Article

Characterization of Freisa Wines from Piedmont (Italy) by Aroma and Element Profile

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Abstract: The main purpose of this study was to characterize Freisa red wines from Piedmont (northern Italy) according to their volatile organic compounds (VOCs) and elemental composition. Moreover, the authors investigated whether it was possible to distinguish among the five different DOCs produced in Piedmont on the basis of these chemical parameters. The VOCs profile of Freisa wines was very similar, and the most abundant species detected were isoamyl alcohol, phenylethyl alcohol, ethyl octanoate, and diethyl succinate. Conversely, elemental composition allowed us to partially distinguish one Freisa DOC with respect to the others. Multivariate statistical analysis applied to elemental composition revealed differences among Freisa wines and other red wines from different regions of Italy. In particular, Freisa wines featured higher concentrations of Cr and Ni metals, which are strongly correlated with the composition of the soil of Piedmont. These two elements are hence good candidates as chemical markers for Freisa wines from Piedmont.

Keywords: volatile organic compounds (VOCs); major and trace elements; multivariate statistical treatment; fingerprinting; Freisa wine

1. Introduction

In 2017 Freisa wine celebrated 500 years of history. The first official witness of this kind of wine dates back to 1517, in an official municipal price list. Among Italian red wines, Freisa is almost exclusively produced in the Piedmont region. Currently, five denominations of controlled origin (DOC) of Piedmont Freisa wines are recognized: Freisa di Chieri (hereafter indicated in the paper as FC), Freisa d'Asti (FA), Monferrato Freisa (MF), Langhe Freisa (LF), and Colli Tortonesi Freisa (CTF), which are related to the area of production. Although Freisa does not belong to the group of most famous "noble" Piedmont wines (such as Barolo and Barbaresco), it is gaining growing popularity among consumers also because of its versatility, since it can be vinified in different ways, ranging from sparkling to still and even sweet wines. Freisa wines are traditionally consumed young, and those that are subjected to aging represent a minor fraction. Information reported on the websites of the most important cellars indicates that fermentation and refinement are usually performed in steel or cement barrels. According to production regulations, the employment of wood barrels is not mandatory even for aged wines, which are often sold under the denomination 'Superior'. Nonetheless, most of the wines belonging to this category spend a variable period of time during refinement and/or aging in oak casks, especially those of the Langhe Freisa DOC. Today, Freisa is cultivated over an area of about 850 hectares, with a year's production of 2.5 million bottles. In a demonstration of the growing interest in this kind of wine, some papers recently appeared in the literature regarding its characterization at various levels. The phenolic composition [1,2] and anthocyanins profile [1,3] of grapes were

investigated, as well as the genetic profile of the Freisa variety [4,5]. Moreover, a recent paper appeared on the physicochemical characterization of Freisa grapes and wines [6]. Herein, the authors studied the mechanical features of berries and the phenolic content of grapes and wines, determining the standard chemical parameters (total acidity, alcohol content, dry net extract, etc.) of wines.

Volatile compounds present in wine are mainly responsible for the so-called bouquet, i.e., the typical fragrance of wine. This results from the occurrence of hundreds of volatile compounds, which belong to different chemical families, including higher alcohols, fatty acids, esters, aldehydes, and terpenes; their concentrations range from ng/L to mg/L. The volatile compounds profile may be obtained by gas-chromatography—mass spectrometry (GC-MS) after an extraction-pre-concentration step. Solid phase microextraction (SPME) is an extraction technique developed by Pawliszyn and coworkers [7] and represents a valuable and highly reproducible tool for the extraction of volatile and semi-volatile analytes from different matrixes. The sample volume required is small, and the coupling with gas chromatography and mass spectrometry (GC-MS) provides high sensitivity. It has been widely employed for the study, among others, of many different food matrixes. In the headspace mode (HS-SPME), analytes are extracted from the gas phase, which is in equilibrium with the liquid sample. This avoids contamination of the fiber by non-volatile substances without lowering the sensitivity of the method.

On the other hand, several mineral elements are present in wine at different concentrations, encompassing major elements such as Na, Ca, and P, minor elements (e.g., Fe, Na, Rb) and trace elements (e.g., Cu, Zn, Cr, Ni). Their content in wine is linked to several factors, the most important being the geographical origin of the wine, i.e., soil composition and, to a lesser extent, grapes and wine treatment and contamination [8]. Elemental analysis may be easily performed by several experimental techniques, such as atomic absorption spectroscopy and inductively coupled plasma optical emission spectroscopy (ICP-OES).

To our knowledge, no paper has already been published concerning volatile compounds analysis and elemental analysis of Freisa wines. In the present paper, we report our results obtained by the above-mentioned techniques for the characterization of DOC Freisa wines produced in Piedmont.

2. Materials and Methods

2.1. Samples and Chemicals

Fifty-five wines were purchased from different wineries and shops, belonging to the five different DOC typologies: Asti (FA: 16 wines), Chieri (FC: 11), Langhe (LF: 13), Monferrato (MF: 9), and Colli Tortonesi (CTF: 6). Figure 1 shows the production areas of the five different wines. The harvest year spans from 2012 to 2017. We purchased 3-octanol compounds at analytical purity degree from Sigma Aldrich F (St. Louis, MO, USA) and used them as we received them, with no further purification as an internal standard and reference. Standard solutions were prepared by dilution of reference compounds in ethanol 95%. Element standard solutions were prepared from concentrated (1000 and 10,000 mg/L) stock solutions (Sigma-Aldrich TraceCERT (St. Louis, MO, USA) and acidified to pH = 1.5 by nitric acid of analytical purity.

