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Impact of Various Prefermentation Treatments on the Pigment, Polyphenol, and Volatile Composition of Industrial Red Wines Made from *Vitis vinifera* cv Maratheftiko

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Abstract: The grape variety Maratheftiko (*V. vinifera* sp.) is one of the most prestigious Cypriot varieties, yet Maratheftiko wines are rather poorly studied with regard to principal quality characteristics, such as the polyphenolic and aromatic profile. The current study was undertaken with the scope to investigate the effect of various common industrial prefermentation treatments on the non-volatile and volatile fractions of Maratheftiko wines, in two consecutive harvests. Different lots of grapes underwent the *saigneé* process, which was also combined with cold maceration, and/or enzyme and tannin addition, and the wines produced on industrial scale were analyzed to portray their profiles of anthocyanins, non-anthocyanin polyphenols, and volatiles. The non-anthocyanin composition was found to be dominated by flavonol glycosides (mainly quercetin 3-*O*-glucuronide), but also caftaric acid. The major anthocyanin pigment in all wines examined was malvidin 3-*O*-glucoside, accompanied by its *p*-coumarate derivative. The principal volatiles determined were isoamyl alcohol, ethyl octanoate, 2-phenylethanol, ethyl caprate, and isoamyl acetate. Principal component analysis revealed that the wines could be clearly discriminated based on vintage year but not based on the different treatments. This study offered novel insights into the composition of Maratheftiko wines and provided some evidence regarding the impact of common enological techniques on their non-volatile and volatile fractions.

Keywords: anthocyanins; Cyprus; grapes; polyphenols; *Vitis vinifera*; volatiles; wines

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1. Introduction

Wine quality is a particularly complex notion, determined by a multitude of physicochemical properties and interactions thereof, such as the alcoholic concentration, the presence of sugars, the pH and acidity, as well as the profile of non-volatile and volatile constituents. All these variables contribute to defining the sensory characteristics of a wine, its stability, ageing potential, and, eventually, its marketability [1–3]. These parameters can be largely affected by genetic (varietal) factors (grape variety) and environmental factors (soil, weather), but they are also intentionally crafted by implementing specific winemaking techniques [4–6].

Volatile aromatic and polyphenolic substances are two of the most significant categories of wine constituents that profoundly affect the sensory attributes and the overall quality [2]. Aroma compounds can derive from the raw material (grapes), prefermentation techniques (carbonic maceration), fermentation (yeast metabolism), and wood-based ageing (barrels or wooden chips), and they belong to several classes, including acids, terpenes, aldehydes, alcohols, esters, ketones, etc. [7,8]. Polyphenolics occur in grapes, mainly in

the skin and seeds, and they are largely extracted during maceration, which is a common technique in red wine vinification. Polyphenols are subdivided into various classes, including simple phenolic acids and flavonoids, and a spectrum of organoleptic properties is ascribed to them, such as color (anthocyanins), astringency (condensed tannins), and mouthfeel [5,9].

In red wine production, maceration is a fundamental process, and the conditions under which it takes place (temperature, time) can influence wine style, quality, and aging period. During maceration, grape compounds diffuse into the fermenting must, enriching the final product with a variety of polyphenols and aromas, and the application of various maceration protocols may provide a directed enrichment by regulating key variables. On this ground, numerous treatments have been studied to provoke selective effects on the extraction of important grape constituents, such as cold-soak prefermentative maceration [10], extended maceration [11], enzyme addition [12], *saignée* [13–15], and combinations thereof.

However, each grape variety possesses peculiar characteristics, and therefore the most appropriate vinification technique to bring out varietal potential is a subject of case experimentation. In this regard, the effect of a given treatment on the wine produced from a given variety merits a thorough investigation. In fact, there is a rather limited number of studies describing the effects of various prefermentation treatments on the quality of wines produced from a specific variety, whereas the appraisal of their efficiency and suitability often relies on the comparison of outcomes from different sources, which could be misleading. Furthermore, studies pertaining to the examination of the effect of prefermentation treatments on both the volatile and non-volatile fractions are quite scarce.

Several native varieties occur in the Cypriot vineyard, yielding quality wines, yet their actual enological potential is largely unexamined [16]. Specifically for the variety Maratheftiko, although it is empirically praised for its quality, data on the analytical aromatic and polyphenolic profiles are limited [17–20]. Moreover, the effect of prefermentation treatments has never been reported for the industrial production of wines from Maratheftiko. Considering all the above, this study had as objectives (i) to perform various prefermentation treatments on must obtained from Maratheftiko grapes and (ii) assess the quality characteristics pertaining to the volatile and polyphenolic composition of the wines produced on an industrial scale. As far as the authors are aware, this is the first examination of the vinification of Maratheftiko grapes and provides unprecedented information with obvious industrial value and prospects.

2. Materials and Methods

2.1. Chemicals and Reagents

Catechin (>98%), *trans*-caffeic acid (≥98%), cyanin chloride (≥90%), quercetin 3-*O*-glucuronide (≥95%), quercetin 3-*O*-galactoside (≥97%), 2-octanol, quercetin (≥95%), and rutin (quercetin 3-*O*-rutinoside) (>94%) were obtained from Sigma-Aldrich (Darmstadt, Germany). All solvents used for chromatography were HPLC grade.

2.2. Grape Handling, Treatments, and Vinification

The grapes used were from the native Cypriot *Vitis vinifera* variety Maratheftiko, harvested at technological maturity. All details concerning vineyard location, cultivation, climatic conditions, and technological attributes of the grapes used have been previously reported [16]. Treatments were applied for two consecutive harvests in 2021 and 2022, and the technological characteristics of the grapes, determined by standard enological analyses according to the OIV protocols [21], are given in Table 1.

Table 1. Enological characteristics of the Maratheftiko musts used for the vinifications.

Parameter	Harvest	
	2021	**2022**
Total acidity (g TAE L^{-1}) *	5.89 ± 0.2	6.53 ± 0.3
pH	3.27 ± 0.01	3.30 ± 0.01
Density (g mL^{-1})	1.095 ± 0.001	1.105 ± 0.001
Potential alcoholic title (% *v*/*v*)	13.0 ± 0.1	14.7 ± 0.2
Reducing sugar concentration (g L^{-1})	222 ± 3	251 ± 4

* TAE stands for "tartaric acid equivalents".

