



Article

Pre-Fermentative Cold Maceration and Native Non-*Saccharomyces* Yeasts as a Tool to Enhance Aroma and Sensory Attributes of Chardonnay Wine

Marko Malićanin ¹, Bojana Danilović ^{2,*} , Sandra Stamenković Stojanović ² , Dragan Cvetković ², Miodrag Lazić ² , Ivana Karabegović ² and Dragiša Savić ²

¹ Faculty of Agriculture, University of Nis, Kosančićeva 4, 37000 Krusevac, Serbia; malicanin.marko@ni.ac.rs

² Faculty of Technology, University of Nis, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia; sandrastamenkovic@tf.ni.ac.rs (S.S.S.); cvetkovic@tf.ni.ac.rs (D.C.); lazic@tf.ni.ac.rs (M.L.); ivana.karabegovic@tf.ni.ac.rs (I.K.); savic@tf.ni.ac.rs (D.S.)

* Correspondence: bojana.danilovic@junis.ni.ac.rs

Abstract: The oenological potential of native strains of *Metschnikowia pulcherrima* B-5 and *Candida famata* WB-1, isolated from blackberries, was investigated in pure and sequential fermentation of Chardonnay grape with commercial *Saccharomyces cerevisiae* QA23. The effect of pre-fermentative cold maceration was also analysed. The fermentations were performed in the pilot-scale trials and the profile of volatile compounds and their sensory characteristics were determined. The application of *C. famata* WB-1 and *M. pulcherrima* B-5 reduced the volatile acidity and increased total polyphenols of the wines, compared to the control samples. Higher alcohols and esters were dominant, while fatty acids and aldehydes and ketones were also detected. Esters had the greatest contribution to sensory characteristics, especially the development of floral and fruity aromas. Most esters were present in lower concentrations in sequential fermentations compared to the pure fermentations with *M. pulcherrima* B-5 or *C. famata* WB-1. Pre-fermentative maceration positively affected both the aroma and the sensory profile. The best sensory score was determined for wines produced using *C. famata* WB-1 in the fermentations without pre-fermentative maceration, and the sequential fermentation of *M. pulcherrima* B-5 with maceration. Native strains of *M. pulcherrima* B-5 and *C. famata* WB-1 have shown great potential for the enhancement of the aromatic and sensory profile of Chardonnay wine.

Keywords: Chardonnay wine; native yeasts; cold pre-fermentation treatment; *Metschnikowia pulcherrima*; *Candida famata*



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1. Introduction

During white wine production, only partial extraction of phenolic compounds from grape skin occurs due to the resistance of cell walls and cytoplasmic membranes to mass transfer. To enhance the transfer of different compounds into the must, different maceration techniques can be used to weaken the cell walls [1]. Cold maceration or cold-soak implies the use of low temperatures (5–15 °C) for several days prior to the fermentation. This process improves the extraction of different compounds from grape skins such as, pigments, tannins, and aroma compounds [2].

Due to the increasing demands of consumers for unique, improved, and characteristic wine aromas and tastes, the application of various non-*Saccharomyces* indigenous yeasts in wine production has gained a lot of interest in recent years [3–6]. The main reason for the involvement of non-*Saccharomyces* yeasts is the resultant increase in the concentrations of acetate esters and glycerol and enhancement of the aroma complexity [7]. As non-*Saccharomyces* yeasts can hardly complete the fermentation, they are usually used in sequential fermentation with *Saccharomyces cerevisiae* [8–10]. In this way, the correct fermentation can be ensured, while wine complexity and concentrations of desirable metabolites can be enhanced [11,12].

The investigation of the application of non-*Saccharomyces* yeasts in winemaking mainly refers to the genera *Hanseniaspora*, *Metschnikowia*, *Candida* and *Torulaspora* [13]. Compounds that form the primary (grape-derived) aroma, have a significant role in the distinctive aroma attributes of the produced wine. During fermentation, both *Saccharomyces* and non-*Saccharomyces* yeasts, induce the development of the final wine aroma by biosynthesis of specific compounds (secondary aroma) and influence primary aroma compounds by the action of specific enzymes [14]. Application of *Candida famata* strains relies on the developed enzymatic system of this yeast as it can contribute to the release of the aroma compounds in wine [15]. The increased production of β -D-glucosidase in *C. famata* promotes the release of monoterpene compounds in wine aroma [14,16]. Additionally, *C. famata* strains have the potential for increased production of glycerol in pure and sequential fermentation with *S. cerevisiae* [17].

The yeast *Metschnikowia pulcherrima* is often present during the first stages of wine-making. The presence of *M. pulcherrima* during wine production is mainly associated with decreased volatile acidity, ethanol and H_2S [18] and increased content of terpenes, phenylethyl alcohol, ethyl octanoate, ethyl acetate and 2-phenylethyl acetate [14]. Increased synthesis of aroma compounds relies on the developed enzymatic system in *M. pulcherrima*. The activity of sulphite-reductase, β -lyase, protease and β -D-glucosidase has been reported during the application of *M. pulcherrima* in pure and sequential wine fermentation [18–21]. Additionally, this yeast can have an impact on biological control during winemaking by inhibition of some yeast strains such as *Candida tropicalis*, *C. albicans*, *Hanseniaspora* spp., *Pichia* spp. and some moulds e.g., *Botrytis cinerea*, *Penicillium* spp., and *Alternaria* spp. [21,22].

Based on the promising potential of native yeast strains in the production of red wine [17], the aim of this paper was to determine the sensory characteristics and volatile compounds of Chardonnay wine produced with two native strains, *M. pulcherrima* B-5 and *C. famata* WB-1, previously isolated from cultivated and wild blackberries, respectively, and specific to the same geographical regions as grape. The yeast strains were selected according to their good oenological characteristics in order to analyse the potential of local non-*Saccharomyces* yeast strains and their eventual contribution to the regional character of the wines. Additionally, this paper will analyse the effect of cold maceration on the characteristics of Chardonnay wine produced with pure and sequential fermentation with native *C. famata* WB-1 or *M. pulcherrima* B-5, and the commercial strain *S. cerevisiae* QA23.

2. Materials and Methods

2.1. Wine Production

The Chardonnay grape variety was manually harvested (approximately 1000 kg of grapes) in the wine subregion Tri Morave, Central Serbia (43°37' N, 21°34' E, continental climate, single Guyot vine training system), in September 2020. The characteristics of the must were: 23.8 Brix, 6.8 g/L total acidity, pH 3.34. The grapes were destemmed and crushed, and after the addition of potassium-metabisulfite (5 mg/L), pectolytic enzyme (Lallzyme Cuvée Blanc, Lallemand, Montreal, QC, Canada, 2 mg/L) and vitamin C (5 mg/L), divided into two lots of pomace (500 kg for each lot). The first part of the pomace was immediately pressed (0.6 bar, pneumatic press, Škrli, Slovenia) and clarified (static sedimentation 24 h at 8–10 °C), and then was divided into five steel microvinificators (50 L). The second half of the pomace (500 kg) was subjected to pre-fermentative maceration for 48 h at the temperature of 4 °C. After maceration, the pomace was pressed, clarified, and divided into another five steel microvinificators (50 L). Inoculation was performed with the native strains *C. famata* WB-1, *M. pulcherrima* B-5 (48 h old yeast culture, to the final cell number of 1×10^6 CFU/mL) or *S. cerevisiae* QA23 (Lallemand, Montreal, QC, Canada, 25 mg/L). Yeast strains used in this research were previously isolated from blackberries and identified by PCR analysis according to their ITS sequence (data not shown). For sequential fermentations, *M. pulcherrima* B-5 and *C. famata* WB-1 were used in the initial fermentation stages, and after the reduction of °Brix for 3 degrees, inoculation with *S. cerevisiae* was done. Fermentations were performed twice in each group of experiments. Yeast nutrient Fermaid

E (Lallemand, Montreal, QC, Canada) was added in the concentration of 40 mg/L. After the reduction of the sugar level to 4 g/L, samples were sulphited (25 mg/L). After two months, fining of the wine was performed with bentonite (1 g/L), then it was filtered using Seitz filter plates K 100 (Pall Seitz, Kriftel, Germany), bottled, and stored for about one month until analysis.

Standard wine analyses were performed according to The International Organization of Vine and Wine [23]. All experiments were performed in triplicate and expressed as the mean value with standard deviation.

