mass range of 30–300 *m/z* in the full scan mode (scan time 0.4 s). Data were collected using the GCMSsolution software ver. 2 (Shimadzu). The tentative identification of aromatic compounds was performed based on mass spectra and confirmed by linear retention index. The mass spectrum of each peak was compared to data provided in the National Institute of Standards and Technology mass spectral library (NIST 05). The peak was correctly identified when the similarity of the spectra was at least 80%. Chromatographic retention data for C_7-C_{30} *n*-alkanes (Supelco) were used to calculate the RI of each compound. Experimental RI results were compared to the retention indices found in the bibliography for similar GC columns. Data for the volatile compounds were calculated by relating their peak areas to the peak area of the internal standard. The contents of volatiles in wines were expressed as μg/L.

Fourier transform infrared spectroscopy

FTIR spectra were measured on a 670-IR spectrometer from Agilent. FTIR measurements were made five times for each wine. An attenuated total reflection (ATR) trough in the form of a horizontal ATR (HATR) Ge crystal with an appropriate geometry (*i.e.*, truncated at 45°) was used in the measurements. This was to ensure a 20-fold internal reflection of the absorbed beam. Additionally, background correction was applied and 24 scans were recorded at the recording of each spectrum. Then, the program averaged the spectra obtained, before and after each measurement the crystal was thoroughly purified using ultra-pure and clear solvents from Sigma-Aldrich. The apparatus was continuously purged with argon before (1 h) and during the entire spectral measurement. FTIR spectra were recorded with a resolution of 1 cm^{-1} and measured in the range of 4000 to 400 cm^{-1}. In the end, the spectra were analyzed with Grams/AI 8.0 software (Thermo Fisher Scientific, Waltham, MA, USA). All samples were measured at room temperature.

Statistical analysis

The effects of grape variety, yeast, MLF, and yeast×MLF interaction on the content of volatile compounds in wines were tested using one-way and two-way analysis of variance (ANOVA). One- and two-way ANOVA was performed with the Statistica 13.3 software (Statsoft, Krakow, Poland).

Next, hierarchical cluster analysis (HCA) with Euclidean distance was applied as an unsupervised classification technique in order to explore the FTIR data structure. The hierarchical cluster analysis and dendrogram were performed with Statistica 13.3 software. Next, principal component analysis (PCA) was applied to verify the similarities and differences among the wines. The PCA was based on the data array of the fingerprints of FTIR spectra of each considered wine, and carried out using OriginPro software (OriginLab, Northampton, MA, USA).

RESULTS AND DISCUSSION

Forty-six volatile compounds belonging to several groups – alcohols (21 compounds), esters (12 compounds), acids (5 compounds), aldehydes (2 compounds), ketones (2 compounds), furan compounds (2 compounds), sulfur compounds (1 compound) and volatile phenols (1 compound) – were identified in red wines produced from Zweigelt and Rondo grape varieties (Table 1). Figure S1 and Figure S2 in the supplementary materials present the chromatograms of volatile compounds of Zweigelt and Rondo wines, respectively. Table 2 and Table 3 present the concentrations of volatile compounds of Zweigelt and Rondo wines, respectively, produced with different yeast and MLF combinations.

The dominating alcohols in the wines produced from the Zweigelt variety were: 3-methylbutan-1-ol, 2-methylpropan-1-ol, and hexan-1-ol (Table 2). The concentrations of 2-(2-ethoxyethoxy)-ethanol, decan-1-ol and (*Z*)-2-hexen-1-ol were the lowest. The major esters were ethyl 2-hydroxypropanoate (ethyl lactate), ethyl octanoate, and 3-methylbutyl acetate, while minor ones were: 2-phenylethyl acetate, hexyl acetate, and methyl octanoate. Among the acids, acetic acid and hexanoic acid were found at the highest concentrations. Propanoic acid was detected only in Z2 LAB-Z5 LAB wines. Benzaldehyde was the most abundant compound in the two detected aldehydes, 4-methyl-3-penten-2-one was the most abundant compound in the two detected ketones, dihydrofuran-2(3*H*)-one was the most abundant in the two detected furan compounds.

The most abundant alcohols in wines produced from the Rondo variety were: 3-methylbutan-1-ol, 2-methylpropan-1-ol, and 2-phenylethanol (Table 3). Rondo wines had the highest concentration of 3-methylbutan-1-ol, similarly to our previous works [Stój *et al*., 2017a,b] and to the work of Liu *et al*. [2017]. Contrary to our findings, Ruocco *et al*. [2019] reported the highest content of 2,3-butanediol among all alcohols. The minor alcohols of Rondo wines were: 3-ethyl-4-methylpentan-1-ol, phenylmethanol, and decan-1-ol (Table 3). The dominating esters were: ethyl 2-hydroxypropanoate, 3-methylbutyl acetate, and ethyl octanoate, and it is in agreement with our previous works [Stój *et al*., 2017a,b]. According to Liu *et al*. [2017], ethyl octanoate and ethyl acetate were the main esters in wines produced from Rondo variety. In turn, Ruocco *et al*. [2019] stated that ethyl 2-hydroxypropanoate and ethyl acetate were the dominating esters in Rondo wines. The dissimilarities between

TABLE 1. Volatile compounds identified in red wines.