Vinifications were performed in stainless steel tanks, and the treatments tested were applied after typical grape processing, including destemming, crushing, and mashing. Mash sulfiting was performed at a final level of 2 g hL^{-1} free SO_2. An overview of the treatments performed is illustrated in Figure 1. The pre-fermentation treatments tested were as follows:

- Treatment C (control)—Fermentation of must was performed in contact with grape pomace for 11 days. Fermentation temperature was maintained at 13–17 °C.
- Treatment CE—Cryoextraction was applied prior to fermentation at 5 °C for 48 h, followed by *saigneé*. The latter technique consisted of removing from the mash a volume of must corresponding to 10% of the total volume. After racking off this volume of must, vinification was carried out as for treatment C.
- Treatment CEE—Cryoextraction was applied prior to fermentation at 5 °C for 48 h, followed by *saigneé* and pectolytic enzyme addition. The enzyme added was a pectolytic preparation (Laffasse HE Grand Cru, LAFFORT OENOLOGIE, Bordeaux, France) and added at a level of 2 g per 100 kg of grapes, according to the manufacturer's specifications. After enzyme addition, vinification was carried out as for treatment C.
- Treatment CEET—Cryoextraction was applied prior to fermentation at 5 °C for 48 h, followed by *saigneé*, pectolytic enzyme addition, and enological tannin addition. The enological tannins used were SUBLITAN VINIF (Martin Vialatte, Thévenet, France), and added at a level of 20 g hL^{-1}. After additions, vinification was carried out as for treatment C.

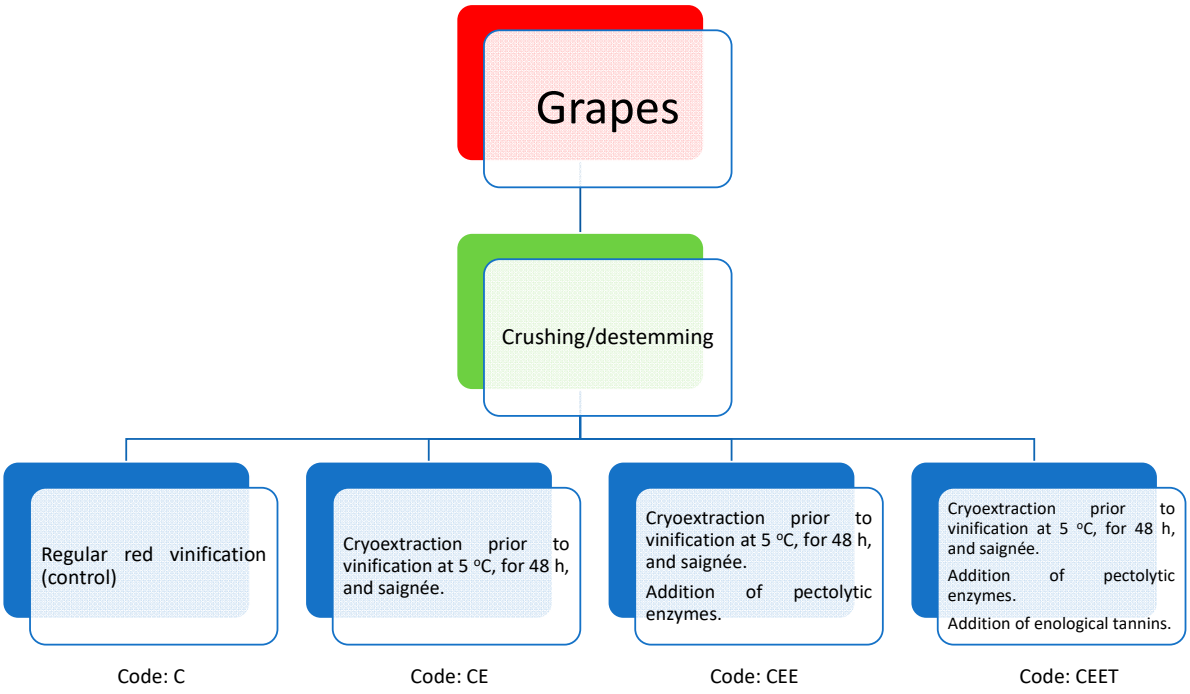

Figure 1. Overview of the prefermentation treatments carried out.

In all cases, mash inoculation was conducted with *S. cerevisiae* (Vitilevure, France) at a level of 15 g hL^{-1}. Pressing was performed with an identical pressing regime, using a hydraulic basket press with a total capacity of 120 L. Vinification was completed by racking off the free-run wines and blending them with the first-press wines (0.8 bars). Sulfite levels were continuously monitored, and, whenever necessary, they were corrected to 20 mg L^{-1} free SO_2. The technological characteristics of the wines produced were determined by standard enological analyses according to the OIV protocols and presented in Table 2.

Table 2. Enological characteristics of the wines produced after implementing the various prefermentation treatments. C, control, CE, cryoextraction and *saignée*; CEE, cryoextraction, *saignée* and enzyme addition; CEET, cryoextraction, *saignée* and enzyme and tannin addition.

Wine	*d* (g mL^{-1})	Alcohol Content (% *v*/*v*)	Titratable Acidity (g L^{-1})	Volatile Acidity (g L^{-1})	pH
Harvest 2021					
C	0.989 ± 0.001	13.1 ± 0.1	6.9 ± 0.2	0.47 ± 0.01	2.96 ± 0.01
CE	0.989 ± 0.001	12.6 ± 0.1	7.6 ± 0.2	0.52 ± 0.02	2.83 ± 0.02
CEE	0.989 ± 0.001	13.1 ± 0.1	7.7 ± 0.1	0.42 ± 0.01	2.70 ± 0.01
CEET	0.988 ± 0.002	13.3 ± 0.1	6.8 ± 0.1	0.52 ± 0.02	3.07 ± 0.02
Harvest 2022					
C	0.998 ± 0.001	15.5 ± 0.2	9.0 ± 0.1	0.49 ± 0.02	2.53 ± 0.01
CE	0.985 ± 0.002	15.1 ± 0.1	7.6 ± 0.1	0.49 ± 0.01	2.87 ± 0.01
CEE	0.999 ± 0.001	13.9 ± 0.1	7.2 ± 0.1	0.57 ± 0.01	3.09 ± 0.02
CEET	0.989 ± 0.001	14.6 ± 0.1	7.7 ± 0.2	0.49 ± 0.01	2.86 ± 0.02

2.3. Sample Preparation and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The volatile profile of the wines investigated was examined using headspace solid-phase microextraction (HS-SPME) by implementing a previously published methodology [22]. An SPME fiber with divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) coating was used (Supelco, Bellefonte, PA, USA), which was preconditioned at 270 °C for 30 min prior to use, according to the manufacturer's specifications. SPME was performed in a 25 mL glass vial on a wine sample (10 mL) containing 3 g of NaCl and spiked with 2-octanol (final concentration 2 mg L^{-1}) as an internal standard. The vial was tightly sealed with a PTFE/silicone septum and maintained at a constant temperature of 40 °C in a water bath, for the whole duration of treatment (40 min extraction and 10 min equilibration), with the fiber being placed over the wine surface (headspace). In all cases, samples were under continuous stirring at 250 rpm.