2.2. Solid-Phase Microextraction

Wine samples were extracted according to the following procedure: 5 mL of wine were placed in a 20 mL glass vial (headspace volume 15 mL) with 1.5 g of sodium chloride and 5 μL of internal standard solution. This solution was prepared by diluting 0.5 mL of 3-octanol (97% *p/p*, *d* = 0.819 g/mL) to a final volume of 400 mL with ethanol, thus yielding a solution concentration of 994 mg/L, and hence a concentration of 0.993 mg/L in the sample. The vial was sealed with an aluminum-coated silicone rubber septum, and headspace extraction was performed with a CAR/PDMS/DVB 30/50 μm fiber (Supelco, Bellefonte, PA, USA) for 4 h at 40 °C under continuous stirring. The fiber was then transferred into the GC inlet.
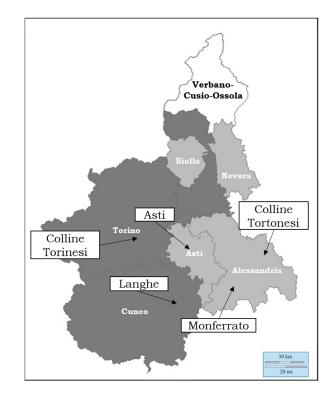
Figure 1. Areas of production of the five different DOCs of Freisa wine in Piedmont.

2.3. GC-MS

A Focus GC Thermo Electronic gas chromatograph with a quadrupole mass spectrometer operated in electron ionization (EI) mode was used for measurements. The GC system was equipped with a 30 m fused silica capillary column (0.25 mm ID; 1 μm film thickness) (Supelco Bellefonte PA). GC conditions were the following: inlet at 200 °C; splitless time: 3 min; initial oven temperature: 40 °C (3 min) then to 250 °C at 4.0 °C/min; GC-MS transfer line at 250 °C; carrier He (5.5 grade of purity) at 1.2 mL/min. The MS ion source temperature was kept at 250 °C. The ionization occurred with an electron kinetic energy of 70 eV. Mass spectra and reconstructed chromatograms (total ion chromatograms [TICs]) were obtained by automatic scanning in the mass range 35–750 u. GC–MS data were processed with the Excalibur 1.4 software. The relative amounts of volatiles (semiquantitative analysis) were obtained by multiplying the area ratio of volatile compound/internal standard by the concentration (μg/L) of the internal standard.

2.4. Acid Digestion

A 10 mL sample of each wine was transferred to a flask, and 16 mL of nitric acid (65%) was carefully added. The resulting solutions were stirred for 12 h until the red fumes ceased completely. The obtained solutions were diluted to 50 mL with high pure water (HPW), i.e., water purified in a Milli-Q system and having a resistivity of 18.2 MΩ·cm, and used for ICP-OES measurements.

2.5. ICP-OES

An Inductively Coupled Plasma Atomic Emission Spectrometer, ICP-OES (Perkin Elmer, model Optima 7000 DV), equipped with an Echelle monochromator, a cyclonic spray chamber, and a Teflon Mira Mist nebulizer, was used to determine the following elements: Al, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Na, Ni, P, Rb, Sr, Ti, and Zn. The instrumental conditions were: plasma power 1.3 kW; sample aspiration rate 15 rpm (approximately 2 mL/min); argon nebulizer flow 0.6 L/min; argon auxiliary flow 0.2 L/min; and argon plasma flow 15 L/min. The signals were measured in triplicate. Sets of instrumental

blank and calibration verification checks were run at frequent intervals during the batch sequences for ICP-OES analyses.

2.6. Statistical Data Treatment

Statistical analyses were conducted using the XlStat 2023 software package, an add-on of Microsoft Excel. The least significant difference (Fisher-LSD) test ($p < 0.05$) and one-way analysis of variance (ANOVA) were used to detect differences in mean values between the Piedmont sites considered (groups). In addition, the correlation among the contents of the elements investigated in wines was studied and expressed by Pearson correlation coefficients.

Afterward, element data were processed using multivariate chemometric techniques, namely hierarchical cluster analysis (HCA) and principal component analysis (PCA) [9,10]. PCA allows representing data from multivariate space with a lower number of new variables, called principal components, that are formed by linear combinations of the original ones. The components are sorted according to the fraction of explained variance of the data, which corresponds to the information. Therefore, the first components bring most of the useful information, excluding noise and spurious ones. With the PCA, it is possible to study the relationship between the samples and the correlations between the variables. HCA can be used to identify groups of variables and samples already obtained by PCA, but it is also not well detectable with the latter since HCA considers all the information contained in the dataset. This method assumes that the nearness of the objects (wine samples), defined by their variables (element content), reflects the similarity of their properties. The objects with the maximum similarity are iteratively arranged into groups, or clusters, more and more populous until all objects are in the same group.

The analytes and samples whose concentrations were below the detection limits in more than 30% of samples (or for more than 30% of analytes) were not included in the treatments. Missing data were estimated using the K-Nearest Neighbors Imputation algorithm, and then the dataset was autoscaled.

3. Results and Discussion

3.1. Volatile Compounds

A characteristic chromatogram of a Freisa wine sample is reported in Figure 2.

Unambiguous identification of 46 analytes was performed by NIST library search, provided that the reverse match index was higher than 800. Alternatively, for doubtful cases, injection of pure standards was performed. Wine volatile compounds may be grouped into three different categories, namely: (a) primary aromas, which are already present in the grape or that are formed in pre-fermentation steps; (b) secondary aromas, which are formed by fermentation and other winemaking procedures; and (c) tertiary aromas, which derive from reactions of the previous ones as a consequence of aging. Table 1 reports the volatile compounds identified and listed according to this criterion of grouping. One of the aims of the present paper was to differentiate the five kinds of Freisa according to their VOCs profile; unfortunately, the preliminary survey of the various spectra was not encouraging from this point of view since all the samples showed very similar results. In fact, four analytes were identified as being the most abundant in all the wines analyzed, i.e., isoamyl alcohol, phenylethyl alcohol, ethyl octanoate and diethyl succinate. However, we herein describe the main features of the compounds found in order to characterize Freisa wine as a whole.