Peak no.	Compound	Similarity (%)	RT (min)	RI exp.	RI lit.	References
	Alcohol					
2	Propanol-1-ol	95	13.50	1051	1044	Mendes *et al.* [2012]
4	2-Methylpropan-1-ol	97	16.31	1122	1100	Mendes *et al.* [2012]
7	Butan-1-ol	96	18.73	1169	1173	Welke *et al.* [2012]
8	3-Methylbutan-1-ol	98	21.79	1228	1210	Mendes *et al.* [2012]
9	Pentan-1-ol	94	23.46	1261	1259	Mallouchos *et al.* [2007]
12	4-Methylpentan-1-ol	95	26.35	1318	1309	Duarte *et al.* [2010]
13	3-Methylpentan-1-ol	97	26.94	1329	1322	Duarte *et al.* [2010]
16	Hexan-1-ol	98	28.20	1353	1361	Mallouchos *et al.* [2007]
17	3-Ethoxypropan-1-ol	93	29.44	1376	1371	Welke *et al.* [2012]
19	(*Z*)-2-Hexen-1-ol	87	31.73	1417	1397	Welke *et al.* [2012]
21	Octen-3-ol	95	33.94	1453	1451	Song *et al.* [2014]
22	Heptan-1-ol	97	34.24	1458	1470	Welke *et al.* [2012]
24	2-Ethylhexan-1-ol	98	36.19	1490	1486	Duarte *et al.* [2010]
25	3-Ethyl-4-methylpentan-1-ol	92	37.14	1508	1509	Welke *et al.* [2012]
27	Butane-2,3-diol	98	38.61	1543	1563	Welke *et al.* [2012]
29	Octan-1-ol	98	39.32	1559	1567	Mallouchos *et al.* [2007]
32	Propane-1,2-diol	90	40.75	1592	1591	Welke *et al.* [2012]
33	2-(2-Ethoxyethoxy)-ethanol	89	41.67	1618	1622	Welke *et al.* [2012]
40	Decan-1-ol	95	46.13	1760	1778	Welke *et al.* [2012]
43	Phenylmethanol	84	49.27	1881	1869	Duarte *et al.* [2010]
44	2-Phenylethanol	97	50.10	1916	1919	Mallouchos *et al.* [2007]
	Ester					
1	Ethyl butanoate	97	13.05	1037	1034	Mallouchos *et al.* [2007]
3	Ethyl 3-methylbutanoate	94	14.43	1078	1066	Duarte *et al*. [2010]
5	3-Methylbutyl acetate	98	16.88	1133	1125	Duarte *et al.* [2010]
10	Hexyl acetate	90	24.16	1275	1272	Mallouchos *et al.* [2007]
14	Ethyl heptanoate	91	27.38	1337	1349	Welke *et al.* [2012]
15	Ethyl 2-hydroxypropanoate	98	27.87	1346	1339	Welke *et al.* [2012]
18	Methyl octanoate	85	30.01	1387	1381	Welke *et al.* [2012]
20	Ethyl octanoate	97	32.85	1435	1429	Welke *et al.* [2012]
30	3-Methylbutyl 2-hydroxypropanoate	97	39.85	1572	1568	Mendes *et al.* [2012]
36	Ethyl decanoate	88	42.38	1639	1643	Welke *et al.* [2012]
38	Diethyl butanedioate	96	43.66	1677	1672	Duarte *et a*l. [2010]
41	2-Phenylethyl acetate	90	47.73	1819	1810	Duarte *et al.* [2010]
	Acid					
23	Acetic acid	98	34.59	1464	1457	Welke *et al.* [2012]
28	Propanoic acid	86	39.02	1552	1536	Welke *et al.* [2012]
31	2-Methylpropanoic acid	97	40.11	1578	1573	Mallouchos *et al.* [2007]

TABLE 1 continued

Peak no.	Compound	Similarity (%)	RT (min)	RI exp.	RI lit.	References
42	Hexanoic acid	96	48.53	1851	1851	Mallouchos *et al*. [2007]
45	Octanoic Acid	95	53.38	2065	2067	Mallouchos *et al.* [2007]
	Aldehyde					
26	Benzaldehyde	91	37.90	1526	1522	Mallouchos *et al.* [2007]
37	4-Methylbenzaldehyde	92	42.78	1651	1638	Duarte *et al.* [2010]
	Ketone					
6	4-Methyl-3-penten-2-one	98	17.56	1146	1139	Jørgensen *et al.* [2000]
11	3-Hydroxybutan-2-one	90	25.39	1299	1289	Mallouchos *et al.* [2007]
	Furan compound					
34	Ethyl 2-furoate	88	41.93	1625	1627	Welke *et al.* [2012]
35	Dihydrofuran-2(3*H*)-one	93	42.22	1634	1627	Mallouchos *et al.* [2007]
	Sulphur compound					
39	3-(Methylsulfanyl)propan-1-ol	96	44.95	1718	1715	Duarte *et al.* [2010]
	Volatile phenol					
46	3,5-Di-*tert*-butylphenol	86	58.08	2305	2310	Shimoda *et al.* [1995]

RT – retention time; RI exp. –retention index experimentally determined; RI lit. –retention index reported in the literature for a CP-Wax columns or equivalent stationary phase. Peak no. correspond to those in the chromatograms in Figures S1 and S2 in the supplementary materials.

concentrations of volatile compounds among studies may have been due to differences in geographical origins of grapes, winemaking, and methods of determination of volatile compounds. Acetic acid was present at the highest concentration among the acids of Rondo wines (Table 3), while propanoic acid was not detected. Similarly, other authors reported that acetic acid was the major acid of wines produced using Rondo grapes [Liu *et al*., 2017; Ruocco *et al.*, 2019] and we also stated this in our previous publications [Stój *et al.*, 2017a,b]. Major volatile compounds within the other classes were: benzaldehyde, 4-methyl-3-penten-2-one, and dihydrofuran-2(3*H*)-one (Table 3).

A one-way ANOVA was used to study the grape variety/yeast/MLF effect on volatile compound profile of wines. Results of this analysis revealed a significant effect of grape variety, yeast, and MLF on the concentrations of 32, 7, and 10 volatile compounds, respectively, belonging to all groups of compounds (Table 4). The grape variety was the factor with a significant impact on the highest number of identified volatile compounds. The two-way ANOVA confirmed the effects of yeast and MLF on volatile compounds in wines (Table 4). The yeast and MLF factors had a significant impact on a greater number of volatile compounds compared to the yeast×MLF interaction, which affected concentrations of only three compounds: 2-phenylethanol, octanoic acid and 3-(methylsulfanyl)propan-1-ol.

In our study, the grape variety effect was significant for most alcohols, including: propanol-1-ol, butan-1-ol, 3-methylbutan-1-ol, pentan-1-ol, 4-methylpentan-1-ol, 3-methylpentan-1-ol, hexan-1-ol, 3-ethoxypropan-1-ol, (*Z*)-2-hexen-1-ol, octen-3-ol, heptan-1-ol, 2-ethylhexan-1-ol, 3-ethyl-4-methylpentan-1-ol, octan-1-ol, decan-1-ol, phenylmethanol, and 2-phenylethanol (Table 4), while Liu *et al.* [2017] reported that the concentration of all determined alcohols was affected by the variety. Higher alcohols are strictly related to yeast metabolism [Blanco *et al*., 2014; Callejon *et al*., 2010]. They could be synthesized by yeast through two mechanisms: the anabolic pathway from glucose or the catabolic pathway from their corresponding amino acids [Liu *et al*., 2017; Stój *et al*., 2017a]. Yeast species and strains (and other factors such as pH, grape juice composition and fermentation temperature) influence the formation of higher alcohols during fermentation [Liu *et al*., 2017]. In our study, concentrations of 3-ethoxypropan-1-ol, (*Z*)-2-hexen-1-ol, phenylmethanol, and 2-phenylethanol were significantly affected by yeast strain used in winemaking. Both Blanco *et al*. [2014] and Tufariello *et al.* [2014] found no differences in phenylmethanol concentration, whereas Callejon *et al*. [2010] and Tufariello *et al.* [2014] found no differences in 2-phenylethanol concentration between red wines produced using different yeast strains. Similarly to our study, Callejon *et al*. [2010] reported a correlation of phenylmethanol concentration with a yeast strain, and Blanco *et al*. [2014] reported a correlation of 2-phenylethanol content with a yeast strain. The yeast strains used in this study did not influence the concentrations of other higher alcohols, such as propanol-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol, hexan-1-ol, heptan-1-ol (Table 4). Blanco *et al.* [2014], Callejon *et al.* [2010], and Tufariello *et al.* [2014] observed the effect of yeast strain or no effect on the concentrations of higher alcohols. Finally, the MLF

TABLE 2. Concentrations of volatile compounds in Zweigelt wines (μg/L) produced with different yeast and malolactic fermentation (MLF) combinations.