2.2. Extraction of Volatile Compounds in Wines by Headspace-Solid Phase Microextraction (HS-SPME)

Carboxen[®]/Polydimetilsiloxane (CAR/PDMS, 85 μ m, Supelco, Bellefonte, PA, USA) fibre inside a SPME manual holder was used for the HS-SPME. The fibre preconditioning was performed according to the manufacturer's instructions. The samples, 20 mL of wine with 3 g of NaCl and a magnetic stirrer bar were inserted in a dark glass vial (30 mL) and covered with a rubber septum and parafilm. The samples were pre-extracted at 55 °C for 15 min. After pre-extraction, the extraction (sorption) was performed for 35 min under the same temperature and stirring regime. The fibre desorption was carried out for 10 min in the inlet at 250 °C (20:1 split mode) and analysed by the GC/MS/FID technique.

2.3. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis and Gas Chromatography-Flame Ionization Detection (GC/FID) of Volatile Compounds in Wines

An Agilent Technologies 7890B gas chromatograph, coupled with a 5977A mass detector, was used for the GC/MS analysis using a weakly polar HP-5MS silica capillary column (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m $\times$ 0.25 mm, 0.25 μ m; Agilent Technologies, USA). Helium at a constant flow rate of 1 cm³ min⁻¹ was used as a carrier gas. The temperature program was set as: 2 min at 40 °C, up to 250 °C by 7 °C/min, and held at 250 °C for 2 min, consuming 34 min for total run time. The MSD transfer line, the ion source, and the quadrupole mass analyser were held at 300 °C, 230 °C and 150 °C, respectively, with 70 eV of ionization voltage. Mass detection was done in the Scan mode (m/z range 25 to 550).

The software MSD ChemStation (version F.01.00.1903) and AMDIS (version 2.70)/NIST MS Search (version 2.0 g) were used for data processing. A homologous series of n-alkanes (C8-C20) was used for determining the retention indices. The experimentally obtained linear retention indices (LRI_{exp}) were compared with those in the literature (LRI_{lit}). The EI mass spectra were compared with data from Willey 6, NIST11 and RTLPEST 3 mass spectra libraries.

2.4. Quantitative HS-SPME Analysis

Quantification was done using an internal standard (IS) method. Five-points calibration curves were constructed by using the mixture of the following standards: phenylethyl alcohol (0.01–0.24 mg/mL) and ethyl dodecanoate (0.02–0.24 mg/mL) containing 2-octanol (IS). The samples were prepared by adding the appropriate volume of the standard mixture to the wine surrogate (12% ethanol and 4 g/L wine acid with pH value of 3.4 adjusted with NaOH solution). Extraction of the volatiles was performed as already described. The blanks were prepared by adding the appropriate volume of IS to the wine surrogate.

Calibration curves were constructed by plotting the ratio of the standards and the relative areas of the IS peaks in the GC/FID chromatograms, and the concentration ratio of the standards and the IS in the calibration solution.

2.5. Sensory Analysis

Sensory analysis was performed by the descriptive method, following the standards ISO 6658, ISO 3591 and OIV, 2015 [24–26], by a sensory panel of 11 officially certified persons from 29 to 51 years old (6 females and 5 males). Smell attributes (complexity, intensity, typicality, black fruit berries, red fruit berries, floral, vegetable, spice and toasted)

and taste attributes (harmony, intensity, typicality, complexity, fullness, structure, acidity, astringency, and duration) were quantified with a ten-point intensity scale (0—not detected, 10—very intense). All the analyses were performed in duplicate, and the results were expressed as the mean value.

2.6. Statistical Analysis

Statistical analysis was performed using SPSS Statistics 21 (IBM, New York, NY, USA). Shapiro–Wilk and Levene’s tests were performed to evaluate the normality of data distribution and homogeneity of variances respectively. To compare the standard quality parameters and aroma profile between wine samples produced by different yeasts or inoculation procedure one-way ANOVA followed by Tukey’s HSD test was used, while the Kruskal–Wallis test followed by Dunn’s test with Bonferroni correction ($p < 0.05$) were applied for variables that did not meet normality criteria. The independent samples *t*-test was used to assess the significance of the effect of the pre-fermentative treatment on the standard quality parameters and the content of volatile compounds in the wine samples.

3. Results

The winemaking potential of the native yeast strains *C. famata* WB-1 and *M. pulcherrima* B-5 isolated from blackberries, and the effect of pre-fermentative maceration on the characteristics of Chardonnay wine were evaluated in pure and sequential fermentation.

3.1. Standard Wine Analysis

The standard analysis of wine produced without maceration indicated that the content of ethanol was in the range 13.58–14.42% (Table 1). *C. famata* WB-1 in pure fermentation without maceration produced significantly lower concentration of ethanol compared to other analysed wines. Wine produced using *M. pulcherrima* B-5 had significantly lower content of total extract with 21.32 and 21.15 g/L in pure and sequential fermentation, respectively. The concentration of the total extract was the highest in wines produced with *C. famata* WB-1 and increased in sequential fermentation from 24.04 to 26.15 g/L. Total acids rated from 6.20 and 6.00 g/L for wines produced with *M. pulcherrima* B-5 and *C. famata* WB-1, respectively, to 6.40 g/L for both wine samples produced in sequential fermentation. Volatile acids were slightly higher in wines produced with *C. famata* strain and ranged from 0.39 to 0.48 g/L. Sequential fermentation with *S. cerevisiae* QA23 significantly reduced the content of reducing sugars. In pure fermentation, both *M. pulcherrima* B-5 and *C. famata* WB-1 had the ability to complete the fermentation to dryness. Additionally, in sequential fermentations, the content of total polyphenols was reduced in comparison to pure fermentation.

Table 1. Physicochemical analysis of wines produced with and without maceration.

Parameter	Process	<i>M. pulcherrima</i> B-5	<i>C. famata</i> WB-1	<i>S. cerevisiae</i> QA23	<i>M. pulcherrima</i> B-5 + <i>S. cerevisiae</i> QA23	<i>C. famata</i> WB-1 + <i>S. cerevisiae</i> QA23
Ethanol, %v/v	NM	14.39 ^{bA} ± 0.03	13.58 ^{aA} ± 0.22	14.32 ^{bA} ± 0.16	14.42 ^{bA} ± 0.12	14.07 ^{bA} ± 0.08
	CM	14.42 ^{bcA} ± 0.08	14.08 ^{aB} ± 0.08	14.48 ^{cA} ± 0.10	14.41 ^{cbA} ± 0.12	14.17 ^{abcA} ± 0.10
Total extract, g/L	NM	21.32 ^{aA} ± 0.08	24.04 ^{cA} ± 0.15	22.22 ^{bA} ± 0.22	21.15 ^{aA} ± 0.15	26.15 ^{dA} ± 0.15
	CM	25.53 ^{aB} ± 0.06	27.75 ^{bB} ± 0.15	25.34 ^{aB} ± 0.25	25.75 ^{aB} ± 0.15	29.85 ^{cB} ± 0.15
Total acids (as tartaric acid) g/L	NM	6.20 ^{aB} ± 0.08	6.00 ^{aA} ± 0.15	6.00 ^{abA} ± 0.15	6.40 ^{acB} ± 0.08	6.40 ^{abB} ± 0.20
	CM	6.00 ^{aA} ± 0.10	5.80 ^{aA} ± 0.20	5.90 ^{aA} ± 0.1	6.10 ^{abA} ± 0.15	6.20 ^{bA} ± 0.1
Volatile acids (as acetic acid)	NM	0.39 ^{aA} ± 0.06	0.42 ^{aA} ± 0.03	0.36 ^{abA} ± 0.02	0.39 ^{aA} ± 0.02	0.48 ^{acA} ± 0.06
	CM	0.38 ^{aA} ± 0.04	0.39 ^{aA} ± 0.04	0.39 ^{aA} ± 0.04	0.36 ^{aA} ± 0.03	0.39 ^{aA} ± 0.01
Reducing sugar g/L	NM	2.45 ^{bA} ± 0.5	3.82 ^{cB} ± 0.10	2.28 ^{bB} ± 0.02	1.50 ^{aA} ± 0.05	5.44 ^{dB} ± 0.04
	CM	2.45 ^{bA} ± 0.04	4.24 ^{eA} ± 0.04	2.60 ^{cB} ± 0.02	2.34 ^{aB} ± 0.02	3.44 ^{dA} ± 0.04
Total polyphenols, g/L	NM	0.386 ^{dA} ± 0.004	0.359 ^{bA} ± 0.001	0.359 ^{bA} ± 0.002	0.348 ^{cA} ± 0.002	0.312 ^{aA} ± 0.002
	CM	0.393 ^{bA} ± 0.003	0.370 ^{abB} ± 0.005	0.373 ^{abB} ± 0.006	0.353 ^{aA} ± 0.002	0.358 ^{aB} ± 0.017
Free SO ₂ , mg/L	NM	26.88 ^{cB} ± 0.02	29.44 ^{dB} ± 0.11	24.72 ^{aB} ± 0.02	25.60 ^{bB} ± 0.02	26.88 ^{cB} ± 0.02
	CM	25.60 ^{dA} ± 0.02	19.20 ^{bA} ± 0.02	21.76 ^{cA} ± 0.06	21.76 ^{cA} ± 0.04	15.36 ^{aA} ± 0.06
Total SO ₂ , mg/L	NM	89.60 ^{cB} ± 0.10	94.72 ^{dB} ± 0.02	128.1 ^{eB} ± 0.21	81.92 ^{bB} ± 0.02	80.65 ^{aB} ± 0.05
	CM	84.72 ^{eA} ± 0.01	71.44 ^{aA} ± 0.04	76.8 ^{cA} ± 0.02	79.36 ^{dA} ± 0.06	72.16 ^{bA} ± 0.04

NM—no maceration; CM—cold maceration; different letters indicate statistically significant differences in the same row (small letters) and in the same column (capital letters) between NM and CM samples ($p < 0.05$).