After the extraction was complete, the fiber was removed from the vial, threaded through the needle, and placed into the injector of the gas chromatograph. GC-MS analysis was accomplished as previously described [22] with an Agilent Technologies (Folsom, CA, USA) gas chromatograph (model 7890A) equipped with a mass selective detector (model 5975C). Chromatography was performed on an Agilent J&W DB-1 (30 m × 320 µm × 0.25 µm) capillary column with helium carrier gas at a flow rate of 1.5 mL min^{-1}. The injector was set in splitless mode at an operating temperature of 240 °C. The column was initially maintained at 40 °C for 5 min, and then heated to 140 °C at a rate of 2 °C min^{-1}. Finally, the column was heated at 240 °C for 10 min, at a rate of 10 °C min^{-1}. The total run time of the analysis was 75 min.

Mass spectrometer settings were source temperature 230 °C, mass range (m/z) 29–350, quadrupole temperature 150 °C, and electron impact acquisition mode (69.9 eV). Compound identification was based on comparing the mass spectra acquired with electron impact mass spectrum libraries NIST11 (NIST, Gaithersburg, MD, USA) and W8N08 (John Wiley & Sons, Inc., Hoboken, NJ, USA) using Agilent Technologies (Folsom, CA, USA) MSD Chemstation software (ver. E.02.00.493). Sample composition was determined from the GC peak regions (without correction factors) by applying the normalization approach,

and mean values from repeated injections were used to calculate the concentration of the compounds detected, which were expressed as mg of 2-octanol equivalents per L of wine.

2.4. Liquid Chromatography Determinations

The tentative identification of *trans*-caftaric acid (caffeoyltartaric acid), a *p*-coumaric acid derivative, and a ferulic acid derivative was accomplished with liquid chromatography-diode array-mass spectrometry (LC-DAD-MS), using the methodology previously described [23]. Similarly, major anthocyanin pigments were tentatively identified using another published methodology [24]. For all non-pigment polyphenols and anthocyanins, quantitative analyses were carried out with external standards using calibration curves ($R^2 > 0.999$) constructed with solutions of concentrations varying from 0 to 50 μg mL^{-1}. *trans*-caftaric acid was quantified as caffeic acid, and the *p*-coumaric acid and ferulic acid derivatives as *p*-coumaric acid and ferulic acid, respectively. All anthocyanins were quantified as cyanin chloride, and the results reported were expressed as cyanin equivalents. Standard solutions were prepared in HPLC-grade methanol shortly prior to analysis.

2.5. Statistical Processing

For volatile analysis, solid-phase microextractions were repeated twice, and GC-MS determinations were performed in triplicate. Polyphenol analyses were performed with the direct injection of wine, also in triplicate. The values were presented as the average ± standard deviation. All calibration curves were computed with linear regression using SigmaPlot™ 12.5 (Systat Software Inc., San Jose, CA, USA). Examination of the normality of the data was performed with the Shapiro–Wilk test. Statistically significant differences were examined with the Kruskal–Wallis test using IBM SPSS Statistics™ 29 (SPSS Inc., Chicago, IL, USA), considering that the data were not normally distributed. Principal component analysis (PCA) was plotted using the online web tool, SRplot (http://www.bioinformatics.com.cn/srplot, accessed on 1 March 2024).

3. Results and Discussion

3.1. Impact of Treatments on Non-Pigment Polyphenols

Typical chromatograms depicting the non-pigment polyphenolic profile of the wines examined are given in Figure 2. In total, eight major compounds could be tentatively identified and quantified, and the results are analytically presented in Table 3. In terms of concentration, quercetin 3-*O*-glucuronide was the predominant constituent, followed by caftaric acid, catechin, the ferulic acid derivative, and rutin (quercetin 3-*O*-rutinoside). This pattern was the same irrespective of the vintage year and the treatment applied.

For quercetin 3-*O*-glucuronide, any treatment performed on the 2021 vintage resulted in significantly lower levels, particularly for the wine produced with the CE treatment. By contrast, in 2022, the vintage CE treatment gave the highest concentration, although it was not statistically different from the control. Regarding caftaric acid, for both vintages 2021 and 2022, control wines had the highest concentration, and no statistical difference was observed between the two years. The same was seen for the treatments CE and CEET, whereas the samples treated with CEE had significantly different concentrations. In this case too, any treatment implemented had a negative impact on caftaric acid concentration. A similar effect was recorded for the ferulic acid derivative, but the effect of different treatments was diversified between the two vintages. To the contrary, catechin concentration was exceptionally enhanced by the CE treatment for the 2022 vintage, but all the other treatments had a negative outcome. On the other hand, the CEE treatment had a positive impact on quercetin concentration, which was almost doubled compared to the control in the 2022 wines.

