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Article

CHEMICAL AND SENSORY DIFFERENTIATION OF NEMEA PDO SUB-ZONES WINES: TWO VINTAGES EXPERIMENT

DIFERENCIAÇÃO QUÍMICA E SENSORIAL DE VINHOS DAS SUBZONAS NEMEA DOP: EXPERIÊNCIA DE DUAS VINDIMAS

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SUMMARY

Theoretical representation of wine is important for producers, with implications in a technical context. Understanding the correlation between wine characteristics and *terroir* involves emphasizing typicality and linking it to sensory interpretation. Within this research, the adaptable nature of the indigenous red grape variety ‘Agiorgtiko’, well-known for its role in producing PDO wines, was examined both chemically and sensorially. Wine PDOs are based on their place of origin and technical product specifications. A total of ten vineyards located in a dry region were carefully chosen to vinify their grapes at three distinct elevation tiers (low 300-600 m, medium 600-900 m, and high 900-1250 m) over two consecutive vintages (2019-2020). Classical oenological analyses were performed based on the International Organisation of Vine and Wine analytical methods. Phenolic content and color parameters were investigated by spectrophotometric methods and volatile compounds of the wine aroma, such as the esters, higher alcohols and acids, were analyzed by GC/MS. Descriptive sensory analysis was carried out by a trained panel for all produced wines. The findings revealed variations in volatile compounds among wines, that were primarily influenced by grape provenance, which in turn shaped unique compositional and sensory characteristics. Multivariate analysis of the analytes determined proved that vineyards located at the sub-zone with higher altitudes were clearly separated from the other two zones. Notably, the presence of some volatile compounds in wines was indeed impacted by grape origin. In essence, this research illustrated how the sensory attributes of ‘Agiorgitiko’ wines could be changed by diverse *terroir* elements within the designated Nemea wine region.

RESUMO

A representação teórica do vinho é importante para os produtores, com implicações num contexto técnico. Compreender a correlação entre as características do vinho e o *terroir* implica enfatizar a tipicidade e associá-la à interpretação sensorial. No âmbito desta investigação, foi examinada a natureza adaptável da casta tinta autóctone ‘Agiorgtiko’, conhecida pelo seu papel na produção de vinhos DOP, tanto química como sensorialmente. Os vinhos DOP baseiam-se no seu local de origem e nas especificações técnicas do produto. Um total de dez vinhas situadas numa região seca foram escolhidas para vinificar as suas uvas em três níveis distintos de altitude (baixo 300-600 m, médio 600-900 m e alto 900-1250 m) ao longo de duas vindimas consecutivas (2019-2020). As análises enológicas clássicas foram realizadas com base nos métodos analíticos da Organização Internacional da Vinha e do Vinho. O conteúdo fenólico e os parâmetros de cor foram investigados por métodos espectrofotométricos e os compostos voláteis do aroma do vinho, como os ésteres, os álcoois superiores e os ácidos foram analisados por GC/MS. A análise sensorial descritiva foi realizada por um painel treinado para todos os vinhos. Os resultados evidenciaram variações nos compostos voláteis entre os vinhos, influenciadas principalmente pela proveniência da uva, que por sua vez moldaram as características composicionais e sensoriais únicas. A análise multivariada dos analitos determinados comprovou que as vinhas localizadas na subzona com altitudes mais elevadas estavam claramente separadas das outras duas zonas. Notavelmente, a presença de certos compostos voláteis nos vinhos foi de facto influenciada pela origem da uva. Na sua essência, esta investigação ilustrou como os atributos sensoriais dos vinhos ‘Agiorgitiko’ podem ser alterados por diversos elementos do *terroir* na região vinícola de Nemea.

Keywords: Agiorgitiko, Nemea PDO wine, sub zones, aroma compounds, sensorial profile.
Palavras-chave: Agiorgitiko, vinho DOP Nemea, subzonas, compostos do aroma, perfil sensorial.