Compound	Z1	Z2	Z3	Z4	Z5	Mean±SD	Z1 LAB	Z2 LAB	Z3 LAB	Z4 LAB	Z5 LAB	Mean±SD
Alcohol												
Propanol-1-ol	56.41	99.01	34.18	84.46	95.78	73.97±27.86	101.21	146.66	68.96	56.19	103.14	95.23±35.20
2-Methylpropan-1-ol	284.23	192.49	163.93	389.60	672.59	340.57±205.51	321.81	276.36	423.62	420.16	447.18	377.83±74.38
Butan-1-ol	6.45	8.39	4.58	nd	15.26	6.94±5.60	13.73	4.16	6.46	6.16	5.75	7.25±3.73
3-Methylbutan-1-ol	2563.09	2261.99	2188.89	3435.22	5011.19	3092.08±1181.67	5050.42	3215.93	3343.36	3591.01	3576.70	3755.48±741.09
Pentan-1-ol	1.89	1.51	2.06	2.19	2.88	2.11±0.50	3.78	2.50	2.73	2.16	2.10	2.66±0.68
4-Methylpentan-1-ol	4.60	4.23	6.32	5.08	8.21	5.69±1.61	8.68	6.74	7.81	4.93	5.44	6.72±1.57
3-Methylpentan-1-ol	6.30	5.85	7.96	7.45	9.66	7.44±1.50	11.45	9.32	9.71	7.22	6.64	8.87±1.95
Hexan-1-ol	205.80	172.87	241.20	270.36	332.39	244.52±61.31	381.30	268.08	281.05	298.70	199.89	285.80±65.20
3-Ethoxypropan-1-ol	0.22	1.22	1.07	0.82	1.57	0.98±0.50	0.50	1.35	1.40	1.17	1.14	1.11±0.36
(*Z*)-2-Hexen-1-ol	nd	0.05	0.26	0.18	nd	0.10±0.12	0.07	0.25	0.18	0.23	nd	0.15±0.11
Octen-3-ol	1.48	1.50	1.93	1.81	2.98	1.94±0.61	2.81	2.35	2.21	1.44	1.66	2.09±0.55
Heptan-1-ol	2.81	2.48	5.63	4.85	5.93	4.34±1.60	6.35	5.12	6.34	6.98	3.78	5.71±1.27
2-Ethylhexan-1-ol	11.95	11.88	25.79	15.52	25.40	18.11±6.99	24.32	18.84	29.47	18.71	11.42	20.55±6.77
3-Ethyl-4-methylpentan-1-ol	2.37	2.23	3.02	2.99	4.48	3.02±0.89	4.64	3.51	3.43	3.48	2.27	3.47±0.84
Butane-2,3-diol	69.81	60.81	37.13	37.82	108.91	62.90±29.42	80.47	37.14	47.80	63.35	38.49	53.45±18.37
Octan-1-ol	12.15	6.09	13.96	9.82	30.19	14.44±9.28	28.98	11.13	15.83	14.07	14.62	16.93±6.95
Propane-1,2-diol	2.14	3.55	0.82	0.87	5.62	2.60±2.02	3.14	0.51	1.11	1.62	0.30	1.34±1.13
2-(2-Ethoxyethoxy)-ethanol	0.32	0.16	0.32	0.29	0.69	0.36±0.20	0.60	0.21	0.35	0.41	0.36	0.38±0.14
Decan-1-ol	0.14	nd	0.27	0.08	0.48	0.19±0.19	nd	0.03	0.19	0.13	nd	0.07±0.09
Phenylmethanol	1.14	0.66	0.17	0.46	0.28	0.54±0.38	0.54	0.18	0.10	0.18	0.14	0.23±0.18
2-Phenylethanol	165.80	143.58	104.27	133.89	327.69	175.05±88.15	221.62	135.84	118.37	187.75	149.87	162.69±41.68
Ester												
Ethyl butanoate	38.82	23.36	36.50	49.30	83.64	46.32±22.81	60.27	48.58	53.47	34.52	33.10	45.99±11.88
Ethyl 3-methylbutanoate	6.87	5.69	4.36	6.21	12.87	7.20±3.30	8.81	6.82	5.31	3.51	7.07	6.30±2.00
3-Methylbutyl acetate	53.96	7.55	37.56	44.86	nd	28.79±23.71	12.92	93.08	107.50	69.76	109.37	78.52±39.95
Hexyl acetate	nd	nd	0.50	0.22	nd	0.14±0.22	nd	0.33	0.16	nd	0.56	0.21±0.24

TABLE 2 continued

Compound	Z1	Z2	Z3	Z4	Z5	Mean±SD	Z1 LAB	Z2 LAB	Z3 LAB	Z4 LAB	Z5 LAB	Mean±SD
Ethyl heptanoate	3.20	4.87	1.07	3.30	3.08	3.10±1.35	4.15	2.43	2.00	1.09	2.35	2.41±1.11
Ethyl 2-hydroxypropanoate	121.21	154.99	456.41	104.51	196.32	206.69±143.95	889.19	773.57	623.82	532.46	670.39	697.89±137.78
Methyl octanoate	0.56	0.21	nd	0.85	nd	0.32±0.37	nd	0.16	0.09	nd	nd	0.03±0.07
Ethyl octanoate	14.65	43.95	48.91	79.09	136.10	64.54±46.07	89.80	47.53	59.08	82.74	66.90	69.21±17.21
3-Methylbutyl 2-hydroxypropanoate	2.74	3.28	9.66	2.16	6.33	4.83±3.14	22.02	15.50	12.01	15.06	17.14	16.35±3.67
Ethyl decanoate	nd	0.31	nd	0.57	1.48	0.47±0.61	1.03	nd	0.46	nd	nd	0.21±0.46
Diethyl butanedioate	44.27	42.33	42.08	27.72	84.13	48.11±21.20	61.72	38.77	41.85	29.03	40.48	42.37±11.93
2-Phenylethyl acetate	nd	nd	0.29	0.25	0.27	0.16±0.15	0.82	nd	0.35	nd	0.26	0.28±0.34
Acid												
Acetic acid	73.80	74.54	211.19	93.53	195.70	129.75±67.96	222.29	242.45	1232.57	295.25	281.45	254.80±31.82
Propanoic acid	nd	nd	nd	nd	nd	nd	nd	0.43	0.37	0.17	0.62	0.32±0.24
2-Methylpropanoic acid	nd	nd	5.13	4.80	nd	1.99±2.72	nd	nd	4.03	nd	nd	0.81±1.80
Hexanoic acid	29.40	33.71	30.08	40.54	88.85	44.51±25.17	44.59	28.65	27.04	46.90	29.29	35.29±9.61
Octanoic Acid	7.43	6.51	5.42	6.76	25.19	10.26±8.38	11.57	5.32	3.47	6.60	2.80	5.95±3.48
Aldehyde												
Benzaldehyde	nd	nd	49.99	nd	77.06	25.41±36.09	7.37	28.86	15.98	32.97	47.02	26.44±15.37
4-Methylbenzaldehyde	0.71	1.72	0.98	1.15	1.42	1.19±0.39	1.40	0.88	0.91	1.24	1.07	1.10±0.22
Ketone												
4-Methyl-3-penten-2-one	297.66	308.21	231.75	559.12	903.98	460.14±277.69	636.30	676.76	380.98	430.74	512.75	527.50±127.64
3-Hydroxybutan-2-one	0.31	0.15	0.84	1.05	nd	0.47±0.45	0.15	0.54	1.09	0.29	0.84	0.58±0.39
Furan compound												
Ethyl 2-furoate	0.52	0.51	0.47	0.59	1.11	0.64±0.27	0.76	0.46	0.50	0.48	0.47	0.53±0.13
Dihydrofuran-2(3*H*)-one	3.95	3.69	6.93	2.65	7.27	4.90±2.07	8.37	4.02	4.82	5.62	4.85	5.54±1.68
Sulphur compound												
3-(Methylsulfanyl)propan-1-ol	1.52	0.95	0.59	1.09	2.00	1.23±0.54	1.97	0.64	1.07	1.17	0.76	1.12±0.52
Volatile phenol												
3,5-Di-*tert*-butylphenol	4.17	3.25	3.61	4.08	9.39	4.90±2.54	6.51	5.63	3.44	3.27	1.81	4.13±1.90

