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Contribution of Grape Skins and Yeast Choice on the Aroma Profiles of Wines Produced from Pinot Noir and Synthetic Grape Musts

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Abstract: The aroma profile is a key component of Pinot noir wine quality, and this is influenced by the diversity, quantity, and typicity of volatile compounds present. Volatile concentrations are largely determined by the grape itself and by microbial communities that produce volatiles during fermentation, either from grape-derived precursors or as byproducts of secondary metabolism. The relative degree of aroma production from grape skins compared to the juice itself, and the impact on different yeasts on this production, has not been investigated for Pinot noir. The influence of fermentation media (Pinot noir juice or synthetic grape must (SGM), with and without inclusion of grape skins) and yeast choice (commercial *Saccharomyces cerevisiae* EC1118, a single vineyard mixed community (MSPC), or uninoculated) on aroma chemistry was determined by measuring 39 volatiles in finished wines using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS). Fermentation medium clearly differentiated the volatile profile of wines with and without yeast, while differences between EC1118 and MSPC wines were only distinct for Pinot noir juice without skins. SGM with skins produced a similar aroma profile to Pinot noir with skins, suggesting that grape skins, and not the pulp, largely determine the aroma of Pinot noir wines.

Keywords: aroma profile; grape skins; Pinot noir; synthetic grape must; wine quality; yeasts

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1. Introduction

High-quality red table wines produced from *Vitis vinifera* L. cv. Pinot noir are renowned for their characteristic volatile profiles, with aromas of red fruits (e.g., cherry and raspberry), florals, spice, and forest floor [1]. The interplay between odorants, derived from a multitude of volatile compound classes, contribute to Pinot noir wine quality and regional differentiation [2,3]. In addition to varietal compounds from grapes and volatiles associated to ageing, yeast-derived odorants, such as acetate and ethyl esters, higher alcohols, and unsaturated volatile fatty acids, are positively correlated to the sensory acceptance of Pinot noir wine and represent the majority of wine aroma compounds [1,3,4].

The dividing line between grape-derived and yeast-derived volatiles is not clear-cut in terms of their biogenesis. For instance, during alcoholic fermentation, yeast converts odorless grape-derived precursors to odiferous volatile compounds, such as polyfunctional thiols [5], and modulates the concentrations of several grape-derived volatiles, including the cleavage of grape-derived C_{13}-norisoprenoids and terpenes from their glycosidic precursors via β-glucosidase enzymes [6,7]. Grape skins play a key role in this release, as increased maceration times during winemaking result in the release of more C_{13}-norisoprenoids and terpenes, since the free and bound forms are mainly present in

the skins [8]. Prolonged pre-fermentation maceration during red winemaking has also shown to increase red and black berry aromas in Merlot wines, with the fruitiest wines obtained by including grape skins and skin extract [9]. These positive fruity characters also show a gradual increase in parallel with yeast fermentative activity, leading to the hypothesis that these aromas were being formed by yeast from inodorous grape skin precursors [9]. Due to the significant impact of yeast in the formation of aroma volatiles from grape skins, fermentation with mixed species of indigenous yeasts may have great potential for elevating bound volatiles, due to the high levels of enzymatic activity in some non-*Saccharomyces* yeast species compared to *Saccharomyces cerevisiae* [10,11].

Given that yeast species and strain modulate levels of both varietal and fermentative volatiles, there has been extensive research comparing volatile formation during spontaneous and inoculated fermentations. Spontaneous fermentations are associated with higher risk of off-flavors and/or stuck and sluggish fermentations [12]; however, the lack of genetic diversity in commercial *S. cerevisiae* strains can result in homogeneous wines lacking complexity or regional characteristics [13]. Ongoing research reinforces the concept that the unique occurrence and composition of yeasts in the environment play a role in characterizing wine-producing regions by influencing wine chemical composition, providing evidence for a microbial component of terroir [14,15]. Thus, isolating and evaluating native yeasts allows winemakers to mimic spontaneous fermentations and preserve regional attributes, while effectively managing quality control during winemaking [14].

Research on the factors influencing the formation of wine aromas is complex since the chemical constituents of the grapes themselves vary greatly based on variety and clone, the growing environment, and viticultural and winemaking practices [16–18]. Therefore, many studies investigating wine volatile formation use SGM for fermentation, as the key constituents supporting yeast growth and metabolism, such as carbon, nitrogen, salts, vitamins, and minerals, can be homogenized across different batches [19]. One of the main drawbacks to SGM is the lack of grape skins, which are essential in red winemaking and have been shown to contain important aroma precursors yielding impact odorants during fermentation [9,20].

Understanding the interactions between grape skins and yeast metabolism towards aroma formation will help to develop new enological tools and strategies for winemakers and enable the formulation of more suitable fermentation media for red wine research. Therefore, the main aim of this research was to compare wines made from Pinot noir juice or SGM, with and without grape skins, to determine the degree of similarity between their aroma profiles and to gain insight into the formation of volatiles derived from the skins versus pulp. The second aim was to characterize the species composition of a native single vineyard yeast community, MSPC, and to evaluate the volatile compounds produced by this community in comparison to a commercial *S. cerevisiae* wine yeast, EC1118, and an uninoculated must. This objective enabled a thorough investigation into the aroma potential of an indigenous community and allowed for the evaluation of yeast metabolism on the degree of aroma formation from combinations of SGM and Pinot noir juice with and without skin contact during fermentation.

2. Materials and Methods

2.1. Chemicals

MilliQ water was used for all experiments (Merck/Millipore Sigma, NZ). Bacteriological agar, casein peptone, dimethyl dicarbonate (≥99.0%), D-fructose, D-glucose, L-tartaric acid, and yeast extract were purchased from Merck/Millipore Sigma (Castle Hill, NSW, Australia). Chelex®100 resin was acquired from Bio-Rad Laboratories, Inc (Richmond, CA, USA), Zymolyase was obtained from MP Biomedicals (Seven Hills, NSW, Australia), and the KAPA2G Fast HotStart ReadyMix PCR kit was purchased from Kapa Biosystems (Wilmington, MA, USA). Sodium chloride (reagent grade, ECP Labchem, Auckland, New Zealand) and argon gas (industrial grade, BOC Gas, Auckland, New Zealand) were used to prepare wine samples for volatile quantification. Deuterated internal standards (pu-

rity of ≥99%), ethyl butyrate-d_3, ethyl hexanoate-d_{11}, ethyl octanoate-d_{15}, 3-methylbutyl acetate-d_3, *n*-hexyl acetate-d_3, 2-phenylethyl acetate-d_3, (±)-linalool-d_3, α-terpineol-d_3, *n*-hexyl-d_{11}-alcohol, 2-phenyl-d_5-ethanol, hexanal-d_{12}, and hexanoic-d_{11} acid, were purchased from CDN Isotopes, Quebec, Canada. Non-deuterated standards, DL-3-octanol (≥99%) and 4-decanol (≥98%), were obtained from Merck/Millipore Sigma (Auckland, New Zealand), and 3,4-dimethylphenol (≥98%) was sourced from Alfa Aesar (Ward Hill, MA, USA).

2.2. Yeast Isolation

Vitis vinifera L. cv. Pinot noir grapes from the 2018 vintage were collected aseptically from a commercial single vineyard site, MSPC, in Marlborough, NZ. Grapes were aseptically hand-destemmed into sterile buckets. Starting must was 21.7 °Brix with a titratable acidity (TA) of 8.8 g L^{-1}, pH 3.1 and yeast assimilable nitrogen (YAN) of 155 mg L^{-1}. Grapes were held at 12 °C overnight then equilibrated to room temperature (~20 °C) to encourage growth of indigenous yeast communities. Upon the decrease of 2 °Brix during spontaneous fermentation, the must was mixed, and a 10-mL sample was transferred to a 20-mL Falcon tube filled with glycerol (15% *v/v*) and stored at −80 °C. A 100 µL aliquot was serially diluted and plated onto yeast extract peptone dextrose (YPD) agar plates (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose, and 20 g L^{-1} agar). After incubation at 28 °C for 48 hours, single colonies were selected at random until there were no further viable cells for culturing in a 96-well plate containing YPD agar (91 total). Isolates were transferred to a 96-well plate containing YPD with 15% glycerol and stored at −80 °C.