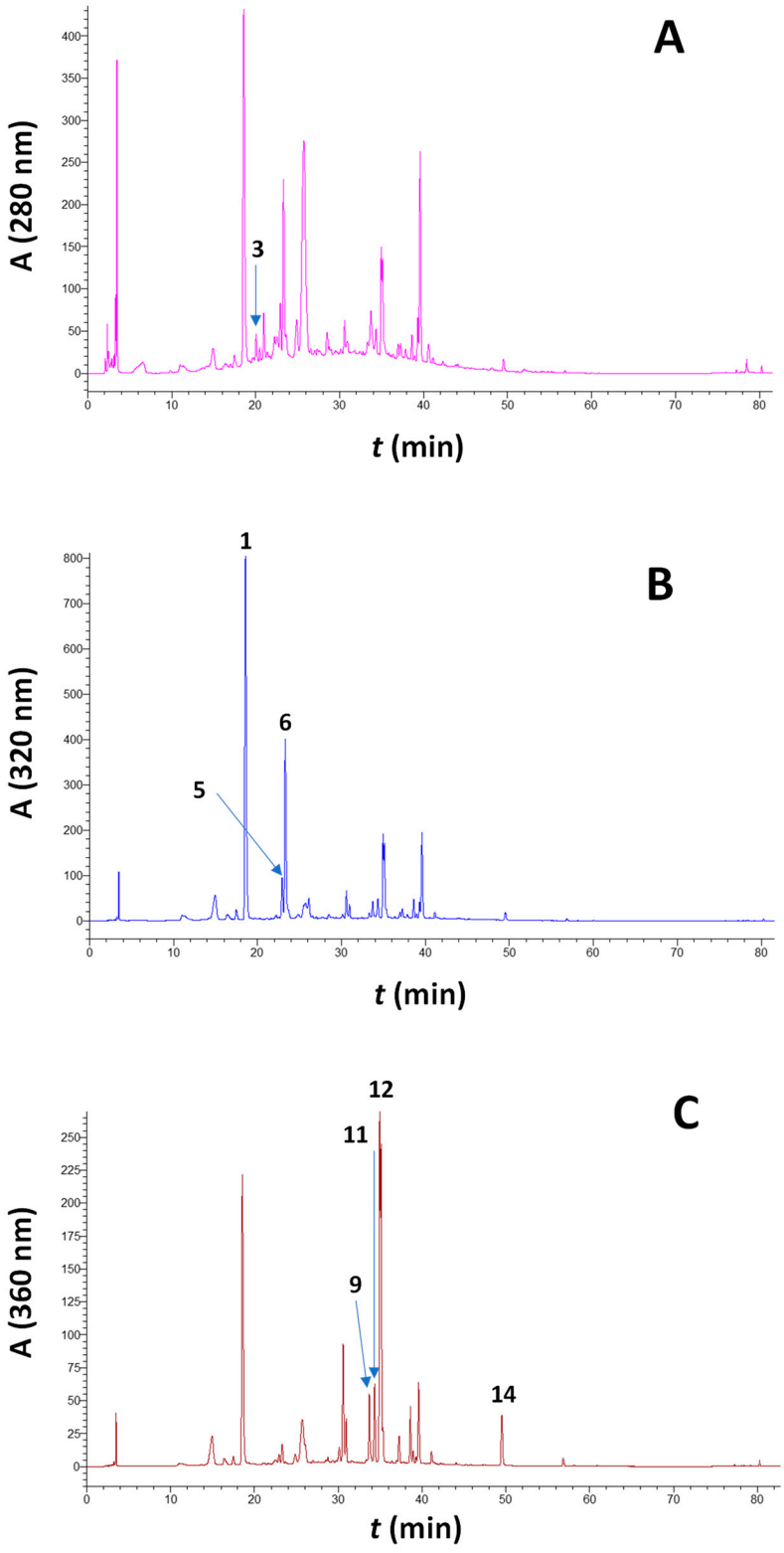

Figure 2. Typical chromatograms of a Maratheftiko wine (control) recorded at 280 (**A**), 320 (**B**) and 360 nm (**C**). Peak assignments: 1, caftaric acid; 3, catechin; 5, *p*-coumaric acid derivative; 6, ferulic acid derivative; 9, rutin; 11, quercetin 3-*O*-galactoside; 12, quercetin 3-*O*-glucuronide; 14, quercetin.

Table 3. Quantitative data on the polyphenolic composition of the wines produced after implementing the various prefermentation treatments. The values given are means of triplicate determination ± standard deviation. C, control, CE, cryoextraction and *saignée*; CEE, cryoextraction, *saignée* and enzyme addition; CEET, cryoextraction, *saignée* and enzyme and tannin addition.

Compound	Year 2021				Year 2022			
	C	CE	CEE	CEET	C	CE	CEE	CEET
Non-pigment polyphenols								
Caftaric acid	87.75 ± 2.24 ^{a}	60.61 ± 2.81 ^{b}	74.81 ± 2.61 ^{c}	76.90 ± 3.46 ^{c}	90.12 ± 5.66 ^{a}	62.14 ± 3.19 ^{b}	54.93 ± 2.58 ^{d}	76.63 ± 5.69 ^{c}
Catechin	53.48 ± 1.22 ^{a}	35.11 ± 0.56 ^{b}	45.63 ± 1.82 ^{c}	29.98 ± 0.86 ^{d}	47.23 ± 1.06 ^{c}	81.86 ± 3.42 ^{e}	25.29 ± 1.03 ^{f}	56.50 ± 4.12 ^{a}
p-coumaric acid derivative	8.51 ± 0.18 ^{a}	6.00 ± 0.11 ^{b}	7.34 ± 0.32 ^{c}	8.00 ± 0.21 ^{a}	8.62 ± 0.45 ^{a}	6.72 ± 0.11 ^{d}	5.54 ± 0.44 ^{e}	7.26 ± 0.52 ^{c}
Ferulic acid derivative	48.71 ± 2.21 ^{a}	29.21 ± 0.87 ^{b}	41.70 ± 0.96 ^{c}	39.69 ± 0.82 ^{c}	49.13 ± 1.06 ^{a}	35.39 ± 1.43 ^{d}	21.87 ± 0.85 ^{e}	36.98 ± 0.88 ^{d}
Rutin	29.09 ± 1.04 ^{a}	13.16 ± 0.35 ^{b}	24.90 ± 0.24 ^{c}	18.69 ± 0.44 ^{d}	12.32 ± 0.67 ^{b}	14.12 ± 0.98 ^{b}	2.52 ± 0.10 ^{e}	8.15 ± 0.32 ^{f}
Quercetin 3-*O*-galactoside	13.92 ± 0.65 ^{a}	6.71 ± 0.23 ^{b}	11.81 ± 0.64 ^{c}	9.22 ± 0.13 ^{d}	9.81 ± 0.84 $^{d, e}$	10.79 ± 0.32 ^{e}	7.32 ± 0.47 ^{b}	8.43 ± 0.44 ^{f}
Quercetin 3-*O*-glucuronide	156.64 ± 7.40 ^{a}	75.69 ± 1.97 ^{b}	133.20 ± 2.47 ^{c}	97.07 ± 3.56 ^{d}	121.17 ± 9.43 ^{c}	130.31 ± 6.44 ^{c}	103.00 ± 5.66 ^{d}	111.93 ± 6.56 ^{d}
Quercetin	4.00 ± 0.24 ^{a}	2.11 ± 0.05 ^{b}	3.34 ± 0.09 ^{c}	2.39 ± 0.06 ^{d}	4.95 ± 0.19 ^{e}	4.21 ± 0.10 ^{a}	9.12 ± 0.52 ^{f}	8.56 ± 0.49 ^{f}
Total	402.11	228.63	342.73	281.94	343.35	345.54	229.59	314.44

Values denoted with superscripted letters (a, b, c, d, e, f) within rows are statistically different ($p < 0.05$).

Considering all the above, it could be argued that substances including caftaric acid, *p*-coumaric acid, and ferulic acid derivatives exhibited insignificant variations between the two vintages for the control wines, but their concentration was negatively affected by any treatment applied. A similar phenomenon was observed for catechin, but for flavonols, there were significant differences between vintages and treatments. Previous studies showed that treatments such as cold soaking did not provoke important changes in phenolic acid, catechin, and flavonol concentrations [25]. On the other hand, cold maceration and *saigneé* treatments were demonstrated to have a differentiated influence on polyphenols. Thus, while there was no benefit for fertaric and coutaric acids and quercetin glucuronide, a positive effect on catechin concentration was seen [14]. Other authors showed that the application of *saigneé* favored the increase in concentration of substances such as caftaric acid, quercetin 3-*O*-glucuronide, and quercetin [15]. To the contrary, *saigneé* had no significant effect on catechin and flavonols such as quercetin 3-*O*-glucuronide, quercetin 3-*O*-galactoside, and quercetin, but only rutin [26]. Likewise, *saigneé* treatment had no effect on caftaric acid, catechin, or quercetin [27]. Furthermore, more recent studies demonstrated a high benefit from the addition of enzymes for flavonols, but for flavanols, no statistically significant difference was found, whereas an important decrease was recorded for phenolic acids [28].