nd – not detected; SD – standard deviation. Z1-Z5 – Zweigelt wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

TABLE 3. Concentrations of volatile compounds in Rondo wines (μg/L) produced with different yeast and malolactic fermentation (MLF) combinations.

Compound	R1	R2	R3	R4	R5	Mean±SD	R1 LAB	R2 LAB	R3 LAB	R4 LAB	R5 LAB	Mean±SD
Alcohol												
Propanol-1-ol	49.20	32.97	55.28	24.88	48.32	42.13±12.67	18.26	46.87	39.06	66.87	66.75	47.56±20.45
2-Methylpropan-1-ol	354.16	276.16	457.58	202.60	288.59	315.82±95.78	204.64	215.66	294.11	500.74	379.76	318.98±123.59
Butan-1-ol	4.18	1.24	2.82	nd	5.01	2.65±2.06	0.13	2.47	3.45	5.22	2.57	2.77±1.84
3-Methylbutan-1-ol	2776.85	2577.03	3611.82	1798.22	2165.92	2585.97±686.64	1869.50	1604.25	2381.25	2796.93	2821.31	2294.65±546.48
Pentan-1-ol	nd	0.96	1.66	0.51	0.33	0.69±0.64	nd	0.30	0.77	1.16	1.10	0.67±0.51
4-Methylpentan-1-ol	nd	0.47	4.13	0.60	1.35	1.31±1.65	2.22	1.28	1.90	1.17	1.78	1.67±0.44
3-Methylpentan-1-ol	3.71	0.91	7.01	1.50	1.56	2.94±2.51	4.33	2.43	3.31	3.23	2.07	3.08±0.88
Hexan-1-ol	97.72	87.73	154.02	66.99	71.45	95.58±34.93	83.96	70.51	106.43	125.72	101.75	97.67±21.25
3-Ethoxypropan-1-ol	0.28	0.59	0.88	0.40	0.42	0.51± 0.23	0.09	0.50	0.67	0.59	0.52	0.47±0.23
(*Z*)-2-Hexen-1-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Octen-3-ol	1.10	1.27	1.83	0.90	0.93	1.21±0.38	1.17	0.97	1.93	1.94	1.62	1.53±0.44
Heptan-1-ol	1.22	1.05	2.59	0.89	1.32	1.42±0.68	1.31	1.08	2.87	2.32	1.87	1.89±0.73
2-Ethylhexan-1-ol	6.65	6.13	12.12	2.36	7.36	6.92±3.49	8.54	3.64	10.58	4.34	9.56	7.33±3.15
3-Ethyl-4-methylpentan-1-ol	0.23	0.22	0.33	0.15	0.13	0.21±0.08	0.19	0.15	0.25	0.28	0.22	0.22±0.05
Butane-2,3-diol	121.13	74.36	37.61	68.27	75.70	75.42±29.88	51.76	58.62	102.65	113.04	61.57	77.53±28.14
Octan-1-ol	0.70	0.67	3.11	0.79	2.22	1.50±1.11	3.82	1.68	6.65	4.79	5.78	4.54±1.92
Propane-1,2-diol	2.65	1.43	0.11	0.82	1.68	1.34±0.95	nd	0.28	2.13	2.37	1.19	1.19±1.06
2-(2-Ethoxyethoxy)-ethanol	0.34	0.31	0.35	0.22	0.37	0.32±0.06	0.13	0.19	0.35	0.28	0.24	0.24±0.08
Decan-1-ol	nd	nd	nd	nd	nd	nd	nd	nd	0.04	nd	nd	0.01±0.02
Phenylmethanol	0.15	0.06	0.07	0.02	0.04	0.07±0.05	0.04	0.03	0.07	0.03	0.08	0.05±0.01
2-Phenylethanol	144.07	75.36	61.75	116.18	176.69	114.81±47.59	90.11	83.79	141.58	146.74	117.70	115.98±28.77
Ester												
Ethyl butanoate	13.98	14.26	29.45	5.51	16.27	15.89±8.63	10.39	13.67	21.49	27.31	18.65	18.30±6.62
Ethyl 3-methylbutanoate	3.83	2.58	2.65	1.82	3.71	2.92±0.84	2.26	1.81	1.92	2.71	2.80	2.30±0.45
3-Methylbutyl acetate	72.26	66.66	39.94	43.11	89.77	62.35±20.86	85.87	80.32	18.75	48.36	118.89	70.44±38.22
Hexyl acetate	0.25	0.46	0.16	nd	nd	0.17±0.19	0.37	0.09	0.12	0.12	0.19	0.18±0.11