Among primary aromas, terpenes represent the most interesting class of compounds. They are appreciably abundant in aromatic vine varieties but represent a considerable factor contributing to the wine aroma for all the other vines. They usually impart floral or fruity nuances to the wines. Their concentration in wines may vary with aging, because of both hydrolysis reactions of their glycosylated form and of oxidation processes [21,22]. The most abundant terpenes found in Freisa wines were citronellol and linalool. Interestingly, traces of limonene, nerolidol and farnesene were detected in a few samples. The overall

concentration of terpenes was very similar among the five DOCs, with a slightly higher value for CTF and LF.
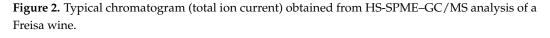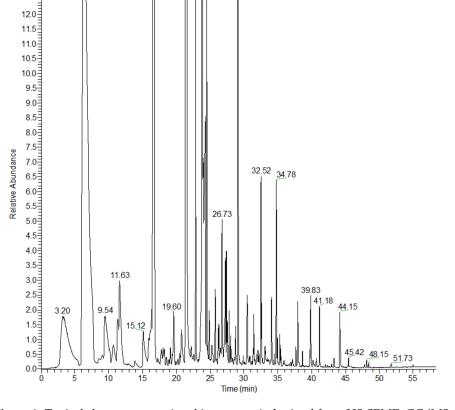

Figure 2. Typical chromatogram (total ion current) obtained from HS-SPME–GC/MS analysis of a Freisa wine.

Table 1. Volatile composition of Freisa wines: odor threshold values (µg/L), concentration (µg/L, mean ± standard deviation) and odor activity values [a].

Compound (µg/L)	Odor Threshold Value	Concentration OAV				
		Asti (FA)	Chieri (FC)	Colli Tortonesi (CTF)	Langhe (LF)	Monferrato (MF)
Varietal Compounds						
Terpenes						
Citronellol	100 [b]	1.97 ± 3.16 *0.0197*	3.06 ± 2.11 *0.0306*	3.11 ± 1.00 *0.0311*	3.55 ± 1.20 *0.0355*	3.74 ± 1.80 *0.0374*
Limonene	200 [c]	4.93 ± 6.10 *0.0246*	7.95 ± 11.8 *0.0398*	2.16 ± 3.36 *0.0108*	5.70 ± 7.41 *0.0285*	0.72 ± 2.16 *0.0036*
Linalool	25 [c]	6.84 ± 6.22 *0.274*	5.20 ± 6.15 *0.208*	3.93 ± 4.34 *0.157*	7.92 ± 4.15 *0.317*	7.67 ± 4.93 *0.307*
Nerolidol	0.1 [d]	0.28 ± 0.83 *2.8*	1.47 ± 2.07 *14.7*	0.67 ± 1.37 *6.7*	0.13 ± 0.33 *1.3*	0.33 ± 0.98 *3.3*
β-Farnesene	0.16 [e]	0.10 ± 0.42 *0.625*	0.26 ± 0.85 *1.625*	0.66 ± 1.62 *4.125*	0.11 ± 0.41 *0.688*	0.24 ± 0.72 *1.5*
Total concentration		14.12	17.94	10.53	17.41	12.7
C6 compounds						
1-hexanol	8000 [c]	0	7.90 ± 15.3 *0.000988*	9.14 ± 8.38 *0.00114*	4.56 ± 8.79 *0.00057*	11.0 ± 22.6 *0.00138*

Compound (µg/L)	Odor Threshold Value	Concentration *OAV*				
		Asti (FA)	Chieri (FC)	Colli Tortonesi (CTF)	Langhe (LF)	Monferrato (MF)
Fermentative compounds						
Esters						
1-Butanol, 3-methyl-, acetate	30 [c]	87.2 ± 81.8 *2.91*	96.5 ± 64.1 *3.22*	45.3 ± 17.5 *1.51*	55.7 ± 31.1 *1.86*	35.2 ± 16.4 *1.17*
Acetic acid, 2-phenylethyl ester	250 [c]	37.6 ± 55.7 *0.15*	24.2 ± 17.1 *0.0968*	21.3 ± 6.73 *0.0852*	19.5 ± 8.33 *0.078*	24.8 ± 5.20 *0.0992*
Acetic acid, hexyl ester	1500 [c]	7.33 ± 14.1 *0.00489*	3.67 ± 8.01 *0.00245*	0.22 ± 0.55 *0.000147*	0.52 ± 1.02 *0.000347*	0.27 ± 0.82 *0.00018*
Benzeneacetic acid, ethyl ester	73 [f]	7.88 ± 6.28 *0.108*	7.95 ± 8.97 *0.109*	7.52 ± 0.84 *0.103*	7.95 ± 2.39 *0.109*	7.92 ± 2.41 *0.108*
Butanedioic acid, diethyl ester	200,000 [c]	518 ± 321 *0.00259*	639 ± 322 *0.00320*	513 ± 238 *0.00256*	591 ± 194 *0.00296*	584 ± 211 *0.00292*
Butanoic acid, ethyl ester	1600 [c]	0.80 ± 0.99 *0.0005*	1.35 ± 1.84 *0.000844*	1.42 ± 1.30 *0.000888*	0.97 ± 0.91 *0.000606*	0.33 ± 0.50 *0.000206*
Decanoic acid, ethyl ester	200 [c]	47.3 ± 37.7 *0.236*	76.3 ± 65.1 *0.382*	73.0 ± 46.4 *0.365*	36.7 ± 23.4 *0.184*	64.3 ± 32.3 *0.322*
Dodecanoic acid, ethyl ester	1500 [c]	3.02 ± 2.04 *0.00201*	4.36 ± 3.90 *0.00291*	3.92 ± 3.29 *0.00261*	2.92 ± 1.97 *0.00195*	8.74 ± 10.1 *0.00583*
Ethyl 9-decanoate	100 [c]	0	0.99 ± 1.99 *0.0099*	2.36 ± 2.89 *0.0236*	1.17 ± 2.85 *0.0117*	1.16 ± 3.48 *0.0116*
Ethyl acetate	7500 [c]	39.4 ± 24.2 *0.00525*	23.2 ± 34.2 *0.00309*	46.3 ± 16 *0.00617*	52.3 ± 17.7 *0.00697*	42.2 ± 13.3 *0.00563*
Hexadecanoic acid, ethyl ester (ethyl palmitate)	1500 [c]	1.91 ± 1.36 *0.00127*	2.07 ± 1.70 *0.00138*	1.44 ± 0.81 *0.00096*	2.65 ± 2.03 *0.00177*	4.25 ± 3.41 *0.00283*
Nonanoic acid, ethyl ester	N/A	1.19 ± 2.03	1.82 ± 2.42	6.09 ± 4.50	2.20 ± 2.68	7.67 ± 4.08
Octanoic acid, 3-methylbutyl ester (isoamyl octanoate)	N/A	2.8 ± 10.6	0.54 ± 1.79	0.72 ± 1.75	1.06 ± 2.14	0.59 ± 1.77
Octanoic acid, ethyl ester	5 [c]	313 ± 194 *62.6*	400 ± 278 *80*	292 ± 162 *58.4*	220 ± 101 *44*	310 ± 114 *62*
Pentadecanoic acid, 3-methylbutyl ester (isoamyl pentadecanoate)	N/A	0	0.044 ± 0.14	0	0.12 ± 0.30	0.37 ± 1.11
Tetradecanoic acid, ethyl ester (ethyl myristate)	2000 [c]	1.39 ± 1.25 *0.000695*	1.56 ± 1.90 *0.00078*	1.04 ± 0.96 *0.00052*	1.42 ± 1.15 *0.00071*	5.03 ± 8.10 *0.00252*
Total concentration		1068	1283	1015	996	1097
Fusel and higher alcohol						
1-Dodecanol	1000 [c]	5.00 ± 5.14 *0.005*	3.35 ± 4.16 *0.00335*	2.70 ± 1.41 *0.0027*	4.86 ± 3.69 *0.00486*	9.10 ± 9.16 *0.0091*
1-Heptanol	300 [c]	1.08 ± 2.36 *0.0036*	3.04 ± 2.44 *0.0101*	2.40 ± 2.15 *0.008*	1.48 ± 0.97 *0.00493*	1.70 ± 0.92 *0.00567*
1-Octanol	N/A	9.56 ± 5.83	17 ± 6.73	13.7 ± 4.97	14.6 ± 6.6	14.1 ± 9.05
3-Heptanol, 2,6-dimethyl-	N/A	4.28 ± 4.11	2.31 ± 4.18	4.86 ± 3.98	3.84 ± 3.79	7.16 ± 5.80
Benzyl alcohol	200,000 [c]	2.97 ± 4.37 *0.0000148*	6.49 ± 6.72 *0.0000324*	4.20 ± 3.94 *0.000021*	2.43 ± 3.56 *0.0000122*	3.38 ± 4.89 *0.0000169*