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a valuable perennial crop grown in the Northern Hemisphere between the parallels of 30° and 50° (Blanco-Ward *et al*., 2021) even in the last years is cultivated in United Kingdom (Biss and Ellis, 2022). Within this region, the optimal temperature, ranging from an average of 12 °C-13 °C to 22 °C-24 °C during the growing season conditions, experienced by the most traditional wine regions (Schultz and Jones, 2010) enable the production of wines of exceptional quality. In Europe, where the production of renowned wines is subject to strict regulations and geographical boundaries (such as designations of origin), viticulture and winemaking play a significant role in contributing to the local economy and enhancing the provision of ecosystem services within the landscape.

The most high-rated European wine regions are located in narrow geographical areas, characterized by environmental conditions well suited to viticulture. In these areas, the interactions among climate, soil, cultivar and human factors influence the wine style and attributes, contributing to the achievement of the high-quality standards and consumer-recognized typicity of the final product. In Greece, more than 60 000 ha are dedicated to the cultivation of grapevines (Hellenic Statistical Authority, 2020), most of which are autochthonous varieties. ‘Agiorgitiko’, a red grape variety native to Greece, is the most widely grown and can mainly be found in the Nemea Protected Designation of Origin (PDO) zone, in the Peloponnese region of southern Greece. This particular grapevine variety covers an extensive area of 3 830 ha, primarily in Nemea, but also in other parts of mainland Greece such as Kavala, Drama, and Attiki (Anderson and Nelgen, 2020). The wine region of Nemea benefits from a Mediterranean climate, characterized by mild winters and hot, dry summers. The majority of the zone is located in the South Central Corinthia district, with smaller portions in Sikyonia and Stymfalia. Informally, the zone is divided into three subzones based on altitude: mountainous (600-850m), semi-mountainous (350-600m), and lowland (250-350m). As a result, the zone experience variations in terrain and climate conditions, which have an impact on the ripening process, microbial ecology, and physicochemical parameters of the grapes (Tassopoulos *et al*., 2021).

Aroma, a crucial quality aspect of wine, is influenced by various types of volatile compounds originating from three primary sources: grapes (primary aroma), alcoholic fermentation (secondary aroma), and maturation-ageing (tertiary aroma) as highlighted by (Guerrini *et al*., 2019). In addition to these sources, factors such as climate, region, soil, grape variety, vineyard management practices, winemaking techniques, and yeasts play significant roles in shaping the ultimate aroma profile and quality of wine, as discussed by Barbará *et al*. (2019). Several researchers have dedicated their efforts to exploring the volatile composition of Greek red and white wines in recent years, shedding light on the intricate interplay of these various factors in determining the sensory characteristics of these wines (Karagiannis and Lanaridis, 2002; Lanaridis *et al*., 2002; Roussis *et al*., 2005; Koussisi *et al*., 2007; Dourtoglou *et al*., 2014; Kanavouras *et al*., 2019; Karimali *et al*., 2020; Lola *et al*., 2023; Marinaki *et al*., 2023). This ongoing research underscores the complexity and multidimensional nature of aroma in wines, highlighting the need for a comprehensive understanding of the influencing factors in order to fully appreciate and evaluate the sensory experience offered by different wine varieties.