TABLE 3 continued

Compound	R1	R2	R3	R4	R5	Mean±SD	R1 LAB	R2 LAB	R3 LAB	R4 LAB	R5 LAB	Mean±SD
Ethyl heptanoate	9.28	2.67	7.42	1.01	3.51	4.78±3.45	3.13	2.16	4.37	3.89	0.90	2.89±1.39
Ethyl 2-hydroxypropanoate	95.55	95.39	127.08	72.82	54.21	89.01±27.41	507.93	388.00	487.93	535.72	536.76	491.27±61.23
Methyl octanoate	0.14	0.18	nd	0.20	nd	0.10±0.10	0.16	nd	nd	nd	nd	0.03±0.07
Ethyl octanoate	12.59	22.69	39.00	15.39	24.07	22.75±10.29	14.35	12.45	45.26	32.63	32.30	27.40±13.82
3-Methylbutyl 2-hydroxypropanoate	1.47	1.28	1.35	1.29	1.25	1.33±0.09	7.85	6.16	10.05	10.96	10.45	9.09±2.03
Ethyl decanoate	0.10	0.07	0.40	0.12	0.38	0.21±0.16	nd	0.10	0.40	0.25	0.31	0.21±0.16
Diethyl butanedioate	20.99	15.44	22.12	13.61	20.65	18.56±3.78	12.88	10.99	29.31	14.14	17.36	16.94±7.29
2-Phenylethyl acetate	nd	0.47	0.15	0.21	0.39	0.24±0.19	0.74	0.16	0.53	0.54	1.18	0.63±0.37
Acid												
Acetic acid	54.19	84.16	76.34	63.63	87.78	73.22±14.10	159.17	161.58	197.76	269.61	213.71	200.37±45.22
Propanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Methylpropanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.71	11.62	3.07±5.04
Hexanoic acid	9.26	5.05	8.42	9.38	15.39	9.50±3.73	4.48	6.94	12.79	12.17	11.97	9.67±3.73
Octanoic Acid	nd	0.12	1.20	0.38	2.04	0.75±0.86	0.16	1.38	3.16	1.29	2.21	1.64±1.12
Aldehyde												
Benzaldehyde	5.56	10.01	14.58	5.37	12.75	9.65±4.15	5.64	8.76	20.43	5.10	9.36	9.86±6.20
4-Methylbenzaldehyde	0.84	0.13	0.35	0.62	0.31	0.45±0.28	0.65	0.19	0.16	0.74	0.90	0.53±0.34
Ketone												
4-Methyl-3-penten-2-one	368.12	229.16	143.72	149.74	272.35	232.62±93.17	179.93	192.16	248.48	305.21	251.76	235.51±50.64
3-Hydroxybutan-2-one	0.15	0.44	0.79	0.19	0.04	0.32±0.30	0.15	nd	0.47	0.61	0.35	0.32±0.24
Furan compound												
Ethyl 2-furoate	0.48	0.45	0.52	0.32	0.53	0.46±0.08	0.27	0.23	0.44	0.33	0.35	0.32±0.08
Dihydrofuran-2(3*H*)-one	6.66	4.13	4.10	3.25	5.29	4.69±1.32	7.57	6.50	12.99	10.44	8.10	9.12±2.60
Sulphur compound												
3-(Methylsulfanyl)propan-1-ol	1.99	0.74	0.90	1.13	1.71	1.30±0.54	1.08	0.82	1.70	1.43	1.09	1.22±0.34
Volatile phenol												
3,5-Di-*tert*-butylphenol	2.23	1.83	1.57	2.00	1.97	1.92±0.24	2.06	1.26	3.95	2.55	2.69	2.50±0.99

nd – not detected; SD – standard deviation. R1-R5 – Rondo wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

TABLE 4. Results of one-way analysis of variance (ANOVA) and two-way ANOVA of Rondo and Zweigelt wines produced with different yeast and malolactic fermentation (MLF) strategies.

Compound	One-way ANOVA			Two-way ANOVA		
	Grape variety effect	Yeast effect	MLF effect	Yeast effect	MLF effect	Yeast×MLF effect
Alcohol						
Propanol-1-ol	***	NS	NS	NS	NS	NS
2-Methylpropan-1-ol	NS	NS	NS	NS	NS	NS
Butan-1-ol	**	NS	NS	NS	NS	NS
3-Methylbutan-1-ol	**	NS	NS	NS	NS	NS
Pentan-1-ol	***	NS	NS	NS	NS	NS
4-Methylpentan-1-ol	***	NS	NS	NS	NS	NS
3-Methylpentan-1-ol	***	NS	NS	NS	NS	NS
Hexan-1-ol	***	NS	NS	NS	NS	NS
3-Ethoxypropan-1-ol	***	***	NS	***	NS	NS
(*Z*)-2-Hexen-1-ol	***	*	NS	*	NS	NS
Octen-3-ol	*	NS	NS	NS	NS	NS
Heptan-1-ol	***	NS	NS	NS	NS	NS
2-Ethylhexan-1-ol	*	NS	NS	NS	NS	NS
3-Ethyl-4-methylpentan-1-ol	***	NS	NS	NS	NS	NS
Butane-2,3-diol	NS	NS	NS	NS	NS	NS
Octan-1-ol	***	NS	NS	NS	NS	NS
Propane-1,2-diol	NS	NS	NS	NS	NS	NS
2-(2-Ethoxyethoxy)-ethanol	NS	NS	NS	NS	NS	NS
Decan-1-ol	*	NS	NS	NS	NS	NS
Phenylmethanol	***	*	*	**	*	NS
2-Phenylethanol	**	**	NS	**	NS	**
Ester						
Ethyl butanoate	***	NS	NS	NS	NS	NS
Ethyl 3-methylbutanoate	***	NS	NS	NS	NS	NS
3-Methylbutyl acetate	NS	NS	*	NS	*	NS
Hexyl acetate	NS	NS	NS	NS	NS	NS
Ethyl heptanoate	NS	NS	*	NS	*	NS
Ethyl 2-hydroxypropanoate	*	NS	***	NS	***	NS
Methyl octanoate	NS	NS	*	NS	*	NS
Ethyl octanoate	**	NS	NS	NS	NS	NS
3-Methylbutyl 2-hydroxypropanoate	***	NS	***	NS	***	NS
Ethyl decanoate	NS	NS	NS	NS	NS	NS
Diethyl butanedioate	***	NS	NS	NS	NS	NS
2-Phenylethyl acetate	NS	NS	NS	NS	NS	NS

TABLE 4 continued

Compound	One-way ANOVA			Two-way ANOVA		
	Grape variety effect	Yeast effect	MLF effect	Yeast effect	MLF effect	Yeast×MLF effect
Acid						
Acetic acid	*	NS	***	NS	***	NS
Propanoic acid	**	NS	**	NS	**	NS
2-Methylpropanoic acid	NS	NS	NS	NS	NS	NS
Hexanoic acid	***	NS	NS	NS	NS	NS
Octanoic acid	***	NS	NS	NS	NS	*
Aldehyde						
Benzaldehyde	**	***	NS	***	NS	NS
4-Methylbenzaldehyde	***	NS	NS	NS	NS	NS
Ketone						
4-Methyl-3-penten-2-one	***	NS	NS	NS	NS	NS
3-Hydroxybutan-2-one	NS	**	NS	***	NS	NS
Furan compound						
Ethyl 2-furoate	***	NS	*	NS	*	NS
Dihydrofuran-2(3*H*)-one	NS	NS	**	NS	**	NS
Sulphur compound						
3-(Methylsulfanyl)propan-1-ol	NS	**	NS	**	NS	**
Volatile phenol						
3,5-Di-*tert*-butylphenol	**	NS	NS	NS	NS	NS

Statistical significance: *significant at $p<0.05$, **significant at $p<0.01$, *** significant at $p<0.001$, NS – not significant.

and yeast×MLF interaction influenced the concentrations of phenylmethanol and 2-phenylethanol, respectively (Table 4). Regarding yeast×MLF interaction, according to Gammacurta *et al*. [2017], 3-methylbutan-1-ol was the only higher alcohol not affected by yeast/LAB combination, while concentrations of other alcohols, such as propan-1-ol, 2-methylpropan-1-ol, and 2-methylbutan-1-ol, differed significantly depending on yeast/LAB combination.