The content of ethanol in wines produced with maceration ranged from 14.08 to 14.48% (Table 1). The lowest content of ethanol was detected in wines produced with *C. famata* WB-1 ranging 14.08 and 14.17% in both pure and sequential fermentations, respectively. Also, wines produced with this strain had the highest content of the total extract. The concentration of the total extract in wines produced with *C. famata* WB-1 increased in sequential fermentation from 27.75 to 29.85 g/L. The content of total acids was in the range 5.80–6.20 g/L and the application of sequential fermentation increased the content of total acids compared to the pure fermentations. Volatile acids content was 0.36–0.39 g/L without a significant difference among the wine samples. The content of reducing sugar in the wines significantly varied between the samples, with the highest value of 4.24 g/L found in wine fermented with *C. famata* WB-1, and the lowest, 2.34 g/L, in sequential fermentation with *M. pulcherrima* B-5 and *S. cerevisiae* QA23. Sequential fermentation decreased the content of reducing sugar in both samples. The content of total polyphenols ranged from 0.353 to 0.393 g/L and decreased with the addition of *S. cerevisiae* QA23 in sequential fermentation.

3.2. Aromatic Compounds

A total number of 27 compounds was detected in the analysed samples produced without maceration (Table 2). The most abundant were esters, which comprised 41% of detected compounds. Aldehydes were not detected in these samples. Alcohols were detected in the highest concentration, especially 3-methyl-1-butanol (125.89–159.62 mg/L) and phenylethyl alcohol (35.85–70.42 mg/L). The application of sequential fermentation increased the total content of alcohols by 15% in the case of fermentation with *C. famata* WB-1 and *M. pulcherrima* B-5, in comparison with pure fermentation. Fermentation with *S. cerevisiae* QA23 produced seven of eight detected higher alcohols in the range 0.7 to 139.68 mg/L, and in a total concentration of 228.47 mg/L. Fermentation with *C. famata* WB-1 produced a higher content of isobutyl alcohol, 3-methyl-1-butanol, and phenyl ethyl alcohol, compared to other two strains. In pure and sequential fermentation, *M. pulcherrima* B-5 and *S. cerevisiae* QA 23 produced 2-methyl-1-butanol but below the odour threshold level (OTL). Only *M. pulcherrima* B-5 and *S. cerevisiae* QA23 in pure fermentation produced (S)-(-)-2-methyl-1-butanol in the concentration of 9.62 and 10 mg/L, respectively, which are below the reported OTL of 30 mg/L. The secondary alcohol S,S-2,3-butanediol was detected only in fermentations with *C. famata* WB-1 and *S. cerevisiae* QA23 in a concentration of 10.27 mg/L which is multiple times lower than the OTL of 668 mg/L.

A total number of 13 esters was detected in analysed samples in the total concentration 32.29–59.17 mg/L. The highest concentration was detected for ethyl acetate (23.73–37.78 mg/L), which gives the wine a fruity and sweet aroma. Ethyl octanoate and decanoate were detected in concentrations high above the OTL, 1.52–2.89 mg/L and 1.82–3.40, respectively. The lowest concentration of ethyl decanoate was detected for *S. cerevisiae* QA23, while sequential fermentation reduced the concentration of this ester produced by *M. pulcherrima* B-5 and *C. famata* WB-1, for 15 and 17%, respectively. Also, the content of most detected esters was reduced in sequential fermentation compared to the fermentation performed only with *M. pulcherrima* B-5 or *C. famata* WB-1. Application of sequential fermentation increased only the content of ethyl octanoate and ethyl-(4E)-decanoate. Methyl-2-methyl octanoate and ethyl 3-methyl pentanoate were detected only in pure fermentation with *S. cerevisiae* QA23. Almost all esters were detected in higher concentrations in fermentation with *C. famata* WB-1 compared to *M. pulcherrima* B-5.

Table 2. Content of volatile compounds (mg/L) in wines produced with and without maceration.