2.3. Molecular Biology Techniques

Restriction fragment length polymorphisms (RFLPs) were used to identify yeast species. DNA was extracted using Zymolyase [21], and part of the ITS rDNA region was amplified following the method in Esteve-Zarzoso et al. [22]. Amplicons were digested using the *Hinf*I and *Hae*III restriction enzymes, and fragment sizes were visualized using gel electrophoresis to group isolates into cohorts. Five individuals in total (1–3 from each cohort) were further analyzed by amplifying and sequencing a fragment of the 26S rDNA gene to determine the species representing each cohort [23]. Amplicons were sequenced using Dye Terminator Sanger Sequencing at the Auckland Genomics Centre, University of Auckland. To characterize *S. cerevisiae* yeast at the stain level, DNA was extracted from each isolate using the Chelex®method [24]. DNA was amplified and genotyped using microsatellite genotyping [25]. Geneious® Peak Scanner software (version 11.1.2) was used to interpret the results returned from GeneScan® (ABI PRISM 310 Genetic Analyzer Applied Biosystems), generated by the Auckland Genomics Centre, University of Auckland. Genotypes were cross-referenced to a *S. cerevisiae* microsatellite genotype database for identification [25].

2.4. Yeast Culture Conditions

Commercial *S. cerevisiae* strain Lalvin EC1118 (Lallemand, Montreal, Canada) was selected for fermentation trials. EC1118 was grown on YPD agar from a −80 °C stock and propagated into liquid YPD at 28 °C with orbital shaking at 150 rpm overnight. To prepare a mixed inoculum of the MSPC indigenous yeast community, isolates stored at −80 °C were separately incubated in three 96 well plates containing liquid YPD at 28 °C until stationary phase (3 days). Each isolate was combined into one mixed culture to simulate the composition of yeasts in the original early-stage ferment. Pre-cultured yeasts were pelleted and washed twice with sterile MilliQ water before inoculation.

2.5. Fermentation Media

SGM was adapted from Harsch et al. [26] with modification to reflect Pinot noir juice amino acid composition (Table S1). The pH was 3.4, YAN was 300 mg L^{-1}, and total sugars were 230 g L^{-1} (23.0 °Brix). Pinot noir grapes, sourced from Marlborough and

Martinborough, NZ, were harvested in 2018 and stored at −30 °C. Grapes were thawed in a sterile bucket at 4 °C for 48 h. Juice was pressed off using a sterile sieve and cheesecloth, and the remaining grape solids were macerated in the juice and homogenized, followed by the addition of dimethyl dicarbonate (DMDC) (20% in ethanol) at 300 μL L^{-1} for sterilization. Grape must was 23.0 °Brix, TA of 7.6 g L^{-1}, pH 3.4, and 182 mg L^{-1} YAN. The bucket was sealed and left overnight at room temperature. To minimize handling time and avoid spoilage, 20 mL aliquots of gently packed and homogenized grape solids were transferred to 50 mL Falcon tubes. DMDC-treated grape juice was divided into sterile tubes as grape juice only or at a ratio of 1:4 grape skins:juice to recreate the original ratio of skins:juice. In addition, 1:4 grape skins:SGM was blended together. The YAN was not standardized across the four experimental media as it was not feasible to ensure that all amino acid concentrations were identical. After blending, media containing grape components were sterilized with 200 μL L^{-1} DMDC at 25 °C overnight. Before and after DMDC additions, aliquots were serially diluted 10, 100, and 1000 times on YPD plates and incubated for 48 h to assess sterilization efficacy.

2.6. Fermentation Trials

Microvinifications of grape juice no skins (JN) and SGM no skins (SN) were fermented in 250 mL sterile flasks with airlocks. Microvinifications with grape juice plus skins (JS) and SGM plus skins (SS) fermented in sterilized glass 250 mL coffee plungers (Zip®) to facilitate cap management, as in commercial red winemaking. Media were warmed to 28 °C for two hours before inoculation to equilibrate to the fermentation temperature being used. *S. cerevisiae* EC1118 (EC) and the MSPC mixed community (MSPC), were inoculated at 2.5 × 10^6 cells mL^{-1} while the control group (N) received no inoculation. Fermentations were conducted in triplicate and kept at 28 °C without shaking. Flasks were manually swirled three times a day, while plungers were plunged three times daily. Fermentations were weighed daily to monitor weight loss as a proxy for fermentation progress [27]. Once fermentations stopped losing weight with a rate of weight loss close to zero, wines with skins were pressed and wines were cold settled overnight at 4 °C. Clear wines were decanted into 50 mL polypropylene Thermo Scientific Nalgene centrifuge tubes and centrifuged at 6000 *g* for 10 mins in a Thermo Scientific Sorvall Lynx4000 Superspeed centrifuge to pellet any remaining solids. Supernatants were transferred to 70 mL sample containers and stored at −80 °C until further analysis.

2.7. Residual Sugar and Alcohol Analysis

Residual sugars, D-glucose and D-fructose, were measured in finished wines using the Megazyme® Auto-Analyser Assay Kit and a SpectraMax® iD3 Plate Reader. Alcohol content was measured as % *v/v* alcohol by volume (ABV) at room temperature by an Anton Paar density meter/Alcolyzer (Austria) with Alcolyzer Wine Method preset.

2.8. Volatile Aroma Compound Quantitation

Volatile compounds were quantified using HS-SPME coupled with GC-MS following the method described in Pinu et al. [28] and developed by Jouanneau et al. [29]. Details on the method validation can be found in Jouanneau et al. [29]. A 10 mL wine sample was mixed with 3.5 g sodium chloride in a 20 mL amber vial (Agilent). Two internal standards mixtures were added: mix one, containing 11 deuterated standards (50 μL) (Table S2), and mix two, containing DL−3-octanol (499 μg L^{-1}), 4-decanol (1005 μg L^{-1}), and 3,4-dimethylphenol (114 μg L^{-1}) (25 μL). Argon gas was used to purge the vial before closing with an Agilent screw cap and PTFE silicone septa. Samples were placed on a Gerstel MPS2 tray (VT32−20) and incubated for 10 mins at 45 °C, with agitation at 700 rpm via a Gerstel Agitator/Stirrer. A DVB/CAR/PDMS fiber (2 cm, 23-gauge, 50/30 μm, SUPELCO, Bellefonte, PA, USA), pre-baked for 5 min (54 mm bakeout penetration) at 250 °C (50 kPa, total flow rate of 14.022 mL/min, and septum purge flow of 2 mL/min), was exposed to the vial headspace (30 mm vial penetration) for 60 mins at 45 °C. The fiber was shifted

to the injection port of an Agilent 7890A GC System coupled to a mass selective detector (5975C inert XL, Santa Clara, CA, USA). Desorption of the analytes was carried out in splitless mode (pressure 27.881 kPa, total flow rate 15 mL/min, and septum purge flow 2 mL/min) for 10 mins at 250 °C (54 mm injection penetration). The carrier gas was helium (27.881 kPa) at a flow rate of 1 mL/min. Analytes were separated on a tandem column composed of a HP−1 ms (30 m × 320 µm internal diameter × 0.25 µm film thickness) and a HP-INNOWax (30 m × 320 µm internal diameter × 0.25 µm film thickness) (Agilent, Santa Clara, CA, USA). The oven temperature was programmed as follows: 40 °C for 5 mins, 200 °C at 2 °C/min for 5 mins, 240 °C at 80 °C/mins for 5 mins, and 40 °C at 80 °C/min for 2 mins. The interface temperature was 250 °C. The ion source, operating in electron impact mode at 70 eV, was held at 230 °C, and the quadrupole temperature was 150 °C. Calibration curves were constructed in model wine (water containing 12% *v/v* ethanol and 5 g L^{-1} L-tartaric acid, pH 3.2), spiked with increasing concentrations of the compound standards. Volatile compounds and internal standards were identified and quantitated using MassHunter Software (Version B.05.00, Agilent, Santa Clara, CA, USA). Peaks were integrated automatically, followed by manual integration to ensure accuracy and comparison of ions according to the NIST library and commercially available reference standards. Response ratios for compounds of interest were calculated using an appropriate internal standard. Linear regression was used to generate the equation for calculating compound concentrations from calibrations curves. Table S2 contains the ions and retention times used to identify each compound (ions used for integration are shown in bold).