Among the various constituents of wine, volatile compounds hold a central role in determining the aroma of wine and consequently its sensory identity. The aroma of wine is the result of a biochemical and technological process (Kotseridis and Baumes, 2000) that involves the contribution of different volatile molecules from grapes, fermentations, reactions related to aging, and occasionally oak and other types of wood. Over 800 volatile compounds, including alcohols, esters, phenols, monoterpenes, norisoprenoids, lactones, aldehydes, and ketones, have been identified to date (Ferreira, 2010). Many of the non-aromatic wine grape varieties contain aroma metabolites in precursor forms, whose presence and occurrence is influenced by grape variety, micro-climate of the vineyard, and vine training methods (Parker *et al*., 2017). When fermentation conditions are held constant, the resulting wine aroma is a result of the interactions between precursors, nutrient levels in the grapes (which are influenced by vineyard factors), and the enzymatic capabilities of yeast. Several studies have indicated that the enzymatic activities of yeasts can also impact the levels of compounds primarily originating from grape varieties, such as terpenes (Slaghenaufi *et al*., 2020; Senses-Ergul and Ozbas, 2016). Due to the uniqueness of the grape variety and its limited cultivation mostly exclusively planted and cultivated in Nemea area, there is lack of published information concerning the chemical and sensorial profile. So far, research has been done in the in the field epiphytic grape microbiome (Kontogiannatos *et al*., 2021; Papadopoulou *et al*., 2022; Kazou *et al*., 2023) as well as to the viticultural techniques in order to improve grape quality (Kondouras *et al*., 2006; Kogkou *et al*., 2017; Alatzas *et al*., 2021)

The Protected Designation of Origin (PDO) designates a specific geographical area that includes one or more grape varieties and allows for certain practices related to farming, date of harvest, and oenology. These technical choices partly contribute to the characteristics and typicality of wines. The focus of this study is on the wine-making variety known as *Vitis vinifera* L. 'Agiorgitiko', which holds a prestigious position in the wine industry. The Nemea PDO region is characterized by diverse topographic features and climatic conditions, ranging from flat areas suitable for viticulture at an elevation of 250 m above sea level (a.s.l.) to steep slopes up to 800 m a.s.l.. The climate in the Nemea area follows a Mediterranean pattern, with a mean temperature of 21.4 °C during the growth period and an average annual rainfall of 809 mm (2009-2018) located in the Nemea region (Tassopoulos *et al*, 2021).

This research paper investigated the volatile and sensory characteristics of 'Agiorgitiko' wines from the PDO Nemea zone in relationship to vineyards located at different altitudes. It is therefore the first attempt to make efforts in this direction and to investigate the possible differences in monovarietal wines between three sub-zones of the Nemea PDO area.

MATERIALS AND METHODS

Vineyards description

The investigation was carried out during the period of 2019-2020 in the PDO Nemea vine-growing zone (37°41′–37°57′ N, 22°28′–22°47′ E, WGS 1984) located in the Peloponnese peninsula in southern Greece, an area renowned for its viticulture and is particularly well known for being the country's largest producer of red wines.

A total of ten vineyards located in a dry region were carefully selected at three distinct elevations over two consecutive vintages (2019-2020) (Table I). All vine plots included in this study, with 'Agiorgitiko' variety and using the same training system, were trained on double cordon and the pruning system was 3 spurs in each cordon, with an average row interval of 2.5 m. These plots were not subjected to irrigation and have an average age of 20 years; all of them were older than 10 years. The selection of vine plots was evenly distributed throughout the PDO zone to encompass all major viticulture areas and capture the various abiotic characteristics present, including soil composition, climate parameters, and topographic factors such as elevation, slope, and aspect.

The altitudinal range for this specific zone begins at an elevation of 300 meters and extends to an impressive height of over 1200 m; nonetheless, it is important to note that there are vineyards that thrive at altitudes reaching up to 850 m in the region known as Asprokambos. Moreover, it is worth mentioning that approximately 50% of this zone is situated at altitudes below 500 m. When considering the climatic conditions, although the overarching classification is Mediterranean, it is evident that significant variations can be observed over relatively short distances, often measured in just a few km. In terms of precipitation, while it is theoretically abundant, averaging around 750 mm annually, it predominantly occurs during the winter months, accounting for approximately 80% of the total rainfall. However, it is important to note that this pattern does not follow any fixed rules, which can significantly influence the vintage and harvest qualities of the grapes. There have been certain years in which rainfall has been notably scarce, such as in 2007 with only 408 mm, 2008 with 515 mm, and 2013 with 541 mm, contrasting with other years in which precipitation levels approached 1000 mm (e.g. 1999 with 908 mm, 2010 with 872 mm, and 2014 with 826 mm). Furthermore, if the strategically significant rainfall is not forthcoming, it seems that the phenomenon of cool nighttime temperatures also plays a crucial role in the overall quality of the produce, rendering the entire situation exceptionally intriguing for those involved in viticulture (Lagouvardos *et al*., 2017)