Compound	Aroma Descriptors	Odour Threshold Level, mg/L	Pre-Fermentative Treatment	<i>C. famata</i> WB-1	<i>C. famata</i> WB-1 + <i>S. cerevisiae</i> QA23	<i>M. pulcherrima</i> B-5	<i>M. pulcherrima</i> B-5 + <i>S. cerevisiae</i> QA23	<i>S. cerevisiae</i> QA23
Alcohols								
Isobutyl alcohol	Fusel, alcohol *	40.00 *	NM	24.51 ^{cA} ±1.12	16.02 ^{bA} ± 0.68	21.42 ^{cA} ±1.79	15.20 ^{abA} ± 0.23	13.02 ^{aA} ± 0.19
			CM	27.97 ^{cB} ±1.05	20.12 ^{aB} ± 0.86	31.56 ^{dB} ±1.64	25.07 ^{bcB} ± 0.58	21.79 ^{abB} ± 0.86
1-Propanol	Fresh, alcohol *	306 *	NM	nd	nd	nd	nd	nd
			CM	nd	nd	nd	nd	8.16 ± 0.22
3-Methyl-1-butanol	Whisky, alcohol **	30.00 **	NM	145.05 ^{bcA} ±8.92	159.62 ^{dA} ±5.12	125.89 ^{aA} ±5.53	156.18 ^{cdB} ±1.18	139.68 ^{abB} ±1.44
			CM	138.84 ^{abA} ±5.21	167.78 ^{cA} ±3.87	140.03 ^{bb} ±1.64	146.40 ^{bA} ±3.38	130.14 ^{aA} ±3.00
2-Methyl-1-butanol	Whisky, malt **	30.00 **	NM	tr	tr	21.04 ^{aB} ± 0.25	22.22 ^{bb} ± 0.34	25.96 ^{cB} ± 0.38
			CM	nd	nd	10.48 ^{aA} ± 0.33	13.10 ^{bA} ± 0.56	13.25 ^{bA} ± 0.43
1-Pentanol	Fruity, balsamic *	80.00 *	NM	21.81 ^{dA} ±1.66	13.50 ^{bb} ± 0.41	24.70 ^{eA} ± 0.79	15.68 ^{cB} ± 0.33	3.53 ^{aA} ± 0.02
			CM	28.46 ^{dB} ± 0.43	10.45 ^{bA} ± 0.34	25.69 ^{cA} ± 0.90	9.15 ^{aA} ± 0.39	8.31 ^{aB} ± 0.19
(S)-(-)-2-Methyl-1-butanol	-	30.00 *	NM	nd	tr	9.62 ^{aA} ± 0.34	tr	10.00 ^{aA} ± 0.14
			CM	nd	nd	10.62 ^{aA} ± 0.89	19.69 ^c ± 0.84	14.69 ^{bb} ± 0.61
Isohexanol	-	-	NM	nd	nd	nd	nd	nd
			CM	nd	tr	3.28 ± 0.09	tr	nd
n-Hexanol	Green grass *	8.00 *	NM	0.99 ^{bA} ± 0.04	1.15 ^{cB} ± 0.03	nd	1.77 ^d ± 0.03	0.70 ^a ± 0.01
			CM	1.94 ^{aA} ± 0.04	0.33 ^{bA} ± 0.01	nd	nd	nd
Phenyl ethyl alcohol	Flower, pollen *	14.00 *	NM	46.83 ^{bb} ±3.87	70.42 ^{cB} ±1.45	44.30 ^{bb} ±1.27	68.16 ^{cB} ± 0.87	35.85 ^{aA} ± 0.66
			CM	36.61 ^{bA} ± 0.71	56.06 ^{cA} ±2.39	26.58 ^{aA} ±2.22	55.17 ^{cA} ±1.81	39.29 ^{bb} ±1.08
S,S-2,3 butanediol	Butter **	668.00 **	NM	nd	10.27 ± 0.44	nd	nd	nd
			CM	nd	nd	nd	nd	nd
Total alcohols			NM	239.19 ^{aA} ± 15.69	270.98 ^{bb} ± 7.97	246.97 ^{aA} ± 9.79	279.21 ^{bA} ± 3.59	228.47 ^{aA} ± 2.76
			CM	233.82 ^{aA} ± 7.35	254.74 ^{cA} ± 7.31	248.24 ^{bA} ± 8.60	268.58 ^{bA} ± 7.47	235.62 ^{aA} ± 6.21
Esters								
Ethyl acetate	Fruity, sweet *	7.500 *	NM	36.28 ^{cA} ±1.57	24.75 ^{aA} ± 0.81	37.78 ^{cA} ± 0.99	29.78 ^{bA} ± 0.61	23.73 ^{aA} ± 0.30
			CM	57.62 ^{cB} ± 0.82	35.27 ^{aB} ±1.15	66.82 ^{dB} ±1.75	47.32 ^{bb} ± 0.97	32.32 ^{ab} ±1.06
Ethyl hexanoate	Fruity, anis *	0.005 *	NM	0.35 ^{eB} ± 0.02	0.26 ^{cB} ± 0.01	0.33 ^{dB} ± 0.01	0.15 ^{aB} ± 0.00	0.23 ^{bb} ± 0.00
			CM	0.09 ^{cA} ± 0.00	0.08 ^{bA} ± 0.00	0.11 ^{dA} ± 0.00	0.08 ^{bA} ± 0.00	0.06 ^{aA} ± 0.00
Ethyl isohexanoate	Fruity #	-	NM	0.17 ^{eB} ± 0.01	0.06 ^{cA} ± 0.00	0.14 ^{dA} ± 0.00	0.05 ^{bA} ± 0.00	0.02 ^{aA} ± 0.00
			CM	0.13 ^{cA} ± 0.00	0.10 ^{bB} ± 0.00	0.18 ^{fb} ± 0.00	0.15 ^{dB} ± 0.00	0.03 ^{aB} ± 0.00
Diethyl succinate	Light fruity *	500 *	NM	nd	nd	nd	nd	nd
			CM	3.60 ^a ± 0.06	3.42 ^a ± 0.15	tr	nd	nd
Ethyl octanoate	Pineapple, pear, floral *	0.002 *	NM	1.88 ^{bb} ± 0.06	2.52 ^{dB} ± 0.11	2.18 ^{cA} ± 0.03	2.89 ^{eb} ± 0.03	1.52 ^{aA} ± 0.03
			CM	1.21 ^{aA} ± 0.04	1.80 ^{cA} ± 0.06	2.27 ^{dA} ± 0.06	2.33 ^{dA} ± 0.05	1.51 ^{bA} ± 0.01
Ethyl decanoate	Fruity, pleasant *	0.200 *	NM	3.36 ^{cB} ± 0.10	2.86 ^{bb} ± 0.09	3.40 ^{cB} ± 0.09	2.81 ^{bA} ± 0.03	1.82 ^{ab} ± 0.03
			CM	1.19 ^{aA} ± 0.04	1.71 ^{bA} ± 0.04	2.14 ^{cA} ± 0.05	2.74 ^{dA} ± 0.09	1.60 ^{bA} ± 0.05
Ethyl dodecanoate	Floral, fruity *	1.500 *	NM	0.22 ^{dB} ± 0.01	0.20 ^{cB} ± 0.00	0.23 ^{dB} ± 0.01	0.17 ^{bb} ± 0.00	0.14 ^{aA} ± 0.00
			CM	0.08 ^{bA} ± 0.00	0.07 ^{aA} ± 0.00	0.14 ^{dA} ± 0.00	0.13 ^{cA} ± 0.00	0.17 ^{eb} ± 0.00
3-Methyl butyl acetate	Banana *	0.03 *	NM	2.60 ^{dB} ± 0.18	2.40 ^{dB} ± 0.08	1.80 ^{bb} ± 0.06	2.10 ^{cB} ± 0.04	1.40 ^{ab} ± 0.02
			CM	1.64 ^{eA} ± 0.05	0.90 ^{cA} ± 0.03	1.07 ^{dA} ± 0.02	0.70 ^{bA} ± 0.02	0.31 ^{aA} ± 0.01
2- Methyl butyl acetate	Blackberry, banana †	1.083 †	NM	11.54 ^{eb} ± 0.37	6.83 ^{dB} ± 0.22	5.31 ^{cB} ± 0.08	2.38 ^{bb} ± 0.05	2.11 ^{ab} ± 0.04
			CM	0.99 ^{dA} ± 0.04	0.26 ^{abA} ± 0.01	0.34 ^{cA} ± 0.03	0.30 ^{bcA} ± 0.01	0.21 ^{aA} ± 0.00
Methyl-2-methyl octanoate	-	-	NM	nd	nd	nd	nd	0.14 ± 0.00
			CM	nd	nd	nd	nd	nd

Table 2. Content of volatile compounds (mg/L) in wines produced with and without maceration.

Compound	Aroma Descriptors	Odour Threshold Level, mg/L	Pre-Fermentative Treatment	<i>C. famata</i> WB-1	<i>C. famata</i> WB-1 + <i>S. cerevisiae</i> QA23	<i>M. pulcherrima</i> B-5	<i>M. pulcherrima</i> B-5 + <i>S. cerevisiae</i> QA23	<i>S. cerevisiae</i> QA23
Ethyl 3-methyl pentanoate	-	-	NM	nd	nd	nd	nd	0.20 ^B ± 0.00
			CM	nd	nd	nd	nd	0.01 ^A ± 0.00
Ethyl-(4 E)-decenoate	-	-	NM	0.02 ^{bA} ± 0.00	0.04 ^{cA} ± 0.00	0.02 ^{bA} ± 0.00	0.04 ^{cB} ± 0.00	0.01 ^{aA} ± 0.00
			CM	0.10 ^{dB} ± 0.00	0.06 ^{cB} ± 0.00	0.02 ^{bA} ± 0.00	0.01 ^{aA} ± 0.00	0.01 ^{aA} ± 0.00
2-phenil-ethyl acetate	Pleasant, floral *	0.25 *	NM	1.62 ^{cB} ± 0.07	1.39 ^{bB} ± 0.04	1.86 ^{dA} ± 0.06	1.44 ^{bA} ± 0.02	0.97 ^{aB} ± 0.02
			CM	1.10 ^{cA} ± 0.04	0.69 ^{aA} ± 0.05	1.92 ^{dA} ± 0.04	1.89 ^{dB} ± 0.06	0.81 ^{bA} ± 0.02
Hexyl acetate	Pleasant, fruity, pear *	0.670 *	NM	nd	nd	nd	nd	nd
			CM	0.03 ± 0.00	nd	tr	nd	nd
Ethyl butanoate	Pineapple, apple, peach **	0.02 **	NM	1.13 ^{cA} ± 0.06	1.01 ^{bB} ± 0.02	nd	0.02 ^a ± 0.00	nd
			CM	1.18 ^{bA} ± 0.03	0.20 ^{aA} ± 0.00	nd	nd	nd
Total esters			NM	59.17^{dA} ± 2.37	42.32^{bA} ± 1.39	53.05^{cA} ± 1.32	41.83^{bA} ± 0.82	32.29^{aA} ± 0.45
			CM	67.96^{dB} ± 1.27	44.50^{bB} ± 1.50	75.01^{eB} ± 1.96	55.65^{cB} ± 1.19	37.04^{aB} ± 1.17
Aldehydes								
Benzaldehyde	Almond *	2.000 *	NM	nd	nd	nd	nd	nd
			CM	0.08 ^a ± 0.00	0.09 ^b ± 0.00	0.10 ^c ± 0.00	0.11 ^d ± 0.00	0.09 ^b ± 0.00
Acids								
Hexanoic acid	Cheese aroma, fatty *	3.000 *	NM	nd	0.66 ± 0.01	nd	nd	nd
			CM	nd	nd	nd	nd	nd
Octanoic acid	Unpleasant, cheese, fatty acid *	0.500 *	NM	4.05 ^{cB} ± 0.15	4.05 ^{cB} ± 0.05	4.43 ^{dB} ± 0.05	1.57 ^{aB} ± 0.01	2.62 ^{bB} ± 0.03
			CM	0.94 ^{bA} ± 0.01	0.83 ^{aA} ± 0.01	1.08 ^{cA} ± 0.01	1.0 ^{bCA} ± 0.03	2.19 ^{dA} ± 0.07
Decanoic acid	Fatty, unpleasant *	15.000 *	NM	2.61 ^c ± 0.09	4.17 ^{eB} ± 0.14	3.06 ^{dB} ± 0.07	1.13 ^{aA} ± 0.02	1.46 ^{bA} ± 0.03
			CM	tr	0.70 ^{aA} ± 0.02	0.84 ^{bA} ± 0.02	1.54 ^{cB} ± 0.05	1.77 ^{dB} ± 0.06
Total acids			NM	6.66^{cB} ± 0.23	8.88^{eB} ± 0.21	7.49^{dB} ± 0.12	2.70^{aB} ± 0.04	4.08^{bB} ± 0.05
			CM	0.94^{aA} ± 0.01	1.53^{bA} ± 0.04	1.92^{cA} ± 0.03	2.54^{dA} ± 0.08	3.96^{eA} ± 0.13
Ketones								
Acetoin	Floral, wet *	150 *	NM	0.48 ^{cA} ± 0.02	0.52 ^{cB} ± 0.01	0.22 ^{bB} ± 0.00	0.56 ^{dB} ± 0.00	0.02 ^{aA} ± 0.00
			CM	0.96 ^{bB} ± 0.05	0.23 ^{cA} ± 0.01	0.11 ^{aA} ± 0.00	0.26 ^{cA} ± 0.01	0.05 ^{aB} ± 0.00