2.9. Statistical Analysis

Statistical tests were performed using IBM® SPSS® 25. *P*-values lower than 0.05 were considered statistically significant. The heatmap to visualize the volatile composition across samples was generated using the heatmap function in R (CRAN; http://cran.r-project.org/ (accessed on 20 August 2021)). Permutational multivariate analysis of variance (PERMANOVA) to estimate the impact of external variables on volatile concentrations was performed using the R-package vegan (2.5−7) [30]. Multivariate statistical ordination analysis and visualization via principal component analysis (PCA) followed the method in Parish et al. [31]. Mardia's criterion was used to identify volatiles of interest [32].

3. Results and Discussion

3.1. Yeast Species and Strain Typing

Indigenous yeasts were isolated from an early-stage Marlborough MSPC Pinot noir fermentation to capture the diversity of yeast species present before an increase in ethanol caused a decrease of less tolerant species. Cohort determination (Figure S1) and Sanger sequencing (Table S3) identified two genera and three species in the MSPC community, *Hanseniaspora uvarum* (21 individuals), *S. cerevisiae* (19 individuals), and *Saccharomyces uvarum* (51 individuals) (Table 1). These species have already been found in early-stage fermentations [15,33]. The 19 *S. cerevisiae* isolates were identified to strain level by microsatellite genotyping. All isolates had identical genotypes (Table S4), matching a strain named CB1. CB1 had previously been isolated from Corbans Winery, Hawke's Bay, NZ, in 2004, indicating its association with the NZ wine industry [25,34]. According to the microsatellite database, CB1 is not a commercial *S. cerevisiae* strain.

Table 1. Yeast species identified in each well of the 96-well MSPC yeast collection plate. *Saccharomyces cerevisiae* (SC); *Saccharomyces uvarum* (SU); *Hanseniaspora uvarum* (HU); Empty well (-).

	1	2	3	4	5	6	7	8	9	10	11	12
A	SC	SU	SU	SU	SC	HU	SU	SU	SU	SU	SU	-
B	SU	SU	SU	HU	SU	SU	SU	SU	SC	HU	SU	SC
C	SU	SU	HU	SU	SC	HU	SU	SU	SU	SC	SU	SU
D	SU	HU	SC	SU	SU	HU	SU	HU	SU	HU	HU	SU
E	HU	SC	HU	SU	SU	SC	HU	HU	SC	HU	SU	HU
F	SU	SU	HU	SU	SC	SU	SU	SC	HU	HU	SC	-
G	SU	SU	SC	SU	SU	SC	SC	SU	SU	SC	HU	-
H	SU	SU	SC	HU	SU	SU	SC	SU	HU	SU	-	-

3.2. Fermentation Progress

Prior to inoculation, the efficacy of the Pinot noir juice and skin sterilization was compared (Table 2). As expected, grape juice treated with DMDC (JN) had lower numbers of viable cells before inoculation (10 CFU mL^{-1}) than media containing grape skins (JS at 1.65×10^4 CFU mL^{-1} and SS at 7.00×10^3 CFU mL^{-1}) (Table 2). Thus, DMDC sterilization efficacy was dependent on the presence of grape solids. This result is consistent with the occurrence of yeasts on grape skins, with skins offering protection to indigenous yeasts during DMDC sterilization [35]. Cumulative weight loss curves showing fermentation progress are presented in Figures S2–S5. Fermentation duration for inoculated ferments was ~262 h, with the exception being JN-EC, which completed after only ~167 h (Table 2). JN-N, JS-N, and SS-N uninoculated controls demonstrated spontaneous fermentation, likely to have been initiated by indigenous yeasts present in the grape material, as predicted from the DMDC efficacy results. These ferments were sluggish, and wines had high residual sugar (>81 g L^{-1}) (Table 2). Since all inoculated ferments began to release CO_2 ~10−20 hours before the non-inoculated controls, there is the assurance that these fermentations were carried out by the yeast/s added, rather than contaminating yeasts. *S. cerevisiae* EC1118 fermentations reached half total weight loss faster than the MSPC community in the same medium (Table 2). None of the MSPC ferments became stuck or sluggish, likely aided by the presence of *S. cerevisiae*, the CB1 strain, in this community [33]. Overall, the MSPC community demonstrated desirable and reproducible fermentation kinetics in the various combinations of juice/medium with and without skins. A 4 × 3 two-way ANOVA with fermentation medium (JN, JS, SN, and SS) and inoculum (EC, MSPC, and N) as between-subject factors revealed a main effect of inoculation scheme, $F(2, 24) = 106$, $p < 0.001$, $\eta_p^2 = 0.898$. This was expected, since the inoculated fermentations had significantly higher sugar/ethanol conversion efficiency than no inoculation (both $p < 0.001$). There was no statistically significant difference in the ethanol produced by the MSPC community compared to EC1118 in any media (Table 2). In general, SN wines exhibited higher concentrations of residual sugar than the other treatments, e.g., average of 7.56 g L^{-1} in SN-MSPC compared to 0.27 g L^{-1} in JN-MSPC, suggesting that SGM alone is missing factors present in grape juice that aid in fermentation. It is likely that the inclusion of natural grape constituents would have supplemented certain nutrients important for yeast metabolism, such as fatty acids [36].

3.3. Analysis of Aroma Compounds Using PERMANOVA and PCA

Mean concentrations of 39 fermentative volatile compounds (acetate esters, ethyl esters of fatty acids, higher alcohols, and volatile fatty acids) and grape-derived compounds (C_6 compounds, cinnamate esters, C_{13}-norisoprenoids, and terpenes) were quantified for four fermentation media (JN, JS, SN, and SS) and three inocula (EC, MSPC, and N) (Table 3). The heatmap in Figure 1 presents an overview of the volatile profiles for each sample while the dendrogram helps to visualize clusters among samples and volatiles.

Table 2. Sterilization efficacy, fermentation duration, total weight loss, ethanol, and residual sugar concentrations of finished wines for each treatment. Mean (n = 3) ± standard deviation. Mean values not connected by the same letter are significantly different (Tukey's HSD, $p < 0.05$). Juice no skins (JN); juice plus skins (JS); SGM no skins (SN); SGM plus skins (SS); EC1118 (EC); MSPC community (MSPC); uninoculated (N); not applicable (-).

	JN-EC	JN-MSPC	JN-N	JS-EC	JS-MSPC	JS-N	SN-EC	SN-MSPC	SN-N	SS-EC	SS-MSPC	SS-N
viable cells after sterilization (CFU mL^{-1})	10	10	10	1.65×10^4	1.65×10^4	1.65×10^4	-	-	-	7.00×10^3	7.00×10^3	7.00×10^3
length of fermentation (h)	167	262	262	262	262	262	262	262	-	262	262	262
fermentation time at half total weight loss (h)	70	70	75	53	60	82	70	70	-	60	70	96
total weight loss (g)	11.8 ± 0.25 d	11.6 ± 0.26 d	4.61 ± 0.35 b	17.8 ± 0.52 e	17.6 ± 0.48 e	10.9 ± 0.41 cd	10.8 ± 0.02 cd	11.0 ± 0.35 cd	0.72 ± 0.26 a	17.1 ± 0.11 e	16.6 ± 0.62 e	9.71 ± 1.02 c
total residual sugar (g L^{-1})	0.10 ± 0.02 a	0.27 ± 0.16 a	113 ± 23.5 c	0.21 ± 0.07 a	0.29 ± 0.25 a	81.4 ± 5.84 b	1.26 ± 0.45 a	7.56 ± 2.78 a	186 ± 9.4 d	0.12 ± 0.05 a	0.10 ± 0.02 a	87.2 ± 4.14 bc
residual D-glucose (g L^{-1})	0.01 ± 0.02 d	0.12 ± 0.14 d	58.4 ± 12.1 b	0.09 ± 0.07 d	0.09 ± 0.01 d	41.6 ± 2.81 c	0.14 ± 0.03 d	0.54 ± 0.47 d	94.2 ± 4.2 a	0.07 ± 0.01 d	0.09 ± 0.06 d	44.0 ± 1.75 c
residual D-fructose (g L^{-1})	0.10 ± 0.03 c	0.14 ± 0.05 c	54.2 ± 11.5 b	0.12 ± 0.03 c	0.20 ± 0.25 c	39.8 ± 3.52 b	1.12 ± 0.48 c	7.02 ± 2.32 c	91.8 ± 5.9 a	0.06 ± 0.05 c	0.02 ± 0.04 c	43.2 ± 2.60 b
ethanol (% *v/v*)	14.4 ± 0.22 e	13.7 ± 0.42 de	5.36 ± 0.19 b	10.9 ± 0.22 c	11.2 ± 0.23 c	4.81 ± 0.15 b	14.3 ± 0.30 de	13.4 ± 0.18 d	1.53 ± 0.09 a	11.3 ± 0.27 c	11.1 ± 0.34 c	4.78 ± 0.05 b

Table 3. Volatile compound concentrations in finished wines for each treatment. Mean ($n = 3$) ± standard deviation. Mean values not connected by the same letter are significantly different (Tukey's HSD, $p < 0.05$). Juice no skins (JN); juice plus skins (JS); SGM no skins (SN); SGM plus skins (SS); EC1118 (EC); MSPC community (MSPC); uninoculated (N); not detected (ND).