Concentrations of half of the esters: ethyl butanoate, ethyl 3-methylbutanoate, ethyl 2-hydroxypropanoate, ethyl octanoate, 3-methylbutyl 2-hydroxypropanoate, and diethyl butanedioate differed significantly according to the grape variety (Table 4). However, Liu *et al*. [2017] found that grape variety significantly impacted concentrations of most esters. Ethyl esters are produced enzymatically by yeast from ethanolysis of acetyl-CoA formed during fatty acid synthesis or degradation [Liu *et al*., 2017; Stój *et al*., 2017a]. The concentrations of any esters were not significantly affected by yeast strain nor by yeast×MLF interaction (Table 4). On the contrary, Gammacurta *et al*. [2014] observed a significant effect of yeast strain on all esters in the red wines studied. Blanco *et al*. [2014] found that the concentrations of several esters (ethyl butanoate, hexyl acetate, 2-phenylethyl acetate, diethyl butanedioate) were independent of yeast strain, while other esters (3-methylbutyl acetate and ethyl octanoate) were yeast strain-dependent. Regarding yeast×MLF interaction, Gammacurta *et al*. [2017] reported that among 40 quantified esters, only seven were not affected by the yeast/LAB combination. Among seven esters, the concentrations of hexyl acetate and ethyl octanoate (also identified in our study) did not depend on the combination of microorganisms. MLF significantly influenced the concentrations of almost half of the esters, *i.e.* 3-methylbutyl acetate (isoamyl acetate), ethyl heptanoate, ethyl 2-hydroxypropanoate (ethyl lactate), methyl octanoate, and 3-methylbutyl 2-hydroxypropanoate (isoamyl lactate). Gammacurta *et al*. [2014] considered that the impact of LAB on esters was controversial because results differed between studies. Several reports showed changes in ester concentrations in wines after MLF with *O. oeni* due to its esterase activity [Brizuela *et al.*, 2018; Diez-Ozaeta *et al.*, 2021; Sumby *et al.*, 2013]. However, Gammacurta *et al*. [2014] found that levels of esters were slightly affected by the LAB. In our study, the ethyl lactate concentration was higher in the wines subjected to induced MLF than in those subjected to spontaneous MLF. Ethyl lactate is one

of the most characteristic aromatic compounds produced during MLF and a marker of LAB activity. Its content increased following MLF [Abrahamse & Bartowsky, 2012; Costello *et al*., 2012; Lasik-Kurdyś *et al*., 2018].

We observed a grape variety effect on most acids, *i.e.* acetic acid, propanoic acid, hexanoic acid, and octanoic acid, whereas Liu *et al*. [2017] found significant differences for almost half of the acids. Fatty acids could be produced *via* anabolic pathways by yeast or during β-oxidation of long-chain fatty acids [Stój *et al*., 2017a]. The concentrations of any acids did not vary depending on yeast strain (Table 4). According to Blanco *et al*. [2014], only the hexanoic acid content of red wines depended on the yeast strain. MLF significantly modulated half of the acid (acetic acid and propanoic acid) contents in our study (Table 4). The concentration of acetic acid was higher in the wines subjected to induced MLF than in those subjected to spontaneous MLF (Table 2 and Table 3). The increase in acetic acid content is due to the metabolism of citric acid [Abrahamse & Bartowsky, 2012; Styger *et al*., 2011]. The octanoic acid concentration was significantly impacted by yeast×MLF interaction (Table 4), which agreed with the results obtained by Gammacurta *et al*. [2017]. Concentrations of 2-methylpropanoic acid and hexanoic acid were not significantly modulated by yeast/LAB combinations, while Gammacurta *et al*. [2017] reported significant differences in the contents of these acids between microorganism combinations.

Regarding the two quantified aldehydes, concentrations of benzaldehyde and 4-methylbenzaldehyde differed significantly between grape varieties (Table 4). Yeast strain significantly impacted the concentration of benzaldehyde, similarly to the work of Tufariello *et al*. [2014] and contrary to the work of Blanco *et al*. [2014]. Neither MLF nor yeast×MLF interaction affected aldehyde concentrations.

The concentration of 4-methyl-3-penten-2-one differed significantly between wines produced from different grape varieties (Table 4). Grape variety did not affect 3-hydroxybutan-2-one (acetoin) concentration, contrary to the report of Liu *et al*. [2017]. The concentration of 3-hydroxybutan-2-one was significantly affected by yeast strain, which agreed with results obtained by Blanco *et al*. [2014]. The concentrations of the two identified ketones did not differ significantly upon the influence of either MLF or yeast×MLF interaction (Table 4). Considering the interactions, Gammacurta *et al*. [2017] observed no significant differences in the concentration of 3-hydroxybutan-2-one. Acetoin is produced from the metabolism of citric acid by lactic acid bacteria [Malherbe *et al*., 2012; Styger *et al*., 2011; Tempère *et al*., 2018]. In our study, there was no trend in its concentration in Zweigelt and Rondo wines subjected to spontaneous and induced MLF. Its concentration was higher in a few wines in which MLF was spontaneous and in a few wines in which MLF was induced. According to López *et al*. [2011], spontaneous MLF resulted in a higher content of acetoine in wines, whereas Malherbe *et al*. [2012] found that acetoin concentration was affected by the bacterial strain used for MLF.

Considering the two quantified furan compounds, grape variety significantly impacted the content of ethyl 2-furoate (Table 4). In turn, the concentration of dihydrofuran-2(3*H*)-one was not influenced by the grape variety, and it is in agreement with the results obtained by Liu *et al*. [2017]. The yeast strain and yeast×MLF interaction did not affect ethyl 2-furoate and dihydrofuran-2(3*H*)-one. Similarly, the yeast did not affect ethyl 2-furoate in studies performed by Callejon *et al*. [2010]. Concentrations of both furan compounds, ethyl 2-furoate and dihydrofuran-2(3*H*)-one (butyrolactone), depend on MLF. Butyrolactone is particularly produced during MLF [Tempère *et al*., 2018]. In our study, the concentration of butyrolactone was higher in most wines subjected to induced MLF. According to López *et al*. [2011], spontaneous MLF resulted in wines with a higher butyrolactone concentration.

The concentration of 3-(methylsulfanyl)propan-1-ol (Table 4) was not affected by grape variety and MLF, but changes in its values were determined as affected by yeast strain and yeast×MLF interaction.

Among the analyzed factors, *i.e*. grape variety, yeast, MLF, yeast×MLF interaction, only grape variety significantly affected the concentration of 3,5-di-*tert*-butylphenol (Table 4).

We showed that in the case of Polish wines produced from grape varieties grown in a cold climate, the grape variety was the factor eliciting a significant impact on the highest number of identified volatile compounds, whereas microorganisms used had a lesser effect. In our study, malic acid was not completely reduced during MLF (spontaneous and induced), similarly to the experiment conducted by Lasik-Kurdyś *et al.* [2018]. Lactic acid has an antimicrobial activity, and at a higher concentration, it can also inhibit LAB. In the case of high-acid musts, a total reduction of malic acid can be impossible [Lasik-Kurdyś *et al.*, 2017].