NM—no maceration; CM—cold maceration; nd—not detected; tr—traces, *—[27], **—[28], #—[29], †—[30]; different letters indicate statistically significant differences in the same row (small letters) and in the same column (capital letters) between NM and CM samples ($p < 0.05$).

Total acids were detected in the concentration range of 2.7–8.88 mg/L. The lowest concentration of acids was present in fermentation with *M. pulcherrima* B-5 and *S. cerevisiae* QA23, 2.7 mg/L. Non-*Saccharomyces* yeasts produced 6.65 and 7.49 mg/L of total acids in pure fermentation with *C. famata* WB-1 and *M. pulcherrima* B-5, respectively. Sequential fermentation with *S. cerevisiae* QA23 increased total acids to 8.88 mg/L for *C. famata* WB-1. In contrast, the addition of *S. cerevisiae* QA23 to the fermentation with *M. pulcherrima* B-5 decreased the content of total acids. Hexanoic, octanoic and decanoic acid were detected in the samples. Among them, the most abundant and significantly higher than OTL was octanoic acid with a concentration from 1.57 mg/L in sequential fermentation of *M. pulcherrima* B-5 and *S. cerevisiae* QA23, to 4.43 mg/L in pure fermentation with *M. pulcherrima* B-5. Hexanoic acid was detected only during sequential fermentation with *C. famata* WB-1 and *S. cerevisiae* QA23 in the concentration of 0.66 mg/L, which is below the OTL of 3 mg/L. Decanoic acid was detected in all samples, but under the OTL of 15 mg/L in all the samples, ranging from 1.13 mg/L in sequential fermentation with *M. pulcherrima* B-5, to 4.17 mg/L in sequential fermentation with *C. famata* WB-1. Among other components, the ketone acetoin was detected in all samples in concentrations from 0.02 mg/mL in the pure fermentation with *S. cerevisiae* QA23, to 0.52 mg/L in sequential fermentation with *C. famata* WB-1.

A total number of 28 compounds was detected in wines produced with the maceration process (Table 2). Nine different alcohols were detected in wines produced with the maceration process in a total concentration of 228.54–268.58 mg/L. The application of sequential fermentation slightly increased the concentration of total alcohols in the samples. The highest concentration of higher alcohols was detected in sequential fermentations with *M. pulcherrima* B-5.

Isobutyl alcohol was present in all fermentations but in the concentration range 20.12–31.56 mg/L, which is lower than the OTL of 40 mg/L. In contrast, 1-propanol was detected only in the sample fermented with *S. cerevisiae* QA23. 3-Methyl-1-butanol, isobutyl alcohol, phenylethyl alcohol and 1-pentanol were present in the highest concentrations among all detected alcohols. The concentration of 3-methyl-1-butanol ranged from 130.14 mg/L to 167.78 mg/L in fermentation with *S. cerevisiae* QA23 and sequential fermentation with *C. famata* WB-1, respectively. Sequential fermentation of *M. pulcherrima* B-5 and *C. famata* WB-1 increased the concentration of this alcohol by 4% and 20%, respectively. 2-Methyl-1-butanol and (S)-(-)-2-Methyl-1-butanol were detected in pure and sequential fermentation with *M. pulcherrima* B-5 and in pure fermentation with *S. cerevisiae* QA23 in concentrations below the OTL.

Esters represent the majority of the detected compounds, and they were detected in a total concentration of 37.04–75.01 mg/L. Application of sequential fermentation caused the concentration reduction of all present esters except ethyl octanoate and ethyl decanoate, which concentration increased in sequential fermentation. The concentrations of most esters were above the OTL. Hexyl acetate and ethyl butanoate were detected only in fermentations with *C. famata* WB-1 in a concentration higher than 0.001 mg/mL. On the other hand, ethyl-3-methyl pentanoate was detected only in pure fermentation with *S. cerevisiae* QA23 in a concentration of 0.01 mg/mL. Higher concentrations of esters (75.01 and 54.75 mg/L) were observed for pure and sequential fermentation, respectively, with *M. pulcherrima* B-5 compared to pure and sequential fermentation with *C. famata* WB-1, (67.96 and 44.5 mg/L, respectively).

Only one aldehyde, benzaldehyde, was detected in wines produced with the maceration process in the concentration 0.08–0.11 mg/L. Sequential fermentation with *M. pulcherrima* B-5 increased the concentration of benzaldehyde by 10%, from 0.10 to 0.11 mg/L, while in the case of *C. famata* WB-1, this increase was 12.5%.

Among acids, octanoic and decanoic acid were detected, both with a fatty unpleasant aroma. Total acids were detected in the range 0.94–3.96 mg/L. The highest concentration of total acids was detected in fermentation with *S. cerevisiae* QA23 (3.96 mg/L) in which high amount of octanoic acid (2.19 mg/L) was detected. Decanoic acid was present in

amounts below the OTL in all samples and it ranged from 0.7 in sequential fermentation with *C. famata* WB-1 to 1.77 in the fermentation with *S. cerevisiae* QA23.

Acetoin was detected in all samples produced with the maceration process in a wide range of concentrations. The highest concentration of 0.96 mg/L was present in pure fermentation with *C. famata* WB-1, while the lowest (0.05 mg/L) was detected in fermentation with *S. cerevisiae* QA23.

According to the OTL level, a total number of 11 compounds was detected in analysed wines with odour activity values OAV > 1 (Table 3), with ethyl octanoate being the most important relative odour contributor with up to 93.8% of relative odour contribution (ROC). Among detected compounds, two alcohols, 3-methyl-1-butanol and phenyl ethyl alcohol, were detected with an OAV > 1 and with a relatively low odour contribution. Among esters, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 3-methyl butyl acetate, 2-methyl butyl acetate, 2-phenyl-ethyl acetate, and ethyl butanoate were present, with the highest contribution by ethyl octanoate. Only one acid, octanoic acid, was present with an OAV > 1 but with odour contribution in the range 0.2–0.7%.

Table 3. Odour activity values (OAVs) and relative odour contribution (ROC) for the aroma compounds in analysed wines produced with and without maceration.