Table 1. *Cont.*

Compound (µg/L)	Odor Threshold Value	Concentration *OAV*				
Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-	N/A	1.73 ± 3.13	0.46 ± 1.53	0.44 ± 1.08	0	0
Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	N/A	1.31 ± 2.21	0.25 ± 0.82	0	0.13 ± 0.48	0
isoamyl alcohol	30,000 [c]	573 ± 230 *0.0191*	695 ± 241 *0.0232*	611 ± 193 *0.0204*	585 ± 119 *0.0195*	543 ± 107 *0.0181*
Phenylethyl Alcohol	14,000 [c]	684 ± 263 *0.0489*	866 ± 409 *0.0619*	824 ± 250 *0.0589*	751 ± 126 *0.0536*	813 ± 189 *0.0581*
Total concentration		1283	1594	1463	1363	1391
Aldehydes						
Benzaldehyde	350 [g]	1.92 ± 5.28 *0.00549*	0.71 ± 1.66 *0.00203*	0	0.86 ± 3.10 *0.00246*	0
Benzeneacetaldehyde	4 [h]	0.63 ± 0.97 *0.158*	1.50 ± 2.45 *0.375*	0.50 ± 0.40 *0.125*	0.46 ± 0.53 *0.115*	0.093 ± 0.28 *0.0233*
Decanal	2 [i]	0	0.86 ± 2.49 *0.43*	0.20 ± 0.48 *0.10*	0.13 ± 0.47 *0.065*	1.03 ± 1.32 *0.515*
Total concentration		2.55	3.07	0.70	1.45	1.12
Ketones						
Cyclohexanone, 4-(1,1-dimethylethyl)-	N/A	2.36 ± 4.48	0.56 ± 1.87	0	0	0
Fatty acids						
Decanoic acid	1000 [c]	99.8 ± 171 *0.0998*	32.1 ± 23.2 *0.0321*	44.7 ± 16 *0.0447*	29.8 ± 14.8 *0.0298*	56.3 ± 23.9 *0.0563*
Dodecanoic acid	10,000 [c]	3.12 ± 3.84 *0.000312*	0.71 ± 1.96 *0.000071*	1.36 ± 1.21 *0.000136*	1.20 ± 0.96 *0.00012*	2.02 ± 2.04 *0.000202*
Nonanoic acid	3000 [c]	15.2 ± 15.6 *0.00507*	12.8 ± 12.6 *0.00427*	24.6 ± 7.85 *0.0082*	16.8 ± 7.69 *0.0056*	25.7 ± 12.5 *0.00857*
Tetradecanoic acid (myristic acid)	10,000 [c]	0.20 ± 0.55 *0.00002*	0.25 ± 0.82 *0.000025*	0.25 ± 0.42 *0.000025*	0.095 ± 0.23 *0.0000095*	0.34 ± 0.74 *0.000034*
Total concentration		118	45.9	70.9	47.9	84.4
Hydrocarbons						
Benzene, 1-methyl-4-(1-methylethenyl)-	N/A	2.89 ± 1.79	1.64 ± 1.66	2.47 ± 1.38	2.94 ± 0.64	3.24 ± 1.93
o-Xylene	N/A	0	0	0.30 ± 0.72	0	0
Undecane, 2,6-dimethyl-	N/A	0.16 ± 0.46	0.30 ± 0.72	0	0.059 ± 0.21	0
Total concentration		3.05	1.94	2.77	3.0	3.24
Phenols						
Phenol, 4-ethyl-	440 [c]	7.95 ± 26.2 *0.0181*	55.9 ± 87.8 *0.127*	86.9 ± 208 *0.198*	23.4 ± 25.8 *0.0532*	23.9 ± 47.3 *0.0543*
Phenol, 4-ethyl-2-methoxy- (4-ethyl guaiacol)	33 [j]	6.67 ± 15.8 *0.202*	34.4 ± 43.4 *1.04*	25.0 ± 46.8 *0.758*	12.3 ± 16.2 *0.373*	21.5 ± 34.1 *0.652*
Total concentration		14.6	90.3	112	35.7	45.4
Aging volatile compounds						