Figure 1 presents the FTIR spectra of the wines. For more straightforward analysis, discussion, and comparison of the tested wines, the spectra were normalized to the maximum at 3327 cm^{-1}. Table 5 presents all the characteristic bands of wine spectra and appropriate vibrations assigned to the functional groups. All spectra of wines (R1-R5, Z1-Z5, R1 LAB-R5 LAB and Z1 LAB-Z5 LAB) had very intense and similar bands (Figure 1). They exhibited bands characteristic of water and ethanol absorption. The broad peak of 3800–3000 cm^{-1} results mainly from stretching vibrations in water molecules, ν(-OH), and in alcohol or phenol molecules [Basalekou *et al*., 2019; Geană *et al*., 2019]. Characteristic bands for vibrations of water molecules are absorption bands with a maximum of about 990 and 1460 cm^{-1} (stretching and deformation – related to the third overtone of these bands) and characteristic deformation bands with a maximum at about 1600 cm^{-1} [Geană *et al*., 2019; Hu *et al*., 2019]. Alcohol-related absorption bands were observed (for all samples) at 2850–3000 cm^{-1} with characteristic peaks at 2937 and 2881 cm^{-1} (Figure 1, Table 5) corresponding to the symmetric and asymmetric stretching vibrations of the CH_2 and CH_3 groups. The build-up of these bands with a broad and noticeably flattened maximum in the range of 2400–2705 cm^{-1} could correspond to a combination of C-H stretching vibrations and overtones of these vibrations originating from molecules of ethanol and partly sugar [Basalekou *et al*., 2019; Geană *et al*., 2019; Hu *et al*., 2019]. Vibrations originating from primary alcohols and glycerol with maximum bands at 1087 and 1050 cm^{-1}, respectively, are related to

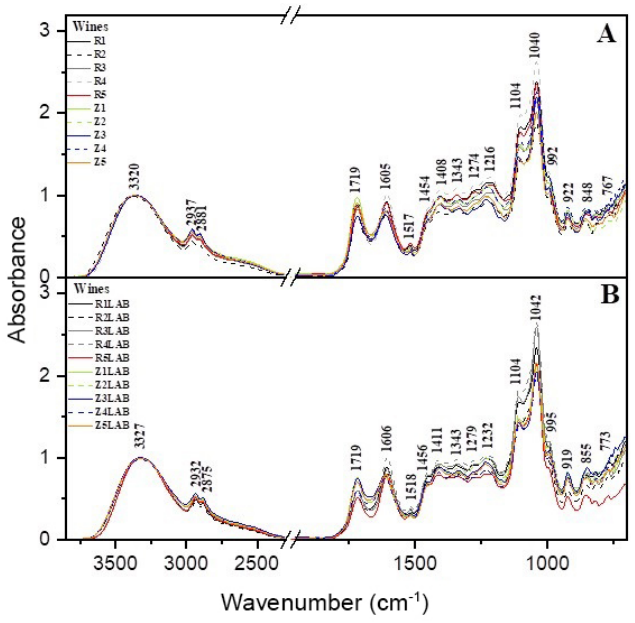

FIGURE 1. Normalized Fourier transform infrared (FTIR) spectra of wines. R1-R5 – Rondo wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous malolactic fermentation (MLF); Z1-Z5 – Zweigelt wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

strong C-O stretching vibrations [Martelo-Vidal *et al*., 2013]. The bands in the 3000–2800 cm^{-1} region were most likely due to stretching vibrations of C–H bonds of hydrocarbons, O–H bonds of carboxylic acids, and asymmetric stretching vibrations of C–H bonds of methyl ($–CH_3$) groups: polyols (glycerol), free phenolic acids and catechins [Geană *et al*., 2019].

The vibration area between 1800–1000 cm^{-1} was characteristic of C-OH stretching, CH_3 and CH_2 deformation, C=C stretching, and C≡N stretching vibrations (Table 5). This area derives from such components as phenols, alcohols, aldehydes, higher alcohols, polyols, acids, sugars, volatile acids and amino acids [Basalekou *et al*., 2020; Hu *et al*., 2019; Versari *et al*., 2014]. The spectral range of about 1850–1590 cm^{-1} was related to the combination of stretching vibrations -OH, $-CH_3$ (first overtone), $-CH_2$, -CH (first overtone) derived from ethanol [Geană *et al*., 2019; Martelo-Vidal *et al*., 2013].

Very interesting vibrations occured in the spectral region of 1580–950 cm^{-1} (Figure 1). In this area, the vibrations show functional groups characteristic for many wine compounds; therefore, there were more considerable differences between the FTIR spectra obtained in this region. Basically, in the region of 1580–950 cm^{-1}, there were vibrations from phenols of the wines tested. The area between 1460 and 1280 cm^{-1} was very complex and provided information about stretching vibrations of the carbonyl group C=O, stretching vibrations C=C, CH_2, and C-H derived from molecules of aldehydes, carboxylic acids, proteins, and esters [Tarantilis *et al*., 2008].

TABLE 5. The maxima of the Fourier transform infrared absorption bands of wines produced with different yeast and malolactic fermentation (MLF) strategies, with assignment of particular vibrations to the respective wine samples. Spectra registered within the range of 700–3700 cm^{-1}.

R1-R5 Z1-Z5	R1 LAB-R5 LAB Z1 LAB-Z5 LAB	Type and origin of vibrations
Wavenumber (cm^{-1})		
3320	3327	ν(-OH) in carboxylic acids
2937	2932	ν_w(-CH) of hydrocarbons $\nu_m(–CH_3)$
2881	2875	
1719	1719	ν_m(-C=O)
1605	1606	δ(-OH) and ν(C=C)
1517	1518	ν(C=C) and ν(C–N)
1454	1456	ν(C=C), $\delta(–CH_3)$, $\delta_m(–CH_2)$ and δ(–CH)
1408	1411	
1343	1343	ν(C=C), $\delta(–CH_2)$
1274	1279	$\delta(-CH_2-)$
1216	1232	ν_m(-C-O) or $\delta_m(-CH_2-)$
1104	1104	ν_{st}(-C-O) and ν_w(O–H) second overtones
1040	1042	ν_m(-C-O)
992	995	δ_w(-HC=CH-, *trans*-) out-of-plane $\delta(-(CH_2)_n-$ and –HC=CH- (*cis*-) (scissor)
922	919	
848	855	
767	773	

ν – stretching vibrations; δ – deformation vibrations; st – strong; m – medium; w – weak; R1-R5 – Rondo wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; Z1-Z5 – Zweigelt wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria

The ester bands showed very characteristic peaks at about 1460–1400 cm^{-1} [Geană *et al*., 2019; Tarantilis *et al*., 2008]. It is also worth emphasizing the bands with maxima of around 1232, 1110–1100, and 1070–990 cm^{-1}, which correspond to C–O and O–H stretching vibrations (second overtone) derived from sugars and organic acids [Ferreiro-González *et al*., 2019; Hu *et al*., 2019; Martelo-Vidal *et al*., 2013; Tarantilis *et al*., 2008].