Compound	Aroma Descriptors	Treatment	<i>C. famata</i> WB-1	<i>C. famata</i> WB-1 + <i>S. cerevisiae</i> QA23	<i>M. pulcherrima</i> B-5	<i>M. pulcherrima</i> B-5 + <i>S. cerevisiae</i> QA23	<i>S. cerevisiae</i> QA23
3-methyl-1-butanol	Whisky, alcohol	NM	4.8 (0.4%)	5.3 (0.4%)	4.2 (0.3%)	5.2 (0.3%)	4.7 (0.5%)
		CM	4.6 (0.6%)	5.6 (0.6%)	4.7 (0.4%)	4.9 (0.4%)	4.3 (0.5%)
Phenyl ethyl alcohol	Flower, pollen	NM	3.3 (0.3%)	5.0 (0.3%)	3.2 (0.2%)	4.9 (0.3%)	2.6 (0.3%)
		CM	2.6 (0.3%)	4.0 (0.4%)	1.9 (0.2%)	3.9 (0.3%)	2.8 (0.3%)
Ethyl acetate	Fruity, sweet	NM	4.8 (0.4%)	3.3 (0.2%)	5.0 (0.4%)	4.0 (0.2%)	3.2 (0.3%)
		CM	7.7 (1%)	4.7 (0.4%)	8.9 (0.7%)	6.3 (0.5%)	4.3 (0.5%)
Ethyl hexanoate	Fruity, anis	NM	70.0 (5.8%)	52.0 (3.5%)	66.0 (5.2%)	30.0 (1.9%)	46.0 (5.2%)
		CM	18.0 (2.3%)	16.0 (1.6%)	22.0 (1.8%)	16.0 (1.3%)	12.0 (1.5%)
Ethyl octanoate	Pineapple, pear, floral	NM	940 (77.8%)	1260 (84.5%)	1090 (86%)	1445 (91.2%)	760 (86.0%)
		CM	605 (79.1%)	900 (31.5%)	1135 (92.3%)	1165 (93.7%)	755 (93.8%)
Ethyl decanoate	Fruity, pleasant	NM	16.8 (1.4%)	14.3 (1%)	17.0 (1.3%)	14.1 (0.9%)	9.1 (1.3%)
		CM	6.0 (0.8%)	8.6 (0.9%)	10.7 (0.9%)	13.7 (1.1%)	8.0 (1%)
3-Methyl butyl acetate	Banana	NM	86.7 (7.2%)	80.0 (5.4%)	60.0 (4.7%)	70.0 (4.4%)	46.7 (5.3%)
		CM	54.7 (7.1%)	30.0 (3.0%)	35.7 (2.9%)	23.3 (1.9%)	10.3 (1.3%)
2-Methyl butyl acetate	Blackberry, banana	NM	10.7 (0.9%)	6.3 (0.4%)	4.9 (0.4%)	2.2 (0.1%)	1.9 (0.2%)
		CM	0.9 (0.1%)	0.2 (<0.1%)	0.3 (<0.1%)	0.3 (<0.1%)	0.2 (<0.1%)
2-phenyl-ethyl acetate	Pleasant, floral	NM	6.5 (0.5%)	5.6 (0.4%)	7.4 (0.6%)	5.8 (0.4%)	3.9 (0.4%)
		CM	4.4 (0.6%)	2.8 (0.3%)	7.7 (0.6%)	7.6 (0.6%)	3.2 (0.4%)
Ethyl butanoate	Pineapple, apple, peach	NM	56.5 (4.7%)	50.5 (3.4%)	0.0	1.0 (<0.1%)	0.0
		CM	59.0 (7.7%)	10.0 (1%)	0.0	0.0	0.0
Octanoic acid	Unpleasant, cheese aroma, fatty acid	NM	8.1 (0.7%)	8.1 (0.5%)	8.9 (0.7%)	3.1 (0.2%)	5.2 (0.6%)
		CM	1.9 (0.3%)	1.7 (0.2%)	2.2 (0.2%)	2.0 (0.2%)	4.4 (0.5%)

NM—no maceration performed; CM—cold maceration; OAVs were expressed as the mean concentration of an aroma compound divided by its odour threshold level; ROC of each compound shown in parentheses was calculated as the ratio of the OAV of the individual compound and the total OAV of each wine.

3.3. Sensory Analysis

Results of the sensory analysis indicated that wine produced with *C. famata* WB-1 and without maceration process had the best overall odour score (7.7, range 7.4 to 8.14) and increased typicality, intensity, and complexity (Figure 1a), compared to other samples produced without maceration. A high level of fruity and floral odour characteristics was observed. On the other hand, lower scores of fruity and flower notes were observed for wines fermented with *M. pulcherrima* B-5 in pure (4.86–5.5) and sequential (3.93–4.71) fermentation (Figure 1a). Sequential fermentation with both native strains indicated lower-level odour characteristics compared to pure fermentations.

The wine produced with *C. famata* WB-1 without maceration had the best taste profile, with a total score of 7.5 for pure, and 7 for sequential, fermentation. The wine produced with *M. pulcherrima* B-5 had much lower scores for all analysed parameters in both pure (5.93–7.14) and sequential (4.86–6.57) fermentation. Fermentation with *S. cerevisiae* QA23 produced a wine with a typicality score of 7.5, but with lower scores in fullness, duration, and intensity.

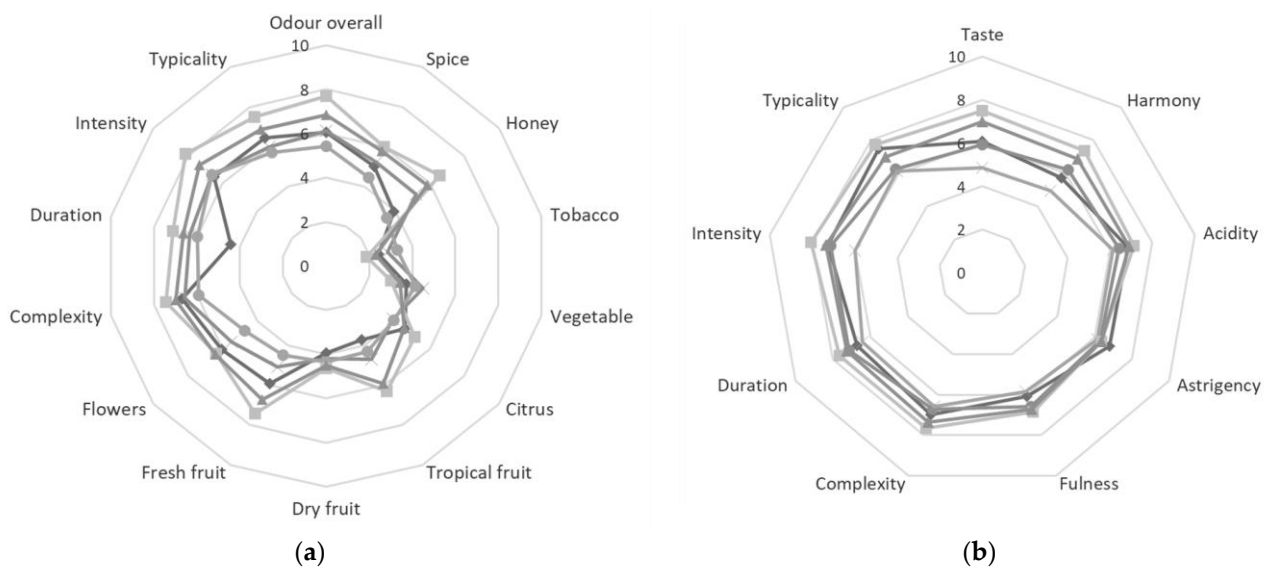


Figure 1. Sensory analysis of (a) smell and (b) taste profile of wines produced without maceration and fermented with *C. famata* WB-1 (■), *C. famata* WB-1 + *S. cerevisiae* QA23 (▲), *M. pulcherrima* B-5 (●), *M. pulcherrima* B-5 + *S. cerevisiae* QA23 (x) and *S. cerevisiae* QA23 (◆).

The maceration process resulted in the change of odour and taste characteristics of wine (Figure 2). The best odour overall (7) and typicality (8.1) score was observed for *S. cerevisiae* QA23, but with a lower level of fruit notes and lack of duration (6.1). The wine produced in sequential fermentation with *M. pulcherrima* B-5 had the best results in floral and fruity notes, and, also, in complexity, duration and intensity (7.5–7.8). Lower values of the latest three characteristics were observed for *C. famata* WB-1 in pure (6.1–6.9) and sequential (6.9–7.1) fermentation.