Table 1. *Cont.*

Compound (µg/L)	Odor Threshold Value	Concentration OAV				
C13-norisoprenoids						
α-Ionone	0.09 [c]	33.5 ± 36.2 *372*	25.4 ± 37.9 *282*	17.8 ± 14.3 *198*	19.7 ± 8.61 *219*	16.6 ± 12.3 *184*
Lactones						
Butyrolactone	1000 [k]	0.22 ± 0.44 *0.00022*	0.52 ± 1.33 *0.00052*	0.30 ± 0.48 *0.0003*	0.035 ± 0.13 *0.000035*	0.028 ± 0.083 *0.000028*
OAV range		*0.0000148–62.6*	*0.000025–80*	*0.000025–58.4*	*0.0000095–44*	*0.000028–62*

[a] Calculated as the ratio concentration/odor threshold value. [b] Retrieved from Ref [11]. [c] Retrieved from Ref [12]. [d] Retrieved from Ref [13]. [e] Retrieved from Ref [14]. [f] Retrieved from Ref [15]. [g] Retrieved from Ref [16]. [h] Retrieved from Ref [17]. [i] Retrieved from Ref [18]. [j] Retrieved from Ref [19] [k] Retrieved from Ref [20]. N/A = not available. OAV are reported in Italics. Organic compounds classes and their total concentrations are reported in bold.

Among primary aromas, we also detected 1-hexanol. High concentrations of alcohols and aldehydes with six carbon atoms do not represent a quality factor for wines since they impart an herbaceous scent [23]. This alcohol was found at low and comparable amounts in three DOCs, namely CT, CTF and MF, with values in the range 8–11 µg/L. Its abundance was about half in LF (around 5 µg/L), while it was absent in the FA samples studied here. However, even if we only performed semi-quantitative analysis, we are quite confident that the concentration of this compound was much lower than the odor detection threshold of 8000 µg/L as reported by Guth [11].

Secondary aromas are the largest class of VOCs in Freisa wines. They are yielded by yeast metabolism and esterification reactions [24]. The most important from the aromatic point of view include the ethylic esters of fatty acids and acetates of alcohols [25]. In terms of primary aromas, the concentration of esters changes during wine aging, but not in a unique way. In fact, according to some authors [26], esters of monocarboxylic acids may undergo hydrolysis with time. Conversely, further esterification reactions take place for dicarboxylic acids, leading to an increasing abundance of esters such as diethyl succinate. This latter compound was one of the four most abundant compounds detected and is responsible for fruit fragrance. The other most abundant ester found was ethyl octanoate, which confers an aroma of grape must.

Among acetates, we detected isoamyl (banana scent), ethyl (pineapple), phenylethyl (rose, honey, tobacco), and hexyl (fruity). Hexanol, found among primary aromas, is considered to be a precursor of this latter ester, yielding it via alcohol acetyltransferase [27]. It is worth noting that ethyl acetate, considered an odor suppressor by some authors [28], was detected in limited amounts in the Freisa wines analyzed. As for varietal aromas, no significant differences were found among the overall ester content of the five DOCs, with a slightly higher concentration found for CTF.

Among higher alcohols, the two most abundant were isoamyl alcohol and phenylethyl alcohol. Higher alcohols are responsible for a great variety of scents, and the two alcohols mentioned above contribute to whiskey and floral nuances, respectively. According to Rapp et al. [29], the contribution to wine aroma is positive only if the overall concentration of this class of compounds is below 300 mg/L. Again, very similar overall concentration values (about 1.3–1.4 mg/L), far lower this limit, were detected for the present five DOCs, and again, the highest value was found for CT wines (ca. 1.5 mg/L). The two terpene alcohols cyclohexanol, 4-(1,1-dimethylethyl)-, cis and trans, could represent good candidates as markers for Asti Freisa wine because they were detected almost exclusively in some of the FA samples analyzed, together with their oxidation product, cyclohexanone, 4-(1,1-dimethylethyl)-. However, further study is necessary to ascertain whether this is not just a case due to the limited number of samples analyzed.

Oxidation of alcohols present in wine may lead to the formation of the corresponding aldehydes [30]. However, we only detected two compounds belonging to this class, namely

benzaldehyde and benzeneacetaldehyde. Overall concentrations ranged from 0.70 µg/L for LF to 3.0 µg/L for CT.

Fatty acids feature unpleasant scents of rancidity. Therefore, their presence in wines is not recommended. They usually undergo esterification reactions with the alcohols present in wine, and hence their concentration is limited by this occurrence. The most abundant was decanoic acid; however, its concentration was largely below the odor threshold of 1000 µg/L, as reported by some authors [25]. Total fatty acids concentration goes from 46 µg/L for CT to 120 for FA.

Volatile phenols found in Freisa wines were 4-ethylphenol and 4-ethyl-2-methoxy-phenol (4-ethyl guaiacol). This class of compounds is yielded by the de-carboxylation of hydroxycinnamic acids present in must by some microorganisms (Brettanomyces yeasts and bacteria) [23,31]. Since these microorganisms are wine contaminants, the above-mentioned phenols represent defects of wines, mainly because of their unpleasant smell. The odor threshold reported in the literature for 4-ethyl guaiacol is 33 µg/L [19] and 440 µg/L for 4-ethyl phenol [12]. Among the wines studied, the concentration values for 4-ethyl phenol were always lower than the threshold; on the other hand, for 4-ethyl guaiacol, only CT wines reached a value comparable to the threshold (34.4 µg/L); this value corresponds to an odor activity value (OAV), calculated as the ratio between the concentration and the odor threshold, of about one, which may be considered negligible [20].