Spectral analysis showed differences in the area for groups associated with alcohols, esters, and acids. It was most likely related to the grape varieties used, however, the main constituents formed during fermentation could also explain the difference. The wines marked as LAB (induced MLF) in the area mentioned above differed from the wines which were left to undergo spontaneous MLF.

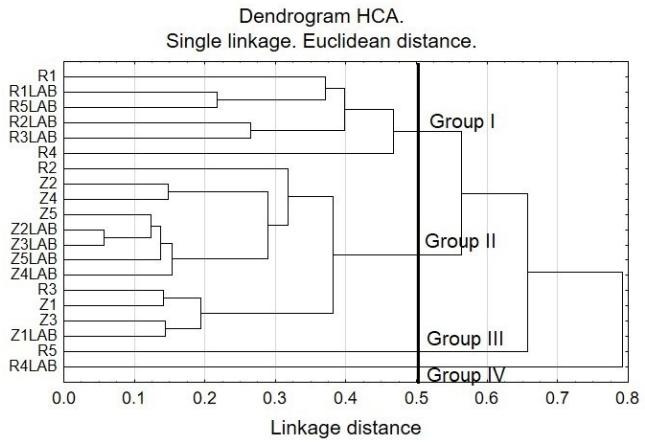

FIGURE 2. Dendrogram of hierarchical cluster analysis (HCA) of wines based on the Fourier transform infrared (FTIR) spectral data for wavenumber range of 1580–950 cm^{-1}. R1-R5 – Rondo wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous malolactic fermentation (MLF); Z1-Z5 – Zweigelt wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

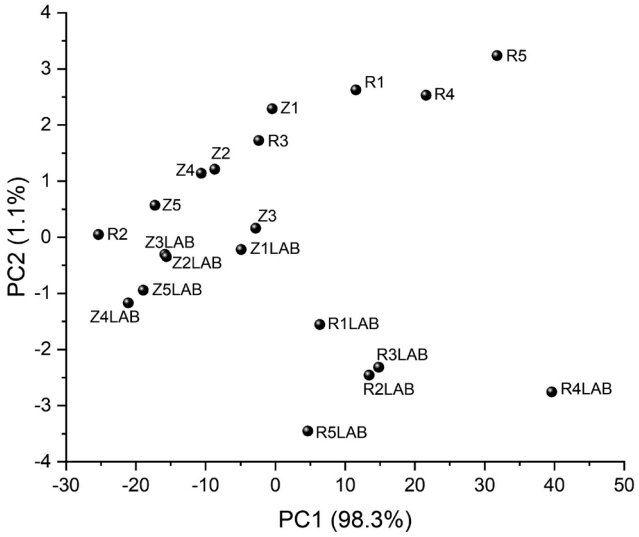

FIGURE 3. The scattered scores plot of principal component analysis (PCA) based on Fourier transform infrared (FTIR) spectra fingerprint of wines. R1-R5 – Rondo wines, in which alcoholic fermentation (AF) was induced using various yeast strains, and the wines were left to undergo spontaneous MLF; Z1-Z5 – Zweigelt wines, in which AF was induced using various yeast strains, and the wines were left to undergo spontaneous malolactic fermentation (MLF); R1 LAB-R5 LAB – Rondo wines, in which AF was induced using various yeast strains (the same strains as in R1-R5 wines), and MLF was carried out by inoculation with lactic acid bacteria; Z1 LAB-Z5 LAB – Zweigelt wines, in which AF was induced using various yeast strains (the same strains as in Z1-Z5 wines), and MLF was carried out by inoculation with lactic acid bacteria.

HCA was performed on FTIR spectra to identify similarities or dissimilarities between the considered samples of wine [Brereton, 2003]. Figure 2 shows the dendrogram obtained from the 20 wine samples. Considering the cut-off of 0.5 dissimilarity units, four clusters were distinguished. The first cluster (Group I) aggregated on the far-left arm of the dendrogram and was formed by six wine samples, while the second cluster (Group II) was the biggest cluster and comprised all samples of Zweigelt wine and two samples of Rondo – R2 and R3. This result suggests these wines have physicochemical properties more similar than the others. The last two Rondo wine samples – R5 and R4 LAB aggregated in third and fourth clusters. The hierarchical cluster analysis showed that the placement of the wine samples on the dendrogram depended on the grape variety and type of MLF (spontaneous or induced).

To more precisely discriminate the relationship between the investigated wines, the 1580–950 cm^{-1} bands were selected as their characteristic spectral fingerprint. The PCA was set as the primary choice for analysis. PCA transforms the original high-dimensional variables into the new low-dimensional variables [Abdi & Williams, 2010; Xu *et al*., 2006]. According to the Scree test criterion, we selected two main components for analysis. Figure 3 shows a two-dimensional scatter plot of the principal components (PC1 and PC2) obtained from the FTIR spectra of different wine samples. The PC1 was the most critical and explained 98.3% of the variance; the second principal component contributed 1.1% to the variance. The first two principal components explained 99.4% of the variance, and only 0.6% of the information was lost. It indicates that the first two principal components expressed 99.4% of all the information. Figure 3 and Figure S3 (supplementary materials) show the relative location of the FTIR spectra in the two-dimensional graph. The location was related to their similarity distance shown by HCA (Figure 2). For example, the close location of Zweigelt wines in the PCA plot was related to their close locations in the HCA graph. Samples R4 LAB and R5, which did not clustered with the other Rondo wines (Figure 2), were obviously distinguished from other samples shown in the two-dimensional PCA graph (Figure 3). Samples of the Zweigelt variety distinguished one another in the same category, as shown on the grouped-scatter plot for all samples (Figure S3). These samples differed only to a slight extent. As it is known, the scatter plot does not classify the objects in every level of distance as accurately as the clustering graph. Still, it can reflect the relationships between the investigated wines. Therefore, the use of complementary analytical methods provides researchers profound knowledge of the wine differences.

CONCLUSIONS

Among the factors studied, the grape variety significantly affected most volatile compounds, most alcohols and acids, and half of the esters. The yeast strains selected in this study had no significant effect on the concentration of either any ester or any acid. MLF significantly influenced the concentration of almost half of esters and acids. Among forty-six quantified compounds, only three were affected by yeast×MLF interaction. Knowledge of the influence of grape, yeast, MLF, and yeast×MLF interaction on wine aroma may help

producers make informed decisions. Further investigations into the effects of the wine matrix on the production of volatile compounds by microorganisms are also required.

The results of FTIR measurements showed differences, most remarkable in the range of 1750–1500 and below 1500 cm^{-1}. These differences are mainly related to various concentrations of volatile compounds, such as alcohols, acids, and esters, in the wines. The results obtained in the experiment may contribute to the expansion of the wine database.

SUPPLEMENTARY MATERIALS

The following are available online at http://journal.pan.olsztyn.pl/Impact-of-Grape-Variety-Yeast-and-Malolactic-Fermentation-on-Volatile-Compounds-and,145665,0,2.html. The concentrations of malic and lactic acids in the final wines; chromatograms of volatile compounds of Zweigelt and Rondo wines, and PCA results.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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