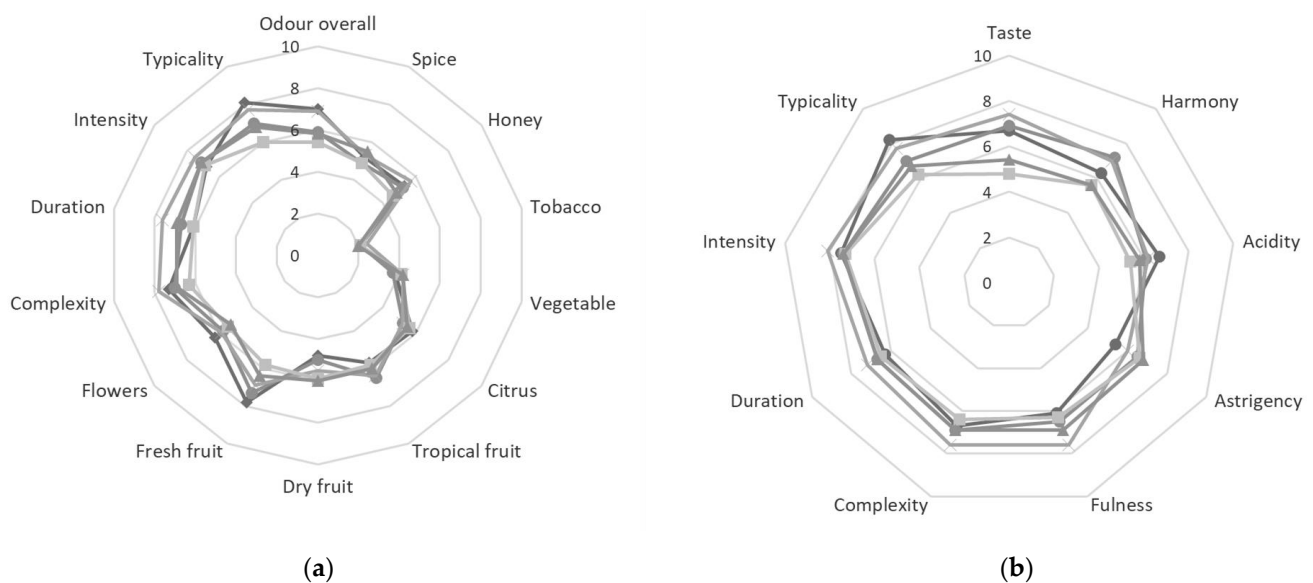


Figure 2. Sensory analysis of (a) smell and (b) taste profile of wines produced with maceration and fermented with *C. famata* WB-1 (■), *C. famata* WB-1 + *S. cerevisiae* QA23 (▲), *M. pulcherrima* B-5 (●), *M. pulcherrima* B-5 + *S. cerevisiae* QA23 (x) and *S. cerevisiae* QA23 (◆).

Fermentation with *C. famata* WB-1 resulted in a wine with lower scores of taste profile characteristics in comparison with *M. pulcherrima* B-5. The overall taste score was 4.8 for pure fermentation and 5.4 for sequential fermentation. Wine produced with *M. pulcherrima* B-5 in sequential fermentation scored 7.4 in overall taste (Figure 2b). Also, this wine had

the best scores for fullness 7.6, complexity 7.6, duration 7.2 and intensity 8.1. Fermentation with *S. cerevisiae* QA23 resulted in good typicality of 8.2, but the lower level of complexity, duration, and intensity of the wine.

4. Discussion

To define the oenological contribution and possible application of two native yeast strains in the production of Chardonnay wines, the effect of *M. pulcherrima* B-5 and *C. famata* WB-1 on the quality, aroma profile and sensory attributes in pure and sequential fermentation was investigated in pilot-scale fermentation trials. Additionally, the pre-fermentative cold maceration process was involved in wine production to enhance wine colour, quality, and complexity. Most of the analysed wine samples had an ethanol content of about 14% volume. Results obtained for the ethanol content correspond to the values already reported (14.2% vol.) for commercial Chardonnay wine [31]. The sequential fermentation of *M. pulcherrima* B-5 with *S. cerevisiae* QA23 did not significantly affect the ethanol content in both processes with and without maceration. Although it is considered that *M. pulcherrima* can produce wines with low ethanol content [18], there are some reports in which this strain did not affect the ethanol content [32] and good fermentation potential of *M. pulcherrima* was stated [9]. This indicates that the ability of *M. pulcherrima* to reduce ethanol content can be strain-dependent [10]. Independent of pre-fermentative treatment, lower ethanol content was observed for all wine samples produced by the *C. famata* WB-1 strain. On the other hand, use of *C. famata* WB-1 in sequential fermentation and without maceration resulted in a significant increase of ethanol content. Although pre-fermentative treatments should not affect the ethanol content, some researches indicated that 10 days of maceration induced a difference in ethanol content by more than 0.5% vol [33]. Reported ethanol content was higher than ethanol content of 11.3 and 12.6% produced by the same strain in red wine Prokupac [17], but these differences are most likely a consequence of the grape variety, initial sugar content and vinification procedure. A significant increase of 13–22% in the dry extract after cold maceration treatment of the must was observed compared to the wine samples produced without pre-fermentative treatment. These results were expected because the extraction of many compounds and minerals from the solid part of grape berries occurs during 48 h of maceration. Additionally, the results are in accordance with the already reported 16% increase of total extract during a 24 h long cold maceration of the Smederevka grape [34]. Total extract represents a complete soluble solids in wine and can contribute to the wine body and structure. In general, total extract content above 20 g/L can be considered good for white wines, while the wines with the content below this value can be considered thin [35]. Total extract was the lowest in the wines produced with *M. pulcherrima* B-5 and was not changed significantly in sequential fermentation, while the highest value was reported for *C. famata* WB-1 independently of the pre-fermentation treatment. This indicates that the application of *C. famata* WB-1 had a positive impact on total extract content and, therefore, on wine quality. Some non-*Saccharomyces* yeast can produce higher amounts of non-volatile compounds, which contribute to the wine taste and take part in total extract content [36].

Total acids were not affected by the maceration process or sequential fermentation. Conversely, the maceration process reduced the values of volatile acidity. This is in accordance with the results for Pedro Ximenez sparkling wines for which the maceration process had no effect on total acidity but induced a decrease of volatile acidity [37]. The results obtained for volatile acidity in wines were a little lower than usual values, which are in the range 500–1000 mg/L [38]. The results correspond to the fact that *M. pulcherrima* is being used in the production of wines with low volatile acidity [18]. The used *C. famata* WB-1 strain has the potential for wine production with low volatile acidity [17]. Total content of polyphenols significantly depended on the used yeast strain and the application of sequential fermentation. The highest total content for polyphenols was found in wines produced by *M. pulcherrima* B-5, regardless of the maceration process. This can be explained by intensive β -glucosidase activity, which promotes the occurrence of phe-

nols and pyranoanthocyanins, and the extraction of phenolic compounds from the grape skin [39]. Sequential fermentation decreased the content of total polyphenols, probably due to the lower concentration of enzymes induced by the fast decline of cell numbers of non-*Saccharomyces* yeasts [36] and the negative effect of *S. cerevisiae* on the growth of *M. pulcherrima* [10]. Application of low temperatures and skin contact during the maceration process induced the increase of total phenolics, in correspondence with the results reported for Sauvignon blanc [40] and Chardonnay wine [41].

The selection of a yeast strain during the winemaking process is of critical importance for obtaining a wine with the desired sensory characteristics. The aroma profile of a wine inevitably depends on the yeast strain selection. Native non-*Saccharomyces* yeasts have proven the ability to modulate and enhance the wine aroma profile [42]. A total number of 28 and 27 different compounds were detected in Chardonnay wines produced with and without the maceration process, respectively. During both vinification processes, with and without maceration, a large number of higher alcohols was synthesized. Yeast produces higher alcohols by conversion of branched chain amino acids [38]. Among higher alcohols, 2-butanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol and phenylethyl alcohol were produced in the highest concentrations. These alcohols were often detected as the most abundant higher alcohols in Chardonnay wine [28,31,43–46]. Generally, isobutanol, 2-methylbutanol and 3-methylbutanol are derived through the decarboxylation and deamination processes of valine, leucine, and isoleucine, respectively [47]. Higher alcohols represent the most important wine aroma contributors, and their biosynthesis is probably species- or strain- dependent [48]. The content of higher alcohols below 300 mg/L contributes to the complexity of wine aroma, while concentration above 400 mg/L can induce a hostile aroma with a strong pungent taste and smell [44,48]. The total content of higher alcohols in analysed wine samples did not exceed the level of 280 mg/L, which can be considered very good for the expression of wine aroma. Lower level of higher alcohols induces the perception of varietal grape aroma compounds [8].