	JN-EC	JN-MSPC	JN-N	JS-EC	JS-MSPC	JS-N	SN-EC	SN-MSPC	SN-N	SS-EC	SS-MSPC	SS-N
Acetate Esters (μg L^{-1})												
isobutyl acetate	67.5 ± 2.3 e	98.6 ± 4.3 f	29 ± 12 d	10.3 ± 0.7 abc	15.1 ± 0.4 bc	21.1 ± 8.6 cd	9.7 ± 3.5 abc	6.2 ± 1.3 ab	ND	6.7 ± 0.4 ab	6.0 ± 0.4 ab	6.1 ± 0.7 ab
isoamyl acetate	738 ± 154 d	747 ± 62 d	66 ± 13 abc	69.7 ± 7.3 abc	140 ± 7.9 abc	66 ± 32 abc	201 ± 46 c	175 ± 27 bc	1.4 ± 0.2 a	67.0 ± 3.6 abc	102 ± 11 abc	24.2 ± 5.6 ab
ethyl phenylacetate	1.72 ± 0.24 cd	1.95 ± 0.45 d	0.21 ± 0.08 ab	5.79 ± 1.42 f	3.67 ± 0.41 e	0.32 ± 0.13 abc	0.20 ± 0.03 ab	0.31 ± 0.10 abc	0.03 ± 0.02 a	2.61 ± 0.74 de	1.53 ± 0.14 bcd	0.18 ± 0.02 ab
β-phenylethyl acetate	76 ± 11 e	75 ± 14 e	13.8 ± 1.1 ab	14.5 ± 0.3 ab	25.4 ± 0.7 bcd	18.0 ± 6.8 abc	44.9 ± 2.2 d	38 ± 10 cd	0.4 ± 0.1 a	13.8 ± 0.7 ab	25.3 ± 1.0 bcd	17 ± 11 ab

Table 3. *Cont.*

	JN-EC	JN-MSPC	JN-N	JS-EC	JS-MSPC	JS-N	SN-EC	SN-MSPC	SN-N	SS-EC	SS-MSPC	SS-N
Alcohols, Aldehydes (μg L^{-1})												
isobutanol	209,866 ± 45,324 d	392,892 ± 38,963 e	19,612 ± 3626 ab	66,983 ± 4624 bc	76,305 ± 4358 c	13,477 ± 504 a	31,436 ± 6436 abc	40,452 ± 389 abc	40 ± 26 a	48,928 ± 2292 abc	33,103 ± 4168 abc	7254 ± 1583 a
1-butanol	14,822 ± 9199 abc	96,376 ± 10,656 e	7766 ± 530 ab	26,947 ± 449 cd	21,768 ± 3274 cd	4259 ± 351 a	32,471 ± 3441 d	20,576 ± 902 bcd	1943 ± 115 a	26,616 ± 3226 cd	18,855 ± 1894 bc	3813 ± 498 a
isoamyl alcohol	416,032 ± 112,652 e	529,378 ± 40,137 f	29,211 ± 2358 ab	180,385 ± 13,726 cd	218,911 ± 8708 d	21,719 ± 922 ab	120,932 ± 12,225 bcd	104,487 ± 5212 abc	23.2 ± 3.9 a	156,540 ± 4185 cd	147,978 ± 18,883 cd	15,135 ± 760 a
phenylethyl alcohol	83,025 ± 8821 e	124,639 ± 17,639 f	19,817 ± 1597 abc	81,886 ± 6220 e	124,654 ± 10,749 f	18,929 ± 1873 abc	29,364 ± 3427 bc	34,830 ± 7805 c	131 ± 28 a	58,624 ± 3592 d	97,529 ± 4470 e	12,289 ± 1927 ab
methionol	5560 ± 1426 e	4619 ± 1300 de	383 ± 168 ab	2269 ± 1052 abc	1738 ± 586 abc	47 ± 37 a	1158 ± 551 abc	1139 ± 387 abc	26.4 ± 0.9 a	2780 ± 1107 cd	2573 ± 646 bcd	111 ± 56 a
benzyl alcohol	1146 ± 16 de	1048 ± 12 de	894 ± 84 d	1332 ± 148 e	1360 ± 187 e	1315 ± 227 e	36.7 ± 7.1 a	76 ± 73 ab	17.5 ± 2.1 a	391 ± 98 bc	431 ± 28 c	435 ± 82 c
benzaldehyde	0.71 ± 0.04 abc	0.56 ± 0.09 abc	1.86 ± 0.35 d	1.131 ± 0.34 cd	0.76 ± 0.20 abc	4.23 ± 0.54 e	0.16 ± 0.07 a	0.24 ± 0.12 ab	0.34 ± 0.19 ab	0.40 ± 0.08 abc	0.26 ± 0.11 ab	0.97 ± 0.35 bc
Fatty Acid Ethyl esters (μg L^{-1})												
ethyl isobutyrate	44 ± 10 bcd	137 ± 33 e	9.7 ± 4.0 ab	44.8 ± 3.3 cd	63 ± 19 d	4.2 ± 0.9 a	5.4 ± 1.8 a	7.2 ± 0.4 a	2.5 ± 3.5 a	26 ± 10 abc	19.9 ± 1.9 abc	2.2 ± 0.3 a
ethyl butanoate	139 ± 21 c	117 ± 7.3 bc	11.5 ± 2.4 a	26.4 ± 1.8 a	41.3 ± 0.4 a	3.5 ± 0.3 a	115 ± 19 bc	88.4 ± 3.2 b	28 ± 36 a	22.4 ± 2.6 a	28.1 ± 3.3 a	4.9 ± 0.2 a
ethyl 2-methyl butanoate	2.89 ± 0.121 b	5.23 ± 0.48 c	0.82 ± 0.511 a	ND	ND	ND	ND	ND	ND	ND	ND	ND
ethyl isovalerate	2.16 ± 0.25 b	5.16 ± 0.49 c	0.04 ± 0.07 a	1.48 ± 0.22 ab	4.48 ± 1.48 c	0.12 ± 0.09 a	0.37 ± 0.13 a	0.34 ± 0.12 a	0.78 ± 1.08 ab	0.74 ± 0.28 ab	1.19 ± 0.24 ab	ND
ethyl hexanoate	172 ± 46 e	130 ± 12 cde	2.5 ± 0.7 a	81.9 ± 8.1 bc	107 ± 8.6 bcd	7.0 ± 1.5 a	148 ± 28 de	156 ± 38 de	0.9 ± 1.0 a	67.4 ± 6.2 b	66.0 ± 5.1 b	1.5 ± 0.3 a
ethyl octanoate	152 ± 45 bcd	92 ± 13 abc	3.4 ± 0.5 a	79 ± 11 ab	91.8 ± 1.8 abc	3.7 ± 0.5 a	256 ± 78 d	200 ± 97 cd	1.7 ± 0.4 a	101 ± 29 abc	66.5 ± 3.4 ab	2.4 ± 0.3 a
ethyl decanoate	24.6 ± 7.1 a	20.2 ± 5.5 a	0.6 ± 0.2 a	21.5 ± 3.5 a	23.9 ± 4.8 a	1.3 ± 0.3 a	199 ± 56 b	208 ± 80 b	0.8 ± 0.3 a	28.0 ± 8.3 a	19.0 ± 7.0 a	0.5 ± 0.2 a