The soils within this particular region are distinctly characterized not only by the abundant presence of clay, which is a fundamental component, but they are also notably marked by the existence of limestone formations that appear to impart unique qualities to the wines produced in this area. As is typically observed in lowland regions, the soils exhibit a higher degree of fertility; however, as one ascends into the hilly terrains, the soil composition transitions to a rockier texture, and in the higher altitudes, the prevalence of limestone contributes to exceptional drainage capabilities, which consequently results in significantly lower yields of agricultural products (Yasoglou *et al*., 2017; Missopolinos, 2015).

Micro- vinification process

'Agiorgitiko' grapes were harvested manually at technological maturity during the 2019 and 2020 vintages from 10 vineyard plots located in the Nemea PDO region. Harvested grapes underwent small-scale vinification at the Laboratory of Enology and Alcoholic Drinks using a common protocol. A total of 75 kg of 'Agiorgitiko' grapes from each vineyard was separated into three portions of 25 kg, and each was destemmed and crushed to provide musts for fermentation in triplicate. The grape musts were transferred to 25 L stainless steel tanks for vinification, 40 mg/L of sulfur dioxide was added, and after 6 h, tanks were inoculated with 250 mg/L of *Saccharomyces cerevisiae* strain HDS 135 (Fermentis, France). 12 h after yeast addition and again after 1/3 of the fermentations achieved, 125 mg/L of diammonium phosphate (DAP) and 125 mg/L of Springferm (Fermentis, France) were added. Maceration was conducted by 'piegage' twice a day, and alcoholic fermentation was performed between 21–23 °C, until sugar depletion, which was determined using an enzymatic analyzer (Y15, Biosystems S.A., Barcelona, Spain). Following this critical fermentation phase, the resulting wines were judiciously separated from the skins and seeds, and the pomace was subsequently subjected to pressing in a hydro fruit press (Pew80, Grifo Marchetti, Piadena Drizzona, Italy). The wines were transferred to 20 L stainless steel tanks and inoculated with Viniflora CH11 (CHR Hansen, Hørsholm, Denmark) for malolactic fermentation (MLF). Once the malic acid concentration was below 0.2 g/L (determined enzymatically; Y15, Biosystems S.A., Barcelona, Spain), MLF was considered complete.. After racking and addition of 50 mg/L of sulfur dioxide, wines were stabilized in a cold room (at about 5 °C) for a period of 2 months. Amber glass bottles of 0.75 L were filled, the headspace was flushed with N_2, and the bottles were closed with a technical stopper (45 mm length, Diam 5). Bottled wines were stored horizontally in cellar conditions (15 ± 2 °C, 65–70% humidity).

Table I

Nemea PDO zone with the three distinct elevation viticulture areas and the 10 study vineyards, ID plots

Region	ID plots	Altitude
R1	7 45	Low 300 - 600 m
R2	24 28 33 40	Medium 600 - 900 m
R3	17 47 68 74	High 900 - 1250 m

Conventional wine analysis

The examination of enological parameters and the determination of chromatic characteristics were conducted subsequent to the process of vinification. The determination of reducing sugars, alcoholic degree, Total Acidity (TA), pH, and Volatile Acidity (VA) were carried out according to the methods of International Organisation of Vine and Wine (OIV, 2018).