3.2. Impact of Treatments on Anthocyanins

In total, six principal anthocyanin pigments were tentatively identified and quantified (Figure 3) in the wines studied. Irrespective of the vintage year and treatment, malvidin 3-*O*-glucoside was by far the predominant pigment, followed by its *p*-coumarate derivative and paeonidin 3-*O*-glucoside (Table 4). This finding was in accordance with a previous investigation on wines made from Maratheftiko [19].

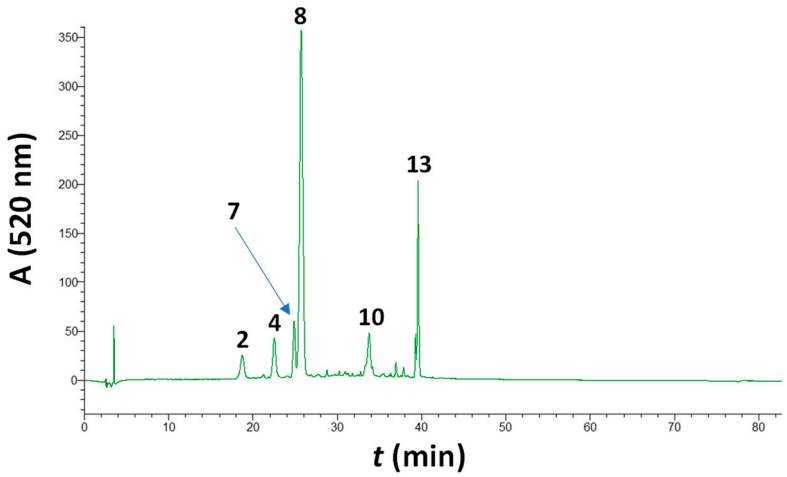

Figure 3. Typical anthocyanin pigment trace of a Maratheftiko wine (control) recorded at 520 nm. Peak assignments: 2, delphinidin 3-*O*-glucoside; 4, petunidin 3-*O*-glucoside; 7, paeonidin 3-*O*-glucoside; 8, malvidin 3-*O*-glucoside; 10, malvidin 3-*O*-glucoside acetate;13, malvidin 3-*O*-glucoside *p*-coumarate.

Table 4. Quantitative data on the polyphenolic composition of the wines produced after implementing the various prefermentation treatments. The values given are means of duplicate determination ± standard deviation. C, control, CE, cryoextraction and *saignée*; CEE, cryoextraction, *saignée* and enzyme addition; CEET, cryoextraction, *saignée* and enzyme and tannin addition.

Compound	Year 2021				Year 2022			
	C	CE	CEE	CEET	C	CE	CEE	CEET
Anthocyanin pigments								
Delphinidin 3-*O*-glucoside	49.34 ± 1.25 ^{a}	18.94 ± 0.58 ^{b}	41.97 ± 1.09 ^{c}	29.13 ± 1.10 ^{d}	63.37 ± 2.83 ^{e}	57.94 ± 1.56 ^{f}	23.43 ± 1.06 ^{g}	57.37 ± 2.22 ^{f}
Petunidin 3-*O*-glucoside	68.93 ± 2.33 ^{a}	32.59 ± 0.94 ^{b}	56.41 ± 1.98 ^{c}	39.11 ± 1.23 ^{d}	90.08 ± 4.00 ^{e}	83.55 ± 2.83 ^{e}	31.08 ± 1.88 ^{b}	70.40 ± 2.69 ^{a}
Paeonidin 3-*O*-glucoside	106.83 ± 3.21 ^{a}	32.45 ± 1.03 ^{b}	89.73 ± 3.33 ^{c}	90.17 ± 2.34 ^{c}	95.87 ± 6.01 ^{c}	143.74 ± 7.77 ^{d}	43.17 ± 2.44 ^{e}	101.06 ± 6.31 ^{a}
Malvidin 3-*O*-glucoside	750.74 ± 14.32 ^{a}	438.29 ± 12.33 ^{b}	631.71 ± 22.11 ^{c}	520.02 ± 12.89 ^{d}	863.52 ± 43.02 ^{e}	928.07 ± 56.04 ^{e}	222.05 ± 12.09 ^{f}	600.00 ± 17.44 ^{c}
Malvidin 3-*O*-glucoside acetate	44.88 ± 1.06 ^{a}	35.08 ± 0.86 ^{b}	36.96 ± 1.55 ^{b}	33.16 ± 0.98 ^{c}	58.40 ± 1.22 ^{d}	61.05 ± 1.99 ^{e}	12.78 ± 0.74 ^{f}	34.85 ± 1.58 ^{b}
Malvidin 3-*O*-glucoside *p*-coumarate	177.01 ± 9.58 ^{a}	114.43 ± 2.69 ^{b}	144.46 ± 3.62 ^{c}	98.13 ± 2.93 ^{d}	194.99 ± 11.53 ^{e}	239.15 ± 11.05 ^{f}	27.68 ± 1.11 ^{g}	128.69 ± 7.56 ^{h}
Total	1197.73	671.78	1001.24	809.72	1366.23	1513.5	360.19	992.37

Values denoted with superscripted letters (a, b, c, d, e, f, g, h) within rows are statistically different ($p < 0.05$).

For the vinification of 2021, any prefermentation treatment deployed resulted in decreased anthocyanin concentration, the most pronounced being CE, which afforded an almost 44% reduction in total anthocyanin concentration. The use of pectolytic enzymes (treatment CEE) resulted in a higher anthocyanin concentration, and compared to the control, the decrease observed was more limited (16%). The addition of enological tannins (treatment CEET) also contributed to a lower anthocyanin concentration, which, compared to the control, was almost 32% lower. To the contrary, the results obtained for the 2022 vinification demonstrated that the application of CE treatment had a positive and statistically significant effect on paeonidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, malvidin 3-*O*-glucoside acetate, and malvidin 3-*O*-glucoside *p*-coumarate (Table 4). Overall, the increase found in the total anthocyanin concentration compared to the control was 8.7%.

On the other hand, similarly to what was observed for the 2021 vintage, the treatments CEE and CEET resulted in 66 and 24% lower anthocyanin concentrations, respectively.

The effects of prefermentation maceration on anthocyanins are an issue that has been tackled by early studies, which showed a rather controversial influence on the individual pigments, depending on temperature and treatment length [29]. More recent examinations revealed that prefermentative cold soaking had a rather negligible effect on anthocyanin concentration [25], while other studies showed either a slight [30] or a more advanced decrease in major anthocyanin concentration upon prefermentation soaking [31]. Results drawn by the application of the *saigneé* technique were in line, revealing a significant decrease in all principal anthocyanins compared to the control [14], while other studies showed no statistical difference in anthocyanin concentration between the control and the *saigneé*-derived wine [26,27].