Freisa wines are usually consumed within a few years after bottling. Hence, the concentration of tertiary aromas was expected to be low, as indeed was found. However, it is remarkable that we found a substance belonging to the class of norisoprenoids. This is a valuable occurrence since norisoprenoids, which are formed by the degradation of carotenoids [32], impart positive notes to the wine. The compound was α-ionone (cedar-wood scent), with concentrations ranging from 16.6 for MF to 33.5 µg/L for FA. Moreover, aging determines the passage from wood to the wine of many types of interesting substances, such as lactones. Here, γ-butyrolactone was detected in all the DOCs studied; however, its concentration is very low and reaches the maximum value for CT wines (0.52 µg/L).

Comparison of the present results with those reported in the literature was not straightforward since very few data are available in the literature concerning the aromatic profile of red wines from Piedmont. This is quite surprising since Piedmont can exhibit a long and established tradition of wine production. Marengo et al. [33] differentiated five different wines, namely Barolo, Barbaresco and three DOCs of Nebbiolo, all deriving from the Nebbiolo grape, according to aging and, partly, to the type of wine, by means of multivariate statistical analysis. However, comparison with Freisa wines leads to obvious conclusions since the wines analyzed by Marengo et al. are aged wines, while Freisa wines are not. The aged wines were rich in tertiary aromas and almost completely devoid of primary aromas; the contrary is true for Freisa. Another study concerning autochthonous wines from Piedmont is the one of Bonino et al. [34]. However, this study is only focused on the determination of varietal aroma compounds of Ruchè, and the wines were not commercial but instead were produced by micro-vinification by the authors. The authors detected a great number (59) of varietal aroma compounds in Ruchè, with average concentrations considerably higher than those found in Freisa wines. Indeed, this difference between Ruchè and Freisa is not unexpected since Freisa by no means can be considered an aromatic wine.

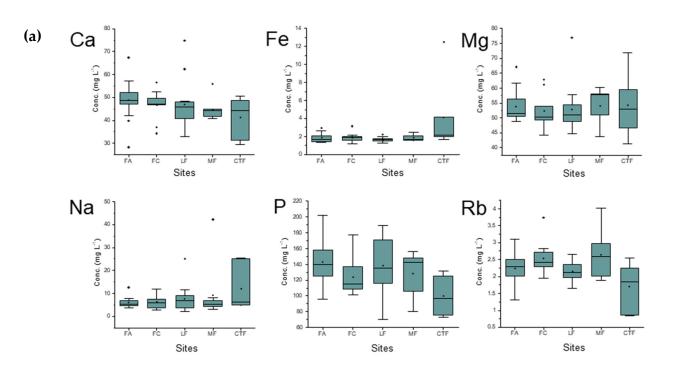*3.2. Major, Minor and Trace Elements*

Table S1—S3 show the mean, standard deviation, and minimum and maximum concentrations for each element determined in FA, FC, CTF, LF, and MF wines. For comparison, Table S4 shows the distribution of major, minor and trace elements in Freisa wines analyzed in this study and in other red wines obtained from vineyards in Italy.

Overall ANOVA showed that there were significant differences in the content of Al ($p < 0.1$), Fe, P and Rb ($p < 0.05$), and Cr and Zn ($p < 0.05$) in Freisa wines collected in different geographical areas of Piedmont. Amongst major and minor elements, only the

phosphorus content showed a highly significant difference by sites, whereas no significant differences among the Ba, Ca, Cu, Mg, Mn, Na, Ni, and Ti content of wines by sites were noted. This can be considered a confirmation of the similar composition of Piedmont Freisa wines: for 53% of the elements considered, there were no significant differences. Above all, a highly significant difference was highlighted for only one element. This finding allows us to hypothesize that Freisa wine can likely be well differentiated from other red wines.

With regard to major and minor elements (Table S1), the most abundant ones were Ca, Mg, and P. The concentrations of all the major elements, with the exception of Na, show lower standard deviations than those found for minor and trace element concentrations in all the areas investigated. The CTF wine samples are characterized by the highest mean concentration and standard deviation for Fe and Na and by the lowest mean concentration for P and Rb. Figure 3a reports the box plots of the major elements in wine samples collected in the five different Piedmont areas. It is possible to notice that CTF samples showed a greater variability for most major elements investigated. Moreover, it is evident that the element characterized by the highest variability was phosphorus. This behavior is likely due to the great importance of this element for the growth of plants which assimilate it in large quantities according to its availability in the soil. In fact, the available fraction of phosphorus strongly depends on the pH and Ca concentration in the soil as well as on the possible addition of fertilizers. All these factors can influence the P content in the wines, and hence, a greater variability for this element is not surprising.