Table 3. *Cont.*

Fatty Acids												
isobutyric acid (mg L^{-1})	2.8 ± 1.6 ab	5.9 ± 1.8 c	ND	6.0 ± 1.1 c	7.3 ± 0.8 c	0.8 ± 0.2 a	ND	ND	ND	3.5 ± 0.8 b	3.1 ± 0.2 ab	ND
isovaleric acid (mg L^{-1})	ND	0.66 ± 0.16 b	ND	0.59 ± 0.36 ab	0.84 ± 0.23 b	0.14 ± 0.05 a	ND	ND	ND	0.53 ± 0.30 ab	0.65 ± 0.17 b	ND
hexanoic acid (mg L^{-1})	1.26 ± 0.14 d	0.65 ± 0.05 b	0.02 ± 0.01 a	1.3 ± 0.1 d	1.0 ± 0.1 c	0.08 ± 0.01 a	1.5 ± 0.1 e	1.20 ± 0.07 d	0.01 ± 0.01 a	1.29 ± 0.09 d	0.95 ± 0.01 c	0.04 ± 0.01 a
octanoic acid (mg L^{-1})	0.57 ± 0.24 ab	0.16 ± 0.07 a	ND	0.45 ± 0.27 ab	0.53 ± 0.28 ab	ND	1.14 ± 0.44 b	1.16 ± 0.43 b	ND	0.66 ± 0.28 ab	0.63 ± 0.16 ab	ND
decanoic acid (μg L^{-1})	1.7 ± 0.4 d	1.2 ± 0.5 abcd	1.2 ± 0.5 abcd	1.5 ± 0.2 bcd	1.6 ± 0.3 cd	1.2 ± 0.3 abcd	0.56 ± 0.03 abc	0.44 ± 0.07 ab	0.20 ± 0.04 a	1.2 ± 0.3 abcd	1.6 ± 0.6 cd	1.3 ± 0.5 bcd
Cinnamates (μg L^{-1})												
ethyl (di)hydrocinnamate	0.22 ± 0.04 cd	0.32 ± 0.02 d	0.06 ± 0.02 a	0.31 ± 0.06 d	0.26 ± 0.02 cd	0.05 ± 0.01 a	0.05 ± 0.01 a	0.04 ± 0.01 a	0.01 ± 0.001 a	0.20 ± 0.02 bc	0.18 ± 0.02 bc	0.09 ± 0.10 ab
ethyl cinnamate (trans)	0.06 ± 0.01 bcde	0.08 ± 0.01 e	0.069 ± 0.01 de	0.07 ± 0.01 de	0.06 ± 0.02 bcde	0.06 ± 0.02 cde	0.04 ± 0.01 abcd	0.03 ± 0.01 ab	0.01 ± 0.002 a	0.04 ± 0.01 abcd	0.04 ± 0.01 abcd	0.03 ± 0.003 abc
C_{13}-norisoprenoids (μg L^{-1})												
β-damascenone	3.1 ± 0.1 c	2.8 ± 0.4 c	2.6 ± 0.3 c	1.2 ± 0.1 b	1.3 ± 0.2 b	1.1 ± 0.2 b	ND	ND	ND	0.17 ± 0.03 a	0.19 ± 0.01 a	0.16 ± 0.02 a
α-ionone	ND	ND	0.007 ± 0.003 a	0.016 ± 0.001 cd	0.026 ± 0.005 d	0.014 ± 0.004 b	ND	ND	ND	0.021 ± 0.006 c	0.022 ± 0.006 cd	0.012 ± 0.003 b
β-ionone	0.11 ± 0.02 c	0.09 ± 0.02 c	0.08 ± 0.01 bc	0.30 ± 0.04 ef	0.34 ± 0.04 f	0.19 ± 0.03 d	0.01 ± 0.001 ab	0.02 ± 0.003 ab	0.01 ± 0.003 a	0.27 ± 0.04 e	0.33 ± 0.02 ef	0.18 ± 0.01 d
Terpenes (μg L^{-1})												
cis/trans-rose oxide	0.16 ± 0.001 ef	0.08 ± 0.01 bcd	0.04 ± 0.004 ab	0.25 ± 0.01 g	0.18 ± 0.04 f	0.06 ± 0.01 abc	0.09 ± 0.01 cd	0.08 ± 0.01 cd	0.12 ± 0.02 de	0.07 ± 0.01 bc	0.05 ± 0.01 abc	0.02 ± 0.002 a
linalool	32.7 ± 2.2 d	30.2 ± 1.1 cd	15.3 ± 1.1 b	27.9 ± 2.5 cd	33.7 ± 4.2 d	15.1 ± 0.5 b	26.1 ± 0.6 c	26.0 ± 0.8 c	8.6 ± 0.7 a	25.6 ± 3.5 c	26.5 ± 1.5 c	15.7 ± 1.0 b
α-terpineol	4.7 ± 0.3 abc	7.0 ± 2.2 cd	5.1 ± 0.3 bc	5.7 ± 1.1 bc	9.7 ± 2.9 d	5.8 ± 0.7 bc	3.5 ± 0.8 abc	2.4 ± 0.3 ab	1.4 ± 0.5 a	3.5 ± 0.6 ab	3.9 ± 0.2 abc	2.4 ± 0.4 ab
β-citronellol	32.1 ± 1.2 e	14.3 ± 0.9 cd	7.9 ± 3.3 bc	53.2 ± 5.1 f	25.7 ± 3.7 e	4.5 ± 1.3 ab	6.1 ± 1.0 ab	0.8 ± 0.3 ab	0.12 ± 0.03 a	30.9 ± 4.1 e	16.6 ± 1.2 d	2.6 ± 1.1 ab
nerol (*cis*-geraniol)	9.4 ± 1.2 ab	6.0 ± 0.9 ab	6.2 ± 1.5 ab	16.4 ± 1.0 c	16.3 ± 4.4 c	10.2 ± 3.2 ab	ND	ND	ND	9.5 ± 2.1 ab	11.1 ± 1.0 bc	5.6 ± 1.0 a
geraniol (*trans*-geraniol)	ND	ND	ND	0.16 ± 0.03 a	0.30 ± 0.13 a	ND	ND	ND	ND	0.31 ± 0.18 a	0.31 ± 0.02 a	ND

Table 3. *Cont.*

C_6-compounds (μg L^{-1})												
hexanal	5.5 ± 1.9 ab	5.3 ± 1.4 a	10.3 ± 2.0 ab	69 ± 12 d	53 ± 13 cd	5.0 ± 1.8 a	7.7 ± 0.98 ab	12.2 ± 0.9 ab	0.60 ± 0.06 a	55.2 ± 24.3 cd	31.4 ± 0.9 bc	12.4 ± 4.6 ab
Trans-2-hexenal	12.4 ± 4.2 ab	21.3 ± 4.1 bc	23.1 ± 2.2 cd	27.4 ± 4.4 cd	32.5 ± 3.2 d	20.6 ± 4.7 bc	5.9 ± 2.1 a	5.6 ± 5.5 a	3.1 ± 0.9 a	9.7 ± 1.5 a	10.4 ± 0.9 a	8.4 ± 2.1 a
hexanol	3237 ± 83 c	3041 ± 410 c	5626 ± 382 d	3676 ± 494 c	3715 ± 154 c	3360 ± 204 c	84.2 ± 10.8 a	87.4 ± 7.1 a	67 ± 28 a	1831 ± 124 b	2155 ± 193 b	1980 ± 148 b
Trans-3-hexen-1-ol	66.0 ± 9.3 cd	46.7 ± 5.6 bc	ND	24.7 ± 2.2 a	40.1 ± 8.3 ab	ND	70 ± 16 d	73.4 ± 7.0 d	ND	25.5 ± 5.4 a	26.4 ± 2.0 a	ND
Cis-3-hexen−1-ol	139 ± 11 e	96 ± 17 d	1.31 ± 0.40 a	61.6 ± 2.7 bcd	90.1 ± 8.5 cd	3.18 ± 0.10 a	139 ± 30 e	143 ± 14 e	ND	54.2 ± 9.0 b	59.8 ± 4.4 bc	0.96 ± 0.22 a