Color and phenolics determinations

The sum of absorbance at 420, 520, and 620 nm in a cell with a path length of 1 cm was used to determine the color intensity (Ribéreau–Gayon *et al*., 2000). To conduct this analysis, a spectrophotometer (UV-1900, Shimadzu Scientific Instruments, Kyoto, Japan) was used. The hue was quantified by calculating the ratio of absorbance between 420 nm and 520 nm (Ribéreau–Gayon *et al*., 2000). Each measurement was performed in triplicate for every replication of the winemaking process.

Total anthocyanins were determined by a spectrophotometric method (McDougall *et al*., 2005) based on SO_2 bleaching. All analyses were performed in triplicate.

Tannin concentration was determined by two different assays. By Bovine Serum Albumin Precipitation Assay (BSA) according to the protein precipitation method (Habertson *et al*., 2003) and by Methylcellulose Precipitation (MCP) assay, developed and validated by Sarneckis *et al*. (2006).

In order to determine the Total Polyphenol Index (TPI) of wine samples, they were diluted and analysed at 280 nm by a spectrophotometer (Ribéreau–Gayon, *et al*., 2000).

Quantitative determination of Volatile compounds

The volatile compounds (higher alcohols, esters, acids) were evaluated through liquid-liquid extraction, as described by Goulioti *et al*. (2023). Initially, wine (40 mL) was spiked with internal standards (3-octanol for alcohols, ethyl heptanoate for esters, and heptanoic acid for acids) to reach a concentration of 10 mg/L before the addition of dichloromethane (5 mL). The mixture was stirred continuously with a magnetic stirrer for 15 min. The organic layer was removed, and a second extraction was carried out with an additional 5 mL of dichloromethane. The organic extracts were combined, centrifuged at 1968 g for 10 min at 4 °C, and the lower organic layer was transferred into a vial and dried with 1.5 g of anhydrous sodium sulfate. After filtration, the organic extract was concentrated to 500 μL under a nitrogen stream. The organic phases containing the aroma compounds were gathered, and the solvent was evaporated under a nitrogen stream to 500 μL. The concentrated extract was then introduced (1 μL) into a GC-MS instrument to identify the aroma substances present. All analyses were conducted in triplicate. The aroma profile assessments were carried out using a gas chromatograph Clarus 5090 (Perkin Elmer, Waltham, MA, USA) with an Agilent J&W capillary (DB WAX) GC column (50 m × 0.25 mm i.d. and

0.25 μm film thickness), along with a Clarus SQ8S MS detector (Perkin Elmer, Waltham, MA, USA) connected to an automatic injector (HTA S.R.L., Brescia, Italy). The injector and MS transfer line temperatures were set at 250 °C and 240 °C, respectively. Pulsed splitless injections for 0.8 min followed by split 1:50 were used. Helium was used as the carrier gas with a flow rate of 1.96 mL/min. The oven conditions were as follows: 40 °C for 2 min; 40 °C to 240 °C at a rate of 5 °C/min; 240 °C for 20 min. The MS spectra were obtained in electron ionization (EI) mode at 70 eV within the range m/z 40–400 using selected ion full ion (SIFI) mode. The concentration of the compounds was quantified by establishing a calibration curve for each compound based on commercial standards using seven concentration levels.