Among the trace elements, the most abundant were Cu and Mn, followed by Al, Sr, Zn, Cr, Ni, Ba, and Ti. In particular, Ti concentrations in Freisa wines showed a great variability as it was present in some wine samples in very high concentration. The assimilation mechanisms of this element by plants are not well understood and, therefore, it is difficult to find an explanation for this behavior. However, contamination of this element can be excluded given that it is not considered a typical pollutant and its source is usually geogenic. Cr and Ni were characterized by a low variability (relative standard deviation percentages were 7.1 and 9.8, respectively). Finally, the overall low concentrations of Al, Cu, Fe, and Zn permit the exclusion of possible contamination by the winemaking process, which could lead to an increase in these elements. Indeed, it is known that these elements can be introduced into the wine due to long contact of the same with materials (aluminum, brass, glass, stainless steel, and wood) from which wine-making machinery, pipes, casks, and barrels used for handling and storing wine are made [35,36]. This is a confirmation that, as the maturation times necessary for Freisa wine are reduced if compared with those of other more mature wines, contamination during the winemaking process is less likely. The FC samples showed lower mean concentrations for Cr, Sr, and Ti than the other Freisa wine samples. Instead, CTF samples were characterized by the highest mean concentrations for Cr, Sr, Ti, and Zn. From the box plots (Figure 3b), it is possible to evidence that CTF samples showed the highest concentrations for Sr while MF samples showed the lowest concentrations for Zn. It is also interesting to observe the opposite trend shown for Mn and Cr distribution among the Freisa wines collected in the different Piedmont sites. In particular, the CTF samples were characterized by the highest mean concentration for Cr and by the lowest concentrations for Mn, while FA samples were characterized by high concentrations for Mn and low concentrations for Cr. It should be kept in mind that Mn, as Cu and Fe, forms stable complexes with amino acids, polyphenols, melanoids and other chelating ligands. This association is an important natural anti-oxidative mechanism that decreases the rate of formation of reactive oxygen species responsible for reactions causing staleness and spoilage of wine [37]. In taking this into account, it is possible to hypothesize that FA samples containing the highest mean concentrations for Mn and Cu also likely have a higher anti-oxidative capacity. Moreover, as the content of Cu and Fe does not exceed the concentration of 1 mg L^{-1} and 7 mg L^{-1}, respectively, it is possible to declare that Freisa wines do not give unpleasant, astringent cupric and ferric tastes, often present when the Cu and Fe content in the wine is higher than the concentrations indicated above [35].
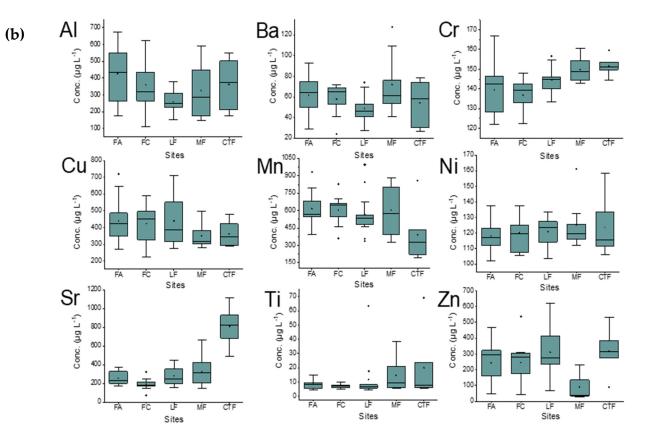
Figure 3. Oxplot of the elements determined in Freisa wine samples from Asti (FA), Chieri (FC), Colli Tortonesi (CTF), Langhe (LF) and Monferrato (MF) sites (**a**) Ca, Fe, Mg, Na, P, and Rb; (**b**) Al, Ba, Cr, Cu, Mn, Ni, Sr, Ti, and Zn.

In all samples, the concentrations of Zn and Cu, considered of special interest due to their toxicity in case of excess, were much lower than the legal threshold limit values established for the Italian wines by the Italian Republic (DM 10/08/2017), namely 5 mg L^{-1} for Zn and 1 mg L^{-1} for Cu [38].

Comparing element contents in Freisa wines and in other Italian red wines, it is evident that Cr and Ni concentrations in our wines were much higher. In particular, Cr concentration is approximately 4 to 10 times higher in Freisa wines than that reported in the other Italian wines. The higher content of these elements is probably due to a relevant natural contribution for chromium and nickel from soils: indeed, Piedmont plains are constituted by sediments that partly derive from serpentinite areas; this, as already reported by other researchers [39–41], causes high background levels of Ni and Cr in soils. It is possible to conclude that the relatively high content of these metals is attributable to the characteristics of the soil on which the vineyards are grown, and they can be considered of natural origin and possible markers for the Freisa wine. Moreover, Freisa wines had a lower content of Ba, Mg, and Zn than the other red Italian wines considered. In comparison with red Italian wines from Southern areas and Sicily, the Freisa wines showed lower Al, Ca, Mn, and Sr concentrations. Finally, the Cu concentration in Freisa wines was higher than in the wines produced in Campania, Tuscany and Veneto but similar to that present in Sicilian wines. Nevertheless, the concentration of most elements in Freisa wines fell in the range of Italian red wines; this happened mainly for Fe, Na, P, Rb, and Ti and partially for the other elements investigated.

3.3. Multivariate Data Analysis

A multivariate study on the analytical data was carried out to obtain a visual representation of the metal distribution in the Freisa wine samples, to find out similarities and correlations among variables (metal contents), and to identify possible clusters among objects (wine samples) which would be more difficult to identify only by a simple comparison of results on chemical composition. Total metal concentrations in wines are useful for characterizing them and classifying them according to geographical origin and assessment of authenticity. Indeed, it is known that the information on metal contents in finished wines is useful for their "fingerprinting", mostly because metals are stable and represent factors affecting wine composition. In particular, several literature studies report that Ba, Ca, Cr, Mg, Mn, Na, Rb, and Sr concentrations are the most discriminatory variables to distinguish the geographical origin of a particular wine and its authenticity [33,35,42–45].