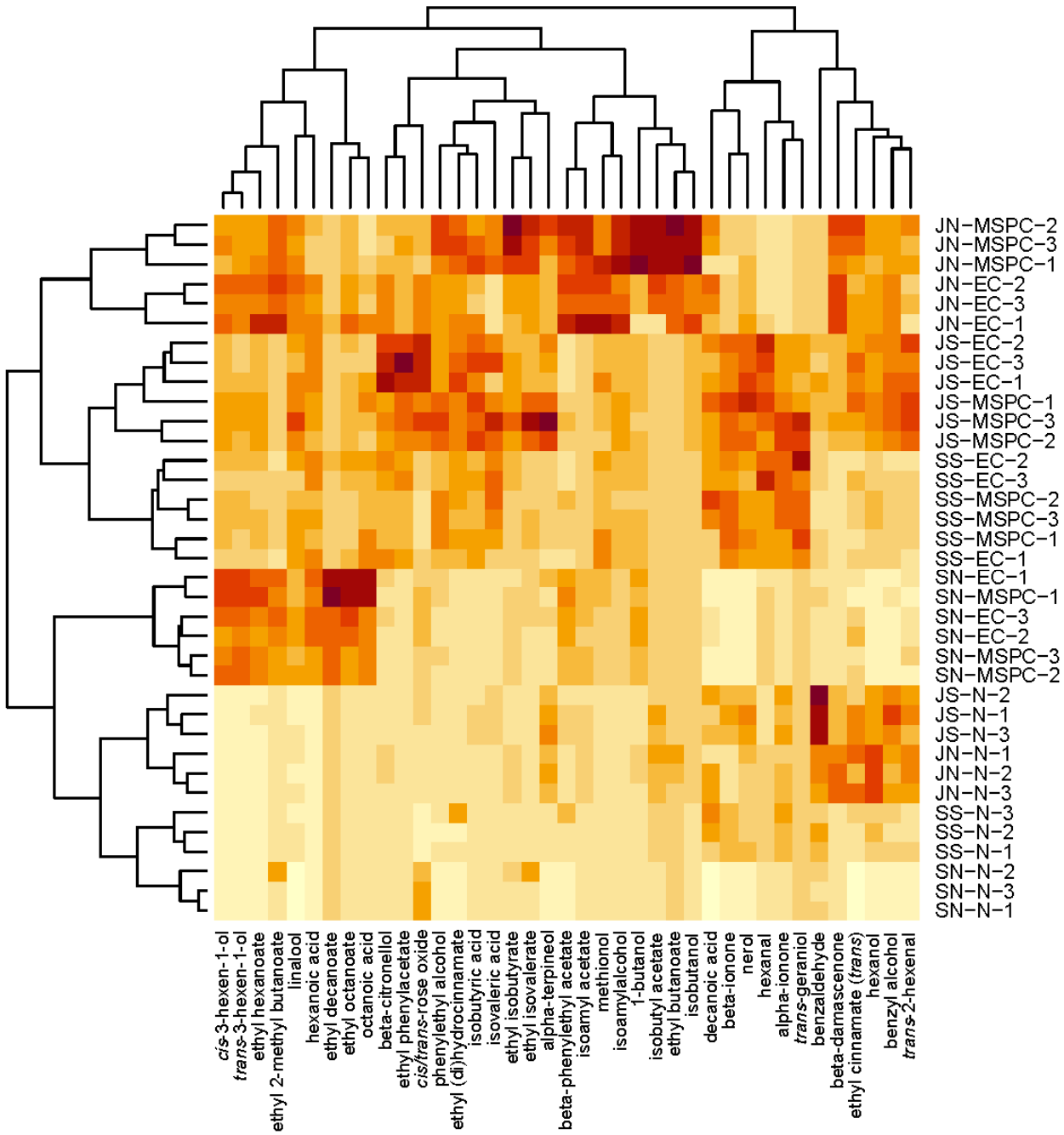

Figure 1. Heatmap and dendrogram to visualize clustering of the fermentation samples based on their volatile composition using 39 aroma compounds. The relative concentrations of each volatile are represented via a chromatic scale with the darkest red corresponding to the highest concentrations and the palest yellow corresponding to the lowest concentrations. Medium (JN, JS, SN, and SS) and inoculation treatments (EC, MSPC, and N).

The heatmap and dendrogram show that the fermentation samples were separated into various clusters based on volatile composition. Samples were clustered together based on the fermentation media (JN, JS, SN, and SS), except for the uninoculated samples, which formed their own cluster. The uninoculated samples were further differentiated by juice (plus or minus skins) and SGM (plus or minus skins). Interestingly, the SN samples with yeast were more closely related to the uninoculated samples than those containing grape juice or skins, while the JS and SS samples were more closely related to the samples

with grape juice only, providing an early indication of the importance of grape skins for volatile concentrations.

A PERMANOVA was applied to the data to determine how much of the variation within the volatile data was explained by the three external variables: fermentation medium, inoculum, and fermentation completion, with an interaction effect between inoculation and medium (Table 4). Over 93% of the variation in the aroma chemistry data was explained by the three factors, as well as the inoculation:medium interaction (Table 4). As suggested by the heatmap and dendrogram, fermentation medium explained 44.5% of the variation in aroma volatiles, while inoculum was accountable for 30.0% (Table 4). Fermentation medium had a more predominant impact on aroma chemistry than inoculation. Only medium and inoculation factors were highlighted on the PCA to assist with the interpretation of the aroma chemistry data, since the impact of fermentation completion was minimal ($p < 0.001$, $R^2 = 2.8\%$).

Table 4. PERMANOVA of the volatile compound concentrations regarding three external variables, medium (JN, JS, SN, and SS), inoculation (EC, MSPC, and N), and fermentation completion (total residual sugars: $\leq$5 g L^{-1}, >5 g L^{-1}). Df = degrees of freedom. Inoculation:medium = interaction between inoculation and fermentation medium factors.

Factor	Df	F-statistic	R^2	*p*-Value
inoculation	2	51.972	0.300	<0.001
medium	3	51.346	0.445	<0.001
fermentation completion	1	9.574	0.028	<0.001
inoculation:medium	5	10.936	0.158	<0.001
residuals	24		0.069	

Next, the 12 treatments were positioned on a PCA biplot based on volatile concentrations (Figure 2a). PC1 (42.7% of the variation) separated the wines by inoculum, while PC2 (36.1%) further separated the samples by fermentation medium. For each set of fermentations, triplicates were tightly clustered together, implying good reproducibility. JN wines were more spread out compared to the other three medium groups, suggesting that the inoculum had a greater impact on aroma production in JN than other media. Uninoculated JS and SS ferments went through spontaneous fermentation to a certain degree, resulting in aroma chemistry profiles that were slightly closer to their respective inoculated ferments (Figure 2a). All uninoculated ferments were tightly clustered, regardless of fermentation medium, indicating that these samples were more alike. Inoculated wines made with EC1118 or MSPC were clustered together for all media except for the JN wines. This shows that medium has a more significant impact on aroma chemistry than yeast. Previous studies have shown that mixed cultures can produce significantly different aroma profiles in grape juice compared to *S. cerevisiae* mono-cultures [37]. Inoculated JN and SN samples were grouped away from the skin-containing JS and SS samples, showing distinctive aroma chemistry profiles (Figure 2a). The significant overlap of JS and SS wines on the PCA indicates that these musts produced wines with comparable aroma chemistry, despite their differing YAN values. Clearly, grape skins significantly modulate wine aroma chemistry, with implications for winemaking. Winemakers could apply clean grape skins, obtained from a ferment with high quality fruit, to a lower quality must to enhance the quality of the aroma profile, as has been shown for dehydrated grape marc additions to bottled red wines [38] and skin supplements to Pinot noir for color improvement [39]. Additionally, the inclusion of skins in SGM is a viable alternative to grape must for research investigating aroma formation.