Sensory assessment

The sensory assessment was conducted by a group of 12 well-trained panelists, consisting of 12 persons, ranging from 25 to 57 years. All participants provided in-formed consent prior to participating in the study. Sensory analysis of wine samples was performed three months after bottling the wine of each harvest. The panelists underwent a comprehensive training program, which included three training sessions and two evaluation sessions, regarding the two vintages wines (2019 and 2020), spanning a period of two weeks. During the initial session, the panelists were presented with a variety of representative ‘Agiorgitiko’ samples in order to familiarize themselves with this particular type of wine. They were then instructed to describe the perceived aroma attributes, as outlined by Nanou *et al*. (2020). In the second session, the panelists received training on the 11 olfactory attributes listed, using appropriate reference standards for each attribute. This training was repeated and the panelists' performance was evaluated. The final two sessions were dedicated to assessing the samples, consisting of ten wines in duplicate, each wine represented each vineyard, following the methodology described by Nanou *et al*. (2020). The wines were presented to the panelists individually, according to a Latin Square Design, with a one-minute break between each sample. The testing took place in separate booths with natural light. Each panelist was provided with 30 mL of wine in ISO wine glasses, covered with plastic Petri dishes and labeled with random three-digit numbers. To ensure consistency, the winemaking replicates for each wine were blended (from each vineyard) equally and allowed to reach room temperature (20–22 °C). Data collection was facilitated using the Compusense20 Cloud software (Compusense, Guelph, ON, Canada). The intensity of sensory attributes (Color Intensity, Color Hue, Aroma Intensity, Red Fruits, Black Fruits, Spices, Earthy, Vegetative, Acidity, Bitterness, Astrigency, Global Mark) was rated on a seven-point scale, ranging from 1 (not perceived) to 10 (very strong).

Data Analysis

All values are presented as the mean and standard deviation. Statistical analyses were performed using Statgraphics Centurion application (version 1.0.1.C). The significance of the results was determined with an unpaired t-test or one-way ANOVA with Tukey’s test. A multivariate statistical data analysis (MVA) of the samples was performed with SIMCA P+ version 15 (Umetrics AB, Umeå, Sweden) and with XLstat (XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel; Addinsoft, Paris, France, 2017).

RESULTS AND DISCUSSION

Wine chemical composition

Table II summarises the basic chemical composition of the wines produced. Wines were fermented to dryness and VA ranged within the legal limits. Alcohol concentration ranged from 12.2 to 13.5 % v/v for wines vinified in 2019 vintage, while 2020 wine samples ranged from 12.4 to 14.5 % v/v. Samples produced in the second year had generally higher alcoholic content with greater variability between them, while wine samples with higher values were highlighted in the wines categorized in the R3 region in both vintages. TA ranged between 6.2 to 7.6 g/L for 2019 wines and 4.9 to 7 g/L for 2020 wines. Both higher and lower concentrations were reported in the R2 category for the first experimental year and in the R3 category for the second year. Following TA, pH values were higher in 2020 vintage wines. Wine samples ID 33 and 24, belonging to R2, and ID74 and 47, belonging to R3, had the pH among 2019 vintage wines, while ID28 from R2 and ID68 from R3 showed the lowest values. In 2020 vintage wines, ID33 from R2, and ID74 and 47 from R3 also had higher pH, and ID45 from R1 had the lowest one with no statistical difference from ID68 from R3. As the winemaking protocol was standardized, these results indicate that climatic, pedological, and topographical variations between the tested sub-regions could be the cause.

The phase of grape ripening is a critical period that impacts the grape composition and, consequently, the characteristics of the wine. This period involves several physical (such as weight, volume, color, and texture) and chemical changes (including pH, acidity, sugar levels, phenolic compounds, and aromatic components).

Table II

Mean concentration (± standard deviation) of enological parameters of ‘Agiorgitiko’ regional wines in two vintages at PDO Nemea