Esters formed during wine production significantly contribute to the final wine aroma. The production of esters depends on various factors involved in the wine production such as: type and characteristics of the grape, yeast strain, vinification method, insoluble components in grape must, must pH, and duration of skin contact [38]. The production of ethyl esters and acetate esters in wine occurs through direct chemical reaction of alcohol and acids and in enzymatic reactions from carbohydrates or amino acids [14]. The total amount of esters detected in the analysed Chardonnay wines ranged from 37.04 to 75.01, and from 32.29 to 59.17 mg/L, for winemaking with and without maceration process, respectively. The maceration process positively affected the total ester concentration in wines produced with both native yeast strains. A similar effect of maceration on total ester concentration was also observed in the production of Sauvignon blanc [40]. Fermentation conducted with both native strains resulted in the increase of ester formation compared to the control fermented with *S. cerevisiae*. This fact can be explained by the developed enzymatic system related to the production of esters in both *M. pulcherrima* and *C. famata* strains [15,18,20,21,49]. Sequential fermentation for both *C. famata* WB-1 and *M. pulcherrima* B-5 induced a decrease in the total ester concentration regardless of the application of the maceration process. Additionally, sequential fermentation had an effect on the composition of esters, rather than on the total ester concentration in the process without maceration. Among esters, ethyl acetate was detected in the highest concentration and high above the OTL. Ethyl acetate concentration was multiple times higher than the concentration of other esters in accordance with the results reported for Chardonnay wines [23,43,45]. Ethyl acetate in relatively high concentrations (more than 120 mg/L), can alter the sensory properties, spoil the bouquet, and express the bitterness in the wine. On the other hand, concentrations of 50–80 mg/L can positively affect the wine aroma and contribute to the olfactory complexity [50]. 3-Methyl butyl acetate was detected in all samples in concentrations high above the OTL contributing to the banana-like and fruity aroma of wine [23]. A high concentration of this ester was already confirmed in Chardonnay wine [43,45,51] and recognized as a difference between

the Chardonnay and some other wines [23]. The production of Chardonnay wine with *M. pulcherrima* B-5 resulted in an increased content of ethyl-octanoate, ethyl acetate and phenyl ethyl acetate, which is characteristic for the use of this yeast strain in winemaking [14]. All these esters contribute to the development of pleasant fruity and floral aromas [44]. Acetate esters express floral and fruity odour [48], while phenyl ethyl acetate, together with 3-methyl butyl acetate, contributes to the wine bouquet [52]. The maceration process increased the total esters concentration with the most intensive effect on the increase of ethyl ester concentration, while all other esters were produced in a lower level compared to the process without maceration. Diethyl succinate was detected only in the fermentation with *C. famata* WB-1 in the wines produced with the maceration process. A similar concentration of diethyl succinate was detected in Australian Chardonnay wines [45]. Diethyl succinate is mainly connected to the aging of wine and increased contact with yeast cells [53,54] and it contributes to the fruity and floral odour [54].

The formation of fatty acids during wine production mainly depends on the fermentation conditions [23]. The total concentration of fatty acids was higher in wines produced without pre-fermentation treatment, which is unlike the results obtained for red wines [52]. Among fatty acids, octanoic and decanoic acid were detected in the process without maceration, while additionally hexanoic acid was detected in one fermentation without maceration. The presence of these acids, although in higher concentrations, was already confirmed for Chardonnay wine [23,55]. In contrast, similar or lower concentrations were reported for Australian Chardonnay wine [43]. Fatty acids C6 to C10 in concentrations of 4–10 mg/L can contribute to a pleasant wine aroma, while concentrations above 20 mg/L are considered undesirable [23].

Aldehydes are mainly formed during wine aging, and they contribute to the sour, almond and vanilla aroma notes [56]. Benzaldehyde gives the almond aroma note, but it was detected in the concentration 0.08–0.11 mg/L, which is below the OTL. Higher levels of benzaldehyde were observed in fermentations with *M. pulcherrima* B-5 compared to *C. famata* WB-1. In the research of benzaldehyde production with various yeast strains, *M. pulcherrima* produced a higher amount of benzaldehyde compared to most strains of *Candida* spp. [57]. Benzaldehyde was not detected in some Chardonnay wines produced in Australia [45], Canada [44] and China [55], while it was detected in the concentration range from 1 to 5 mg/L in some other Chardonnay wines [58].

Ketones contribute to the floral and fruity wine odour [46]. Among ketones, only acetoin was detected in all produced wines. This compound can be considered relatively odourless in the level below the threshold, while concentrations above 150 mg/L may cause an unpleasant buttery aroma [48]. The presence of this component was not reported in the research of aroma compounds in different Chardonnay wines [43,44,46]. On the other hand, the detected concentrations were much lower than 2.6 mg/L reported for Chardonnay wine in China [23]. Sequential fermentation increased the concentration of acetoin in wines. Additionally, fermentation with native strains *M. pulcherrima* B-5 and *C. famata* WB-1 resulted in a higher amount of acetoin compared to *S. cerevisiae*. The higher production of acetoin for various non-*Saccharomyces* yeasts including *M. pulcherrima* in comparison with *S. cerevisiae* has been already reported [48].

According to the OAV value, 11 compounds were identified as odour contributors. Most of them belong to the esters, which are known as the main drivers in the wine odour definition. Ethyl hexanoate, ethyl octanoate, ethyl decanoate, 3-methyl butyl acetate and ethyl butanoate jointly contributed with 97.7–98.4% to the global aroma of analysed wines. Similar results have been reported for Chardonnay wines in China [23]. Although, octanoic acid had OAV values higher than 1, the contribution to the relative odour of wine was low (<1%), so it had neglectable effect on the overall sensory attributes.

As the compounds that mostly contribute to the aroma have fruity and flowery notes, their intensive expression in the smell profile of analysed wines can be expected. Descriptive sensory analysis performed by a sensory panel indicated that application of native strains *M. pulcherrima* B-5 and *C. famata* WB-1 induced the improvement of smell

and taste profile of Chardonnay wine. According to the ROC values, the global aroma of analysed wines was dominated by fermentative aroma namely, ethyl esters of fatty acids. This induced the expression of fruity and floral notes which were observed in the sensory profile of wines. Other researchers have stated that an increase in floral and fruity aromas is probably a result of esters concentration and composition [40]. The wine produced with *C. famata* WB-1 without the maceration process had the best overall score for taste and smell profile with increased typicality, intensity, and complexity. Compared to other wines, it had higher relative odour contributions of 3-methyl-butyl acetate and ethyl octanoate, both responsible for the fruity wine aroma [27,30]. Application of cold pre-fermentative maceration shifted the scores to the advantage of *M. pulcherrima* B-5, so the best results were achieved for sequential fermentation with *M. pulcherrima* B-5. Taking into consideration relative odour contribution, ethyl octanoate, ethyl decanoate, ethyl hexanoate and 3-methyl-butyl-acetate with fruity aromas derived 98% of total wine aroma. Wines produced without maceration had more intense honey and spicy taste compared to the ones produced with maceration. On the other hand, the maceration process led to the increase of dry fruit, citrus, and tropical fruit notes in most of the wines. A similar effect of the maceration process to the increase of floral notes and tropical fruit has already been reported for Chardonnay wines from the Marlborough region of New Zealand [40].

5. Conclusions

Non-*Saccharomyces* yeasts produce a wider range of volatile compounds due to the more developed enzymatic system compared to *Saccharomyces* yeasts. As many of the oenological characteristics of yeasts are strain-dependent, the screening for new, indigenous non-*Saccharomyces* yeasts is of great importance in oenology. In compliance with this statement, the aromatic profiles of analysed wines produced with the native strains *M. pulcherrima* B-5 and *C. famata* WB-1 were shown to be qualitatively and quantitatively different from the Chardonnay wine produced with *S. cerevisiae* QA23. Also, cold pre-fermentative maceration was capable of modifying both the volatile composition of wines and the sensory characteristics. Maceration induced the decrease of total higher alcohols and increased the total ester concentration. Higher levels of floral and fruity notes were observed in wines produced with native strains in correspondence with increased ester concentration contributing to the overall wine attraction. Maceration increased the fullness and intensity of taste and flowery and fruity smell notes, especially in the wines produced with *M. pulcherrima* in pure and sequential fermentation. Both native strains *M. pulcherrima* B-5 and *C. famata* WB-1 isolated from blackberries possess great potential for the production of Chardonnay wine with improved sensory and aroma profiles.

Use of native non-*Saccharomyces* strains isolated from different microbial communities is an innovative biotechnological approach that satisfies new trends in winemaking and allows development of new winemaking practices. This approach has some limitations, which mainly relate to the microbial community development in mixed cultures during the winemaking process. To overcome this challenge, analysis of interrelationships between the strains and consistency of inoculum should be performed in further research. For industrial production, a set of tests should be performed in a scaled-up process to confirm the presented positive impacts of *C. famata* WB-1 and *M. pulcherrima* B-5. Nevertheless, the increase of the quality and sensory characteristics of the produced wines fully justifies the need for further research and investment in industrial application of these native strains. The practical industrial application of the analysed strains could lead to the development of new wine styles with improved quality and distinguished aromas.

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