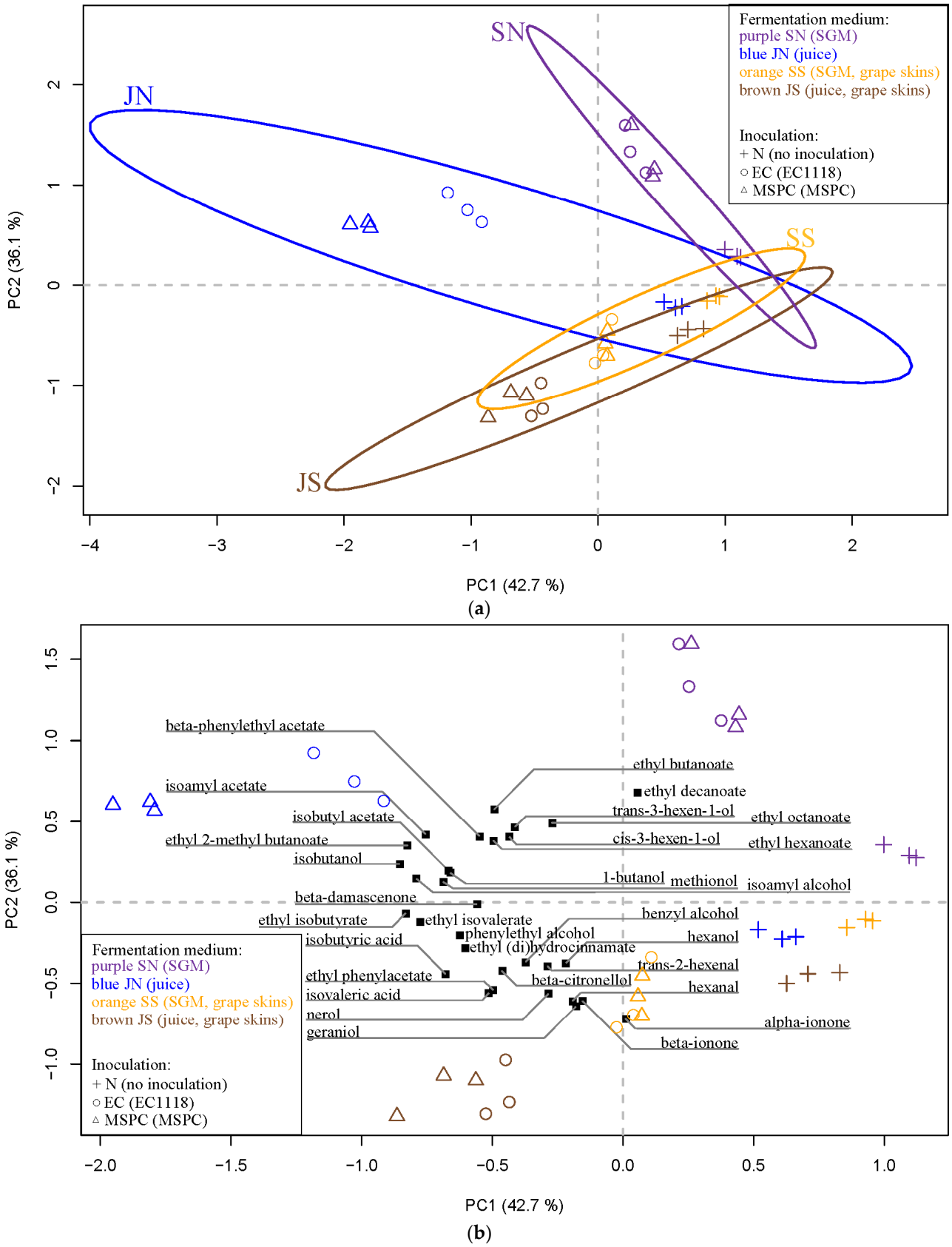

Figure 2. PCA biplots of principal component 1 (PC1) versus principal component 2 (PC2) of the fermentation samples showing the location of medium (JN, JS, SN, and SS) and inoculation treatments (EC, MSPC, and N) based on the wine volatile quantification results. Different symbols represent the medium and inoculum treatments; (**a**) circled by 95% confidence ellipses; (**b**) inclusion of volatiles determined to be important by Mardia's Criterion.

Volatile compounds associated to the main differences between treatments were chosen through Mardia's criterion (Figure 2b). Of the 39 volatiles quantitated, 31 compounds were identified as key drivers behind the variation in external variables (Figure 2b). In general, acetate and ethyl esters and higher alcohols were more associated with the JN and SN groups without skins, while grape-derived C_6-compounds, terpenes, and the α-ionone and β-ionone C_{13}-norisprenoids were more associated with JS- and SS-containing grape skins (Figure 2b). This observation corresponds to the association of fermentative compounds with grape pulp and varietal compounds with grape skins [40].

3.4. Overview of the Aroma Profile for Each Treatment by Compound Class

3.4.1. Acetate Esters and Higher Alcohols

Six higher alcohols were identified and quantified in all 12 treatments with isoamyl alcohol being the most abundant (Table 3). Concentrations of 180 mg L^{-1} for JS-EC and 219 mg L^{-1} for JS-MSPC were comparable to the range of 220–310 mg L^{-1} given in previous reports for Pinot noir wines [41,42]. Following this, its esterification product, isoamyl acetate, was present in much higher quantities than other esters. Isobutanol and isobutyl acetate were both more abundant in JN-EC and JN-MSPC than other wine samples (Table 3), suggesting that there are more available precursors in grape juice than SGM and musts-containing skins. On the PCA biplot, higher alcohols were jointly distributed in the top-right and bottom-left quadrants, while their corresponding acetate esters, such as isobutyl acetate, β-phenylethyl acetate, and isoamyl acetate, showed proximity to them (Figure 2b). Since yeast produces acetate esters from higher alcohols through esterification [43], the correlations between concentrations for these two classes were expected (Table 3) [44,45].

Most higher alcohols concentrations were significantly impacted by fermentation medium (JN, JS, SN, and SS) and inoculum (EC, MSPC, and N). In contrast, benzyl alcohol levels were fundamentally impacted by the medium (Table 3). Benzyl alcohol and its derivatives were reported to be determined by the corresponding glycoside precursors, e.g. β-D-glucopyranosides [46]. It is thought that the glycosidically bound form of benzyl alcohol can increase the free form primarily through acid hydrolysis at wine pH; therefore, the yeast metabolic activities in EC and MSPC groups did not seem to vary significantly in benzyl alcohol concentrations (Table 3).

As expected, acetate esters were higher in the inoculated groups than the non-inoculated controls (Table 3). A similar pattern of variation for different acetate ester concentrations was seen across treatments (Table 3), confirming the key role of yeast, but also media and of course nitrogen composition, in their formation [43]. MSPC produced more isoamyl acetate and β-phenylethyl acetate than EC1118 in SS and JS (Table 3). The effect of inoculum on acetate ester concentrations was dependent on the medium, highlighting the important role of biological transformation in acetate ester production.

3.4.2. Fatty Acids Ethyl Esters and Fatty Acids

Four fatty acids, isobutyric acid, isovaleric acid, hexanoic acid, and octanoic acid, ranged from 0.52 to 6.0 mg L^{-1} in inoculated groups (Table 3), similar to concentrations found previously in Pinot noir wines [41,42]. $C_{6–10}$ volatile fatty acids can contribute to cheese, sweaty, and rancid odors at high levels, whereas levels below their sensory thresholds can elevate overall complexity [47]. EC and MSPC wines contained comparable levels of volatile fatty acids, suggesting that the wild yeasts were unlikely to have had a negative sensory impact on the wines.

Medium-chain fatty acids (MCFAs), hexanoic (C_6), octanoic (C_8), and decanoic acid (C_{10}), are directly associated with the fermentative metabolism of yeasts [48]. MCFAs were found in the SN group of wines (Table 3). MCFAs and their ethyl esters have been experimentally verified to be produced during fermentation in a lipid-free synthetic medium [49]. Fatty acids and their ethyl esters showed close concurrence. For instance, two fatty acids selected by Mardia's criterion, isovaleric acid and isobutyric acid, were closely positioned with their ethyl ester products, ethyl isovalerate and ethyl isobutyrate

(Figure 2b). Although the fermentation treatments had similar impacts on fatty acids and their ethyl ester derivatives, fatty acids were much higher in concentration than their ethyl ester products, in line with a previous report [50]. However, the fatty acids did not exhibit the same trends in concentration as their ethyl esters (Table 3). For instance, decanoic acid concentration was low in the SN group but its ethyl ester product, ethyl decanoate, was found at relatively high concentrations in the same wines (Table 3). Diaz-Maroto et al. [50] also found that ethyl ester concentrations were independent of the corresponding fatty acid levels. At the end of fermentation, esterification molar ratios have been reported to be low and quite different from each other [50], consistent with the quantities of the fatty acids and ethyl esters in this study. The rate of ethyl ester formation is generally slow and can take years to reach equilibrium [50]. As a result, the quantities of the ethyl ester in young wines should be primarily dependent on the forward reaction rate of the esterification, instead of the equilibrium constant. The rate of esterification might be affected by external factors including wine pH, the temperature of fermentation and storage, and the intrinsic chemical mechanisms [50].