Vintage	Region	ID	Alcoholic Degree (% v/v)	pH	Titratable Acidity (g tartaric acid/L)	Glucose and Fructose (g/L)	Volatile Acidity (g acetic acid/L)
2019	**R1**	**7**	12.8±0.1ab	3.45±0.04bc	7.0±0.2ab	0.2±0.1c	0.6±0.1a
		45	12.7±0.2ab	3.38±0.10bc	7.2±0.1ab	0.6±0.2a	0.5±0.1abc
	R2	**33**	12.2±0.6b	3.66±0.01a	6.2±0.2c	0.0±0.0c	0.5±0.1c
		28	13.4±0.8ab	3.37±0.05d	7.6±0.1a	0.5±0.1ab	0.5±0.1abc
		40	12.2±0.1b	3.46±0.03bc	7.1±0.2ab	0.1±0.1bc	0.4±0.1e
		24	12.9±0.7ab	3.72±0.02a	6.7±0.1bc	0.3±0.3abc	0.5±0.1c
	R3	**68**	13.3±0.1ab	3.27±0.04d	6.5±0.3bc	0.6±0.2a	0.6±0.1a
		74	13.3±0.4ab	3.74±0.01a	6.9±0.2b	0.7±0.1a	0.5±0.1bc
		17	12.9±0.6ab	3.51±0.03b	6.5±0.2bc	0.2±0.1bc	0.4±0.1d
		47	13.5±0.1a	3.64±0.06a	7.0±0.5ab	0.7±0.1a	0.5±0.1ab
2020	**R1**	**7**	13.0±0.1bc	3.49±0.02bc	6.2±0.1bc	0.1±0.1d	0.6±0.1a
		45	12.9±0.2bcd	3.25±0.05f	6.5±0.1ab	0.1±0.0d	0.6±0.1bc
	R2	**33**	12.6±0.2de	3.79±0.02a	4.9±0.1e	0.4±0.1bc	0.5±0.1bc
		28	13.0±0.2bc	3.44±0.05cd	5.8±0.4cd	0.1±0.1d	0.4±0.1d
		40	12.6±0.2cde	3.36±0.02de	6.1±0.1bc	0.2±0.1cd	0.6±0.1b
		24	13.3±0.2 b	3.52±0.07bc	6.1±0.5bc	0.1±0.1d	0.5±0.1cd
	R3	**68**	14.5±0.3a	3.31±0.01ef	7.0±0.2a	0.5±0.2b	0.4±0.1d
		74	12.4±0.1e	3.78±0.02a	5.4±0.1de	0.9±0.1a	0.7±0.1a
		17	12.8±0.0cd	3.57±0.02b	5.5±0.1de	0.1±0.1d	0.5±0.1d
		47	12.7±0.1cde	3.77±0.02a	5.2±0.1e	0.2±0.1a	0.6±0.1a

*Values followed by different letters are significantly different according to the post-hoc test ($p<0.05$).

These modifications are influenced by environmental factors, climatic conditions, such as temperature, humidity, sunlight and precipitation, and agricultural practices applied to the vineyard. The sites examined in this research differed primarily in terms of altitude, orientation (south and west-facing), soil composition, vineyard management techniques, vine phenology, and agricultural interventions (Tassopoulos *et al.*, 2021). These key factors' variations played a significant role in shaping the characteristics of the three distinct *terroirs*. Concerning the technological aspects, the grape's sugar content across all vineyards met the requirements specified in the production regulations to achieve an alcohol content ranging between 12.5 % and 13.3 % by volume, depending on the desired wine style. Moreover, the total acidity levels surpassed the minimum threshold of 4.5 g/L established by the production regulations.

Color and phenolic characteristics

Evaluation of the ‘Agiorgitiko’ wines produced by the different Nemea subzones regarding the phenolic content is presented in Table III. Regarding the results of color intensity and total anthocyanins an increasing level was observed in the wines produced by vineyards located in the higher altitudes (R3). Moreover, wines from the R3 recorded higher tannin concentrations, according the MCP and BSA assays than those from R1 and R2 areas. The same trend was observed for the TPI. Finally, it should be mentioned that during the 2020 vintage higher levels of color and phenolic characteristics were observed than during the 2019 vintage.