By contrast, other recent investigations suggested a statistically significant increase in principal pigments and total anthocyanin concentration upon prefermentation cold soaking followed by extended (>10 days) maceration [15,32]. The addition of pectolytic enzymes has also been shown to exert a beneficial effect with regard to increasing anthocyanin concentration in combination with cold maceration [33]. However, in spite of the temporal increase in anthocyanin concentration in must, other authors reported no significant difference between the control wine and the enzyme-treated one after the end of fermentation [28]. The rather non-significant influence of enzyme addition has also been observed by earlier investigations [34,35].

Regarding the effect of tannin addition, the effect recorded was a significant decrease in anthocyanin concentration, with the exception of paeonidin 3-*O*-glucoside for the 2022 vintage. Since there is a scarcity of relevant information available in the literature, the results obtained could be characterized as inconclusive. It is recommended that more thorough investigations be conducted to assess the actual effect of tannin addition on red wine quality.

3.3. Impact of Treatments on the Volatile Profile

In total, 22 volatiles could be reliably identified and quantified by GC-MS in the wines examined. From a quantitative perspective, the principal compounds were isoamyl alcohol, ethyl caprate, ethyl octanoate, 2-phenylethanol, and isoamyl acetate (Table 5). Some volatiles, such as nonanoic acid, β-damascenone, menthol, and nerol, were absent from all samples of the 2022 vintage, indicating a strong year-to-year variation. To the contrary, 3-methyl-3-heptanone, ethyl hexanoate, 2-phenylethanol, ethyl octanoate, 2-phenylethyl acetate, and ethyl caprate occurred in all wines and might be typical of Maratheftiko. On the other hand, isoamyl formate had the highest concentration in some samples, irrespective of the vintage, whereas in other samples, no existence of this substance was found. Other authors have reported 2-methyl propanol, 2-methyl butanol, and 3-methyl butanol as some of the major volatiles in Maratheftiko wines [18], but none of them were detected in the samples analyzed.

Based on the data presented in Table 5, it could be supported that the aromatic profile was mostly year-related and not treatment-related. All wines originating from the 2021 vintage displayed a richer volatile composition as opposed to those from the 2022 vintage, which were deprived of some substances. Considering the major compounds, the effect of the various prefermentation treatments implemented had a rather controversial outcome. Thus, while the control samples of both the 2021 and 2022 vintages were the richest in isoamyl alcohol, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl caprate, in the 2022 samples the concentration of isoamyl alcohol was shown to be boosted by the CEET treatment. The same was observed for 2-phenyl ethanol, menthol, and nonanoic acid in 2021 wines. Likewise, the CEE treatment favored only the formation of isoamyl formate in 2021 vintage and 5-methyl-3-heptanone and 2-phenylethanol in 2022. Based on these findings, no consistent trend regarding the influence of treatments could be detected, but in most cases, it was clear that control samples were more enriched in volatile compounds.

Table 5. Quantitative data on the volatile composition of the wines produced after implementing the various prefermentation treatments. The values given are means of duplicate determination ± standard deviation. C, control, CE, cryoextraction and *saignée*; CEE, cryoextraction, *saignée* and enzyme addition; CEET, cryoextraction, *saignée* and enzyme and tannin addition.

Compound	CAS Number	Retention Time (min)	Year 2021				Year 2022			
			C	CE	CEE	CEET	C	CE	CEE	CEET
Isoamyl alcohol	123-51-3	3.11	5.83 ± 0.98 ^{a}	4.46 ± 0.21 ^{b}	2.66 ± 0.09 ^{c}	2.82 ± 0.15 ^{c}	Nd ^{d}	2.83 ± 0.11 ^{c}	1.68 ± 0.09 ^{e}	3.45 ± 0.12 ^{f}
Isoamyl formate	110-45-2	3.60	Nd ^{a}	Nd ^{a}	5.46 ± 0.12 ^{b}	Nd ^{a}	8.43 ± 0.23 ^{c}	3.39 ± 0.13 ^{d}	4.01 ± 0.08 ^{e}	Nd ^{a}
1,3-butanediol	107-88-0	4.78	Nd ^{a}	Nd ^{a}	0.04 ± 0.00 ^{b}	Nd ^{a}	Nd ^{a}	Nd ^{a}	0.03 ± 0.00 ^{c}	Nd ^{a}
Isoamyl acetate	123-92-2	7.08	0.78 ± 0.01 ^{a}	0.58 ± 0.01 ^{b}	0.46 ± 0.01 ^{c}	0.52 ± 0.01 ^{d}	0.98 ± 0.03 ^{e}	Nd ^{f}	0.21 ± 0.00 ^{g}	0.37 ± 0.00 ^{h}
5-methyl-3-heptanone	541-85-5	10.01	0.16 ± 0.00 ^{a}	0.12 ± 0.00 ^{b}	0.08 ± 0.01 ^{c}	0.17 ± 0.00 ^{d}	0.06 ± 0.00 ^{e}	0.12 ± 0.01 ^{b}	0.13 ± 0.00 ^{f}	0.08 ± 0.00 ^{c}
Methoxy phenyloxime	1000222-86-6	10.48	Nd ^{a}	Nd ^{a}	0.08 ± 0.00 ^{b}	0.03 ± 0.00 ^{c}	0.09 ± 0.00 ^{d}	Nd ^{a}	Nd ^{a}	0.02 ± 0.00 ^{e}
Ethyl hexanoate	123-66-0	13.81	0.38 ± 0.01 ^{a}	0.11 ± 0.00 ^{b}	0.20 ± 0.00 ^{c}	0.11 ± 0.00 ^{b}	0.22 ± 0.00 ^{d}	0.14 ± 0.00 ^{e}	0.06 ± 0.00 ^{f}	0.09 ± 0.00 ^{g}
Hexyl acetate	142-92-7	14.69	0.04 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{c}	0.02 ± 0.00 ^{c}	0.03 ± 0.00 ^{d}	0.04 ± 0.00 ^{a}	Nd ^{e}	0.01 ± 0.00 ^{b}
D-limonene	5989-27-5	15.60	0.03 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	Nd ^{c}	Nd ^{c}	Nd ^{c}	Nd ^{c}	Nd ^{c}	0.01 ± 0.00 ^{b}
2-phenylethanol	60-12-8	20.49	1.36 ± 0.05 $^{a, b}$	1.29 ± 0.07 ^{a}	1.33 ± 0.08 $^{a, b}$	1.45 ± 0.05 ^{b}	0.49 ± 0.00 ^{c}	0.55 ± 0.01 ^{d}	0.67 ± 0.02 ^{e}	0.53 ± 0.00 ^{f}
Diethyl succinate	123-25-1	25.36	0.01 ± 0.00 ^{a}	Nd ^{b}	0.01 ± 0.00 ^{a}	Nd ^{b}	Nd ^{b}	Nd ^{b}	0.02 ± 0.00 ^{c}	0.01 ± 0.00 ^{a}
(±)-menthol	89-78-1	25.41	Nd ^{a}	0.06 ± 0.00 ^{b}	Nd ^{a}	1.70 ± 0.03 ^{c}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}
Ethyl octanoate	106-32-1	27.43	1.44 ± 0.01 ^{a}	0.72 ± 0.02 ^{b}	0.83 ± 0.03 ^{c}	0.74 ± 0.01 ^{b}	1.39 ± 0.03 ^{d}	0.51 ± 0.01 ^{e}	0.10 ± 0.00 ^{f}	0.28 ± 0.00 ^{g}
Octanoic acid	124-07-2	28.74	0.33 ± 0.02 ^{a}	0.22 ± 0.00 ^{b}	0.24 ± 0.01 ^{c}	0.23 ± 0.00 ^{c}	0.03 ± 0.00 ^{d}	0.01 ± 0.00 ^{e}	Nd ^{f}	0.07 ± 0.00 ^{g}
2-phenylethyl acetate	103-45-7	29.85	0.30 ± 0.00 ^{a}	4.24 ± 0.11 ^{b}	0.33 ± 0.01 ^{c}	0.58 ± 0.01 ^{d}	0.17 ± 0.00 ^{e}	0.19 ± 0.00 ^{f}	0.09 ± 0.00 ^{g}	0.12 ± 0.00 ^{h}
Nonanoic acid	112-05-0	34.35	0.05 ± 0.00 ^{a}	0.16 ± 0.01 ^{b}	0.14 ± 0.00 ^{c}	0.18 ± 0.00 ^{d}	Nd ^{e}	Nd ^{e}	Nd ^{e}	Nd ^{e}
β-damascenone	23726-93-4	38.37	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	Nd ^{b}	Nd ^{b}	Nd ^{b}	Nd ^{b}
Nerol	106-25-2	39.06	Nd ^{a}	0.01 ± 0.00 ^{b}	Nd ^{a}	0.01 ± 0.00 ^{b}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}
Ethyl 9-decenoate	67233-91-4	39.58	0.04 ± 0.00 ^{a}	0.29 ± 0.01 ^{b}	0.03 ± 0.00 ^{c}	0.16 ± 0.00 ^{d}	0.03 ± 0.00 ^{c}	Nd ^{e}	0.01 ± 0.00 ^{f}	0.02 ± 0.00 ^{g}
Ethyl caprate	110-38-3	40.53	1.46 ± 0.01 ^{a}	0.90 ± 0.02 ^{b}	1.09 ± 0.06 ^{c}	0.60 ± 0.01 ^{d}	1.16 ± 0.04 ^{c}	0.46 ± 0.01 ^{e}	0.05 ± 0.00 ^{f}	0.14 ± 0.00 ^{g}
Isoamyl octanoate	2035-99-6	43.41	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	Nd ^{c}	Nd ^{c}
Ethyl dodecanoate	106-33-2	52.80	0.13 ± 0.01 ^{a}	0.09 ± 0.00 ^{b}	0.11 ± 0.00 ^{c}	0.13 ± 0.00 ^{a}	0.10 ± 0.00 ^{d}	0.04 ± 0.00 ^{e}	Nd ^{f}	Nd ^{f}