A few ethyl esters, such as ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, were produced in greater amounts in JN and SN, than their counterpart media containing grape skins (Table 3). Ethyl esters of fatty acids are formed from the esterification of ethanol with fatty acids, such as butyric acid and hexanoic acid, which are derived from the metabolism of amino acids [50]. The replacement of a certain portion of the liquid medium with grape solids may have reduced the total amino acid content of the medium [51], leading to a lower production of fatty acids and thus ethyl esters. For cinnamate esters, higher concentrations were obtained in samples produced from media containing grape material (JN, JS, and SS) that went through fermentation with either EC or MSPC (Table 3).

3.4.3. C_{13}-Norisoprenoids and Terpenes

C_{13}-norisoprenoids and terpenes share similar degradation pathways, including acid hydrolysis and enzymatic breakdown from their glycoside precursors into the volatile forms [6,7,52]; therefore, both grape and fermentation conditions, such as pH and yeasts, influence their quantities in wine [16,53]. C_{13}-norisprenoids are considered to be important for Pinot noir regional typicity [54], whereas terpenes appear to have enhancing or suppressing effects on Pinot noir aroma [1]. C_{13}-norisoprenoid and terpene concentrations were distinguished by both PC1 and PC2 (Figure 2b). Two C_{13}-norisoprenoids, α-ionone and β-ionone, and three terpenes, nerol, geraniol, and β-citronellol, clustered around the lower left-hand quadrant and were associated with fermentations containing grape skins. Overall, the concentrations of the three terpenes and three C_{13}-norisoprenoids selected by Mardia's criterion did not show the same trends across the fermentation samples, despite their similar degradation pathways (Figure 2b) [6,7]. JS wines had the highest terpene and ionone production amongst the four media types, followed by JN and SS (Table 3). As expected, SN wines were distinguished by the absence of C_{13}-norisoprenoids and terpenes, regardless of inoculation scheme, since SGM does not contain any precursors (Table 3). SS and JS resulted in generally higher quantities of C_{13}-norisoprenoids and terpenes than their analogs without added grape solids, confirming the strong link between the grape skin component and the release of free terpenes and C_{13}-norisoprenoids [8,53].

A negative association between no inoculation and the three C_{13}-norisoprenoids was also suggested by the long distance between them on the PCA (Figure 2b). The lowest concentration of C_{13}-norisoprenoids was also seen in uninoculated samples, showing that free C_{13}-norisoprenoids released during fermentation account for the majority of these volatiles in the final wines (Table 3). Previous reports have highlighted the positive correlation between yeast metabolic activities and α-ionone, β-damascenone, and β-ionone during fermentation [7]. In this study, MSPC generally produced more C_{13}-norisoprenoids, particularly the two ionones, than EC1118 and no inoculation. *H. uvarum* has previously been reported to have higher β-glucosidase activity than other yeasts, such as *Metschnikowia pul-*

cherrima, *Kluyveromyces thermotolerans*, and *S. cerevisiae* [16,55,56]. Therefore, the *H. uvarum* yeast of the MSPC yeast population may have high β-glucosidase activity, explaining the greater production of C_{13}-norisoprenoids in MSPC wines [46].

A positive correlation between the two ionone isomer concentrations was seen (Figure 2b), suggesting that the effects of two fixed factors, fermentation medium and inoculum, on the ionone concentrations were similar. Concentrations of α-ionone and β-ionone have previously been shown to correlate in other wine varieties [57,58], suggesting that the ionone isomers might be closely associated in terms of their formation pathways. β-Ionone is the oxidative cleavage product of β-carotene, while δ-carotene can give rise to α-ionone and 6,10-dimethyl-3,5,9-undecatrien-2-one (pseudoionone) [59]. Although α-ionone and β-ionone are not derived from the same carotenoid substrate, these two C_{13} compounds are formed from their corresponding carotenoid substrates, both cyclic in chemical structure, via the symmetrical enzymatic cleavage at the 9,10 (9′,10′) positions of the substrate molecules [59]. It could be hypothesized that the quantities of the two ionones, including both glycoside and free forms, are proportionally related in the grape medium. Ionone glycosides can be effectively hydrolyzed into free forms at wine conditions, for example, 90–100% of the hydrolysis of glycosidically bound compounds via enzymatic catalysis [60]. Thus, free ionones in wine could be primarily determined by their total quantities in the original medium.

3.4.4. C_6-Compounds

C_6-alcohols and C_6-aldehydes were mainly separated by PC2, thus highlighting the significant impact of fermentation medium on C_6 compound concentrations (Figure 2b). The origin of C_6-alcohols and C_6-aldehydes in wine can be traced back to the lipids in grape skins and seeds, cleaved by grape berry endogenous enzymes [61,62]. C_6-compounds found in SN samples could be linked to the long-chain unsaturated fatty acid compositions, principally oleic acid (70%) and linoleic, palmitic, and stearic acids (30%) found in Tween 80 [63], a component in the SGM (Table S1). The occurrence of C_6-aldehydes and the corresponding alcohols has previously been reported in chemically defined media fermented by *S. cerevisiae* yeasts [64,65], where long-chain unsaturated fatty acids, such as linoleic acid, were considered to be precursors for the biosynthesis of C_6-compounds during fermentation [61,62]. SN wines had high levels of *trans*-3-hexen-1-ol and *cis*-3-hexen-1-ol, above all three other medium groups (Table 3). The yeast impact on C_6-compound levels was highly dependent on the fermentation medium (Table 3), where both long-chain unsaturated fatty acids in the fermentation medium and yeast metabolic activities had impacts on the production of C_6-compounds, in line with observations in previous reports [64,65].

4. Conclusions

The volatile concentrations quantitated via HS-SPME coupled with GC-MS was used to characterize the aroma production of SGM and Pinot noir wines, made with or without skins, while comparing volatile formation between a commercial yeast strain, a mixed population from a Pinot noir vineyard, and non-inoculated (mostly wild). Remarkably, SGM plus skins produced a very similar aroma profile to Pinot noir juice and skins, with C_{13}-norisoprenoids, terpenes, and cinnamates detected at levels comparable to that of fermentations in grape must. However, SGM plus grape skins is not chemically defined, hence a drawback of this medium is that the volatile release will still be influenced by the composition of the added skins, which will vary across different batches. Future work should explore the application of spent grape skins, from fermentations with high quality Pinot noir grapes, to ferments with lower quality grapes to enhance the overall quality of the aroma profile, as well as wine color stability. The MSPC indigenous yeasts performed well during fermentation and produced aromas that were comparable to EC1118 in all but the Pinot noir juice medium. Marked increases of desirable compounds in Pinot noir, including C_{13}-norisoprenoids and esters, were seen in the wines produced by the MSPC co-cultures. Native yeasts may add regional distinctiveness to finished wines and can produce

more aromatic compounds important to Pinot noir wine quality. From the perspective of the wine industry, the application of indigenous yeasts, as well as the recycling of high-quality grape skins, to the production of Pinot noir are options that may have great promise for securing Pinot noir quality with confidence.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fermentation7030168/s1, Table S1: Formulation of synthetic grape must (SGM) simulating Pinot noir grape juice, Table S2: Ions and retention times (RTs) used to identify aroma compounds and internal standards, Table S3: Species identities of five MSPC isolates (from 96-well positions A3, B3, C3, G2 and H2) selected for Sanger sequencing to represent the three different cohorts identified for MSPC from restriction fragment length polymorphisms (RFLPs) of the ITS1-5.8S rDNA-ITS2 region, Table S4: Microsatellite genotypes of the 19 *S. cerevisiae* isolates from the MSPC community, Figure S1: title, Cohort determination of the MSPC single vineyard community using the amplification of the ITS1-5.8S rDNA-ITS2 region, followed by RFLP analysis using *Hinf*I (A) and *Hae*III (B), Figure S2: Cumulative weight loss (g) over time (h) of fermentations in Pinot noir juice without grape skins (JN), Figure S3: Cumulative weight loss (g) over time (h) of fermentations in Pinot noir juice with grape skins (JS), Figure S4: Cumulative weight loss (g) over time (h) of fermentations in synthetic grape medium (SGM) without grape skins (SN), Figure S5: Cumulative weight loss (g) over time (h) of fermentations in synthetic grape medium (SGM) with grape skins (SS).

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