Volatile compounds concentration

Higher alcohols, esters, and volatile fatty acids are the three main groups into which thirteen volatile compounds found in ‘Agiorgitiko’ wines can be categorized. These compounds were identified in the wines produced in the three Nemea subzones over two consecutive vintages. The concentrations of each compound in the wines and the total concentration of the chemical families are detailed in Table IV. Higher alcohols, which are essential compounds derived from fermentation, play a significant role in enhancing the secondary aroma of wines. Their presence in concentrations below 300 mg/L can positively contribute to the complexity of the bouquet, while concentrations exceeding 400 mg/L can have a negative impact on wine quality (Ayestaran *et al.*, 2019). It was found that alcohols have the highest content among the volatile components present in all wines, as depicted in Table IV. The subtotal concentration of higher alcohols ranged from 286 mg/L to 335 mg/L, contributing favorably to the aroma of the wines in both vintages.

Table III

Mean concentration (± standard deviation) of chromatic and phenolic characteristics of “Agiorgitiko” regional wines in two vintages at PDO Nemea.

Vintage	Region	ID	Color Intensity	Color Hue	Total Anthocyanins (mg/L)	Total Phenolic Index	MCP (catechin mg/L)	BSA (catechin mg/L)
2019	**R1**	**7**	6.3±0.7b	0.6±0.1cde	251±54 bc	41±4cd	1061±172cd	283±26bcd
		45	5.3±1.0b	0.7±0.1bcd	216±14 c	33±3e	1212±161cd	234±9cde
	R2	**33**	5.6±0.1b	0.9±0.1a	322±9 ab	31±4e	858±75d	171±28e
		28	9.4±2.0a	0.5±0.1e	259±35 bc	41±2cd	1330±69c	305±50bc
		40	5.6±0.7b	0.6±0.1de	308±41 ab	38±2cde	1320±101c	316±62b
		24	5.5±0.2b	0.9±0.0a	325±24 ab	43±4c	1430±82bc	318±17b
	R3	**68**	10.6±0.2a	0.6±0.1de	299±65 bc	53±2a	1755±236b	445±11a
		74	5.2±0.7b	0.7±0.1bc	276±19 bc	44±2bc	1777±239ab	323±46b
		17	5.8±0.3b	0.8±0.1ab	236±45 bc	34±4de	1177±144cd	221±11de
		47	9.2±1.1a	0.7±0.1bcd	392±27 a	51±3ab	2131±151a	412±30a
2020	**R1**	**7**	6.8±0.1bc	0.7±0.1cd	285±4 de	35±4c	922±47de	161±2f
		45	7.3±0.1b	0.6±0.1f	346±26 b	32±2cd	861±48e	186±2c
	R2	**33**	4.6±0.0e	0.9±0.1	258±12 ef	34±1c	751±72ef	172±3e
		28	6.9±0.3b	0.6±0.1ef	339±6 b	42±2b	858±51e	146±2g
		40	6.7±0.1bc	0.6±0.1def	306±9 cd	41±2b	556±21f	201±3b
		24	6.7±0.3bc	0.6±0.1def	243±6 f	26±2d	788±24ef	176±1de
	R3	**68**	11.9±0.5a	0.6±0.1def	434±6 a	56±1a	2358±222a	171±9e
		74	7.2±0.1b	0.8±0.1b	336±1 bc	51±2a	1529±67b	184±1cd
		17	5.5±0.1d	0.7±0.1cde	318±2 bc	31±2cd	1380±46bc	177±2cde
		47	6.2±0.2c	0.7±0.1bc	340±14 b	54±3a	1182±143cd	212±2a

*Values followed by different letters are significantly different according to the post-hoc test ($p<0.05$).

The volatile fraction primarily consisted of isoamyl alcohol, 2-phenylethanol, and 1-hexanol in all wine samples, potentially acting as significant odorants, especially considering the variations observed in their concentrations among wines from different sub-regions. The levels of higher alcohols in the final wines can be ascribed to the YAN concentration in the must (Lola et al., 2022), which is influenced by the diverse soil and climatic conditions present in the vineyard (van Leeuwen et al., 2020). Notably, C6 compounds, such as 1-hexanol and (Z)-3-hexenol, play a role in imparting green and herbaceous aromas to