Values denoted with superscripted letters (a, b, c, d, e, f, g, h) within rows are statistically different ($p < 0.05$).

3.4. Discrimination through Principal Component Analysis (PCA) and Multivariate Correlation Analysis (MCA)

To gain a better insight into the results and provide a more credible interpretation of the effect of vintage year and treatments, principal component analysis was carried out. The relevant plots were prepared with SRPlot [36], and they are displayed in Figures 4 and 5.

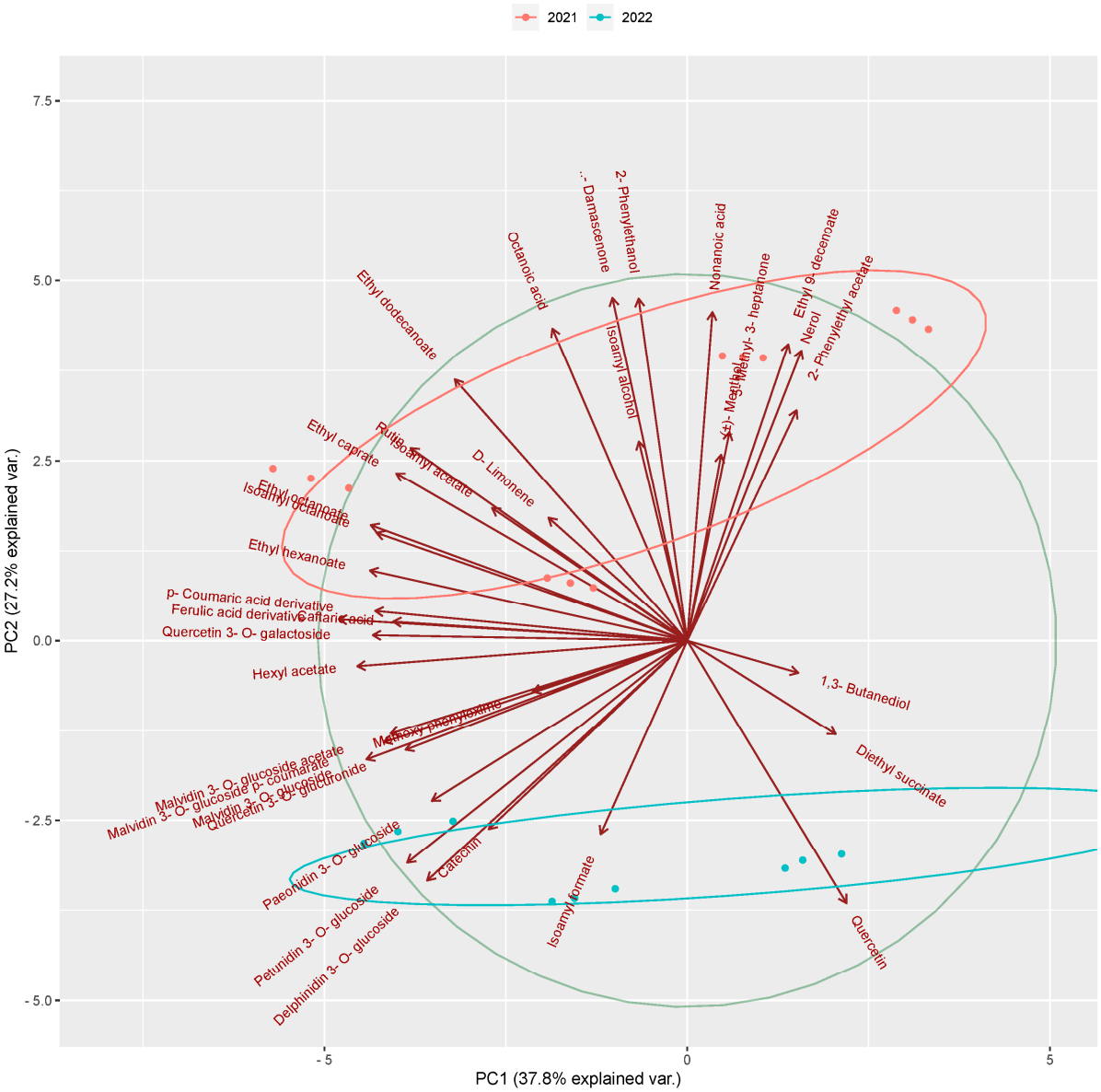

Figure 4. Projection of the wines produced by the various winemaking technologies tested, according to their polyphenolic and volatile composition, in the space defined by the first and second PCs. Vintage-based differentiation (eclipses define the 95% confidence intervals of year).