Figure 4a shows the combined plot of PC1 vs. PC2 obtained by PCA. In general, even if no net grouping is present, it is possible to evidence that (i) most CTF samples (83%) show negative scores on PC1 and positive scores on PC2; (ii) most FA samples (71%) show positive scores on PC1 and negative scores on PC2; (iii) most LF samples (69%) are characterized by negative scores on PC1 and PC2; and (iv) MF and FC samples are dispersed along PC1 and assume both positive and negative values on PC2. It is interesting to note the opposite positions occupied by CTF and FA samples, characterized by higher concentrations of Sr, Cr, Ti, and Zn and by P, Cu, and Mn, respectively. In particular, FA samples are aligned with the direction of the Cu and P vectors due to their higher concentration in these samples. Instead, the LF samples are not strongly influenced by the variables identified by the vectors to indicate how these are characterized by a low content of all the determined elements. Overall, except for the CTF samples, the high similarity found for all the other samples allows us to hypothesize that Freisa wine has similar content of elements regardless of the Piedmont collection area, and this might represent a simplification for evaluating its authenticity compared to other Italian and non-Italian red wines.
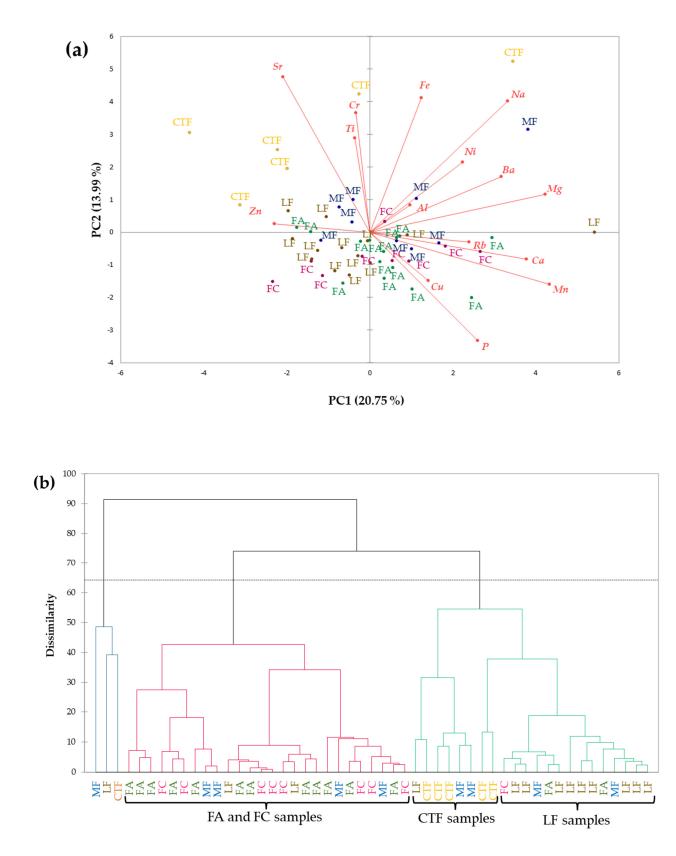
Figure 4. Chemometric processing. (**a**) Biplot on PC1 and PC2 obtained considering Freisa wine samples. (**b**) Dendrogram obtained by HCA in R-mode (i.e., studying the clustering of samples).

The results of the CA are reported as a dendogram in Figure 4b. At a dissimilarity level of 74%, it is possible to identify three clusters: (i) the first on the right composed by

all the CTF samples and most of the LF samples (77%); (ii) the second at the center, larger in size and composed by most of the FA and FC samples (86% and 89% respectively), and finally (iii) the third on the left in which three Freisa wine samples are grouped, belonging to the MF, LF, and CTF sites and characterized by a higher content of elements such as Ni, Ba, Fe, Na, and Mg, as it is also evident from their separate position in the PCA plot shown in Figure 4a. At a lower dissimilarity level, the first cluster shows a further separation between CTF and LF samples. Finally, MF samples are equally distributed among the three clusters (four in the first, four in the second, and one in the third). Therefore, the CA confirms the results obtained by PCA, and in particular allows to distinguish the CTF and LF samples from FA and FC samples.

To evaluate if the element composition of the Freisa wines is useful for their characterization and classification according to geographical origin, the dataset obtained considering the mean element concentrations determined in the Freisa wines (this study) and in the red Italian wines reported in Table S4 was treated by PCA. From the PCA plot (Figure 5), it is apparent how most wine samples taken from the same geographical area tend to group in well-defined clusters. In particular, Freisa wine samples have negative scores on PC1 and are all grouped in a very compact cluster, meaning that these wine samples have quite characteristic elemental composition. On the opposite side of the plot, we find Southern and Sicilian wine samples characterized by positive scores on PC1. Finally, the Northern wine samples are more scattered and arranged along PC1 and are characterized by negative scores on PC2. It is interesting to observe that the Freisa wine cluster is strongly influenced by Cr and Ni variables showing high and negative loadings on PC1. It is possible, therefore, to assess Cr and Ni as possible markers for Freisa wine for their high discriminant capacity to distinguish this wine from other red Italian wines. These two elements are also strongly correlated to each other, showing the highest Pearson correlation coefficient (0.980) in the correlation matrix calculated. This correlation could be explained on the basis of their common origin deriving from the high content of these elements in the Piedmont soils of the investigated sites, as discussed in Section 3.2. Finally, the Sicilian and Calabrian wines are mainly influenced by Na, Al, Sr, and Mg variables, while most of the Northern wine samples are negatively influenced by Cu.
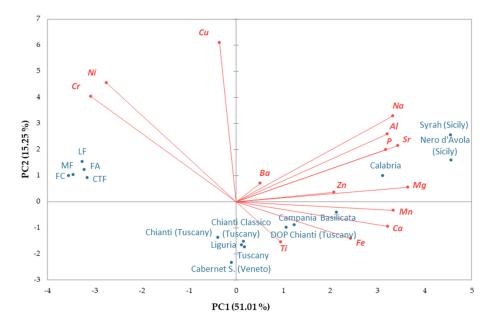

Figure 5. Biplot on PC1 and PC2 obtained considering Freisa wine samples and other Italian red wines.

4. Conclusions

To the best of our knowledge, this is the first study aimed at characterizing Freisa wines from Piedmont northern Italy according to their aromatic profile and elemental composition, and to highlight differences among the five DOCs produced in Piedmont on the basis of these parameters. Our results indicate that the VOCs composition is very similar among the five different DOCs, and no differentiation is possible. The VOCs profile of Freisa is typical of wines deriving from not-aromatic vines and that are consumed quite young, since they display a low but not negligible content of primary aromas, while the content of tertiary aromas such as esters is lower with respect to aged wines such as Barolo and Barbaresco.

Chemometric analysis of the elemental composition allowed us to partly distinguish CTF samples from the other Freisa wines. For the first time, in this study, it was assessed whether the element composition of the Freisa wine allows us to distinguish it from other Italian wines. The combination of ICP-OES analysis with chemometrics has allowed us to identify Cr and Ni as potential markers of Freisa wine. Further studies will have to be performed to increase the dataset in order to develop models with high capability to discriminate Freisa red wines from samples of other Italian wines.

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