The plot explained 65% of the variance. The first principal component (PC1) accounted for 37.8% of the variance and was negatively correlated with most identified compounds (non-pigment polyphenols, anthocyanins, and volatile components). The second principal component (PC2) accounted for 27.2% of the variance. Most volatile compounds and non-pigment polyphenols were positively correlated with PC2, whereas anthocyanins were negatively correlated.

Based on the examined parameters, it can be seen that the samples were separated according to vintage year but not according to treatment. Wines from 2022 vintage clustered on the negative side of PC2, possibly due to their higher content of quercetin, as well as anthocyanins, compared to wines from 2021. On the contrary, wines from 2021 grouped on the positive side of PC1, probably due to their increased content of volatile components. As regards treatments, only control sample (C) could be separated from the treated ones, while no clear distinction could be seen amongst treatments. The overlap between the three examined treatments bespeaks that there are no differences between the wines derived

from the three treatments, in terms of polyphenols, anthocyanins, and volatile components.

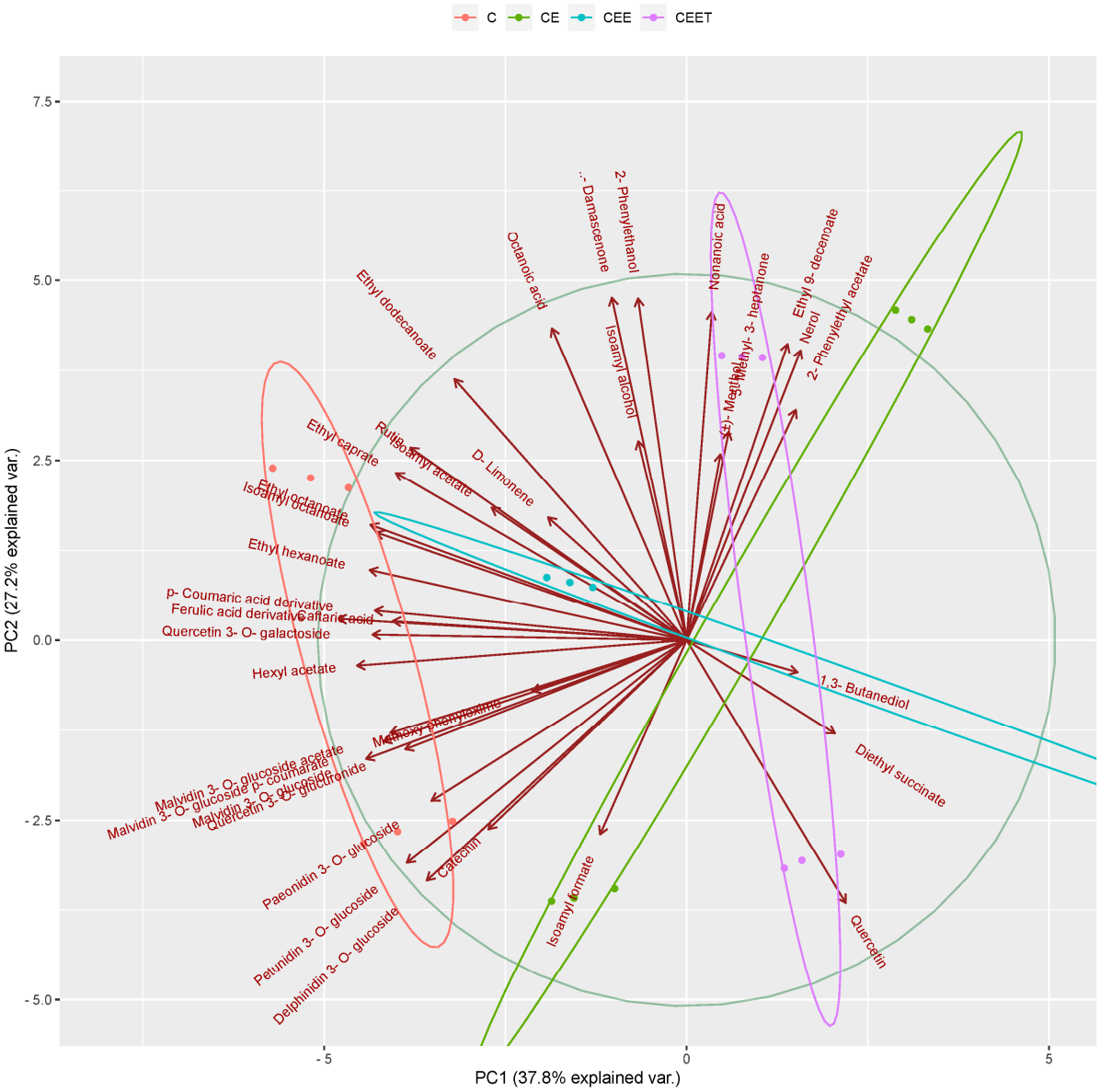

Figure 5. Projection of the wines produced by the various winemaking technologies tested, according to their polyphenolic and volatile composition, in the space defined by the first and second PCs. Prefermentation treatment-based differentiation (eclipses define the 95% confidence intervals of treatment).

4. Conclusions

The study presented herein provided for the first time analytical information concerning the impact of various prefermentation treatments of Maratheftiko grapes on the wines produced. The treatments, performed on an industrial scale, were shown in many instances to negatively affect some anthocyanin pigments, polyphenols, and aromatic constituents. These findings merit profound investigation to fully assess the validity and usefulness of these treatments. Based on the analytical profile of anthocyanins, polyphenols, and aromatic compounds, the principal component analysis carried out revealed that wine distinction may be clear on the basis of vintage year, whereas discrimination based on the various treatments was not evident. The analytical data generated from this study were heretofore unreported and shed more light onto polyphenolic and volatile composition of Maratheftiko wines. On the other hand, the effect of various prefermentation treatments was rather inconclusive. Thus,

future examinations should focus on scrutinizing the implementation of these techniques to clarify their contribution to producing high-quality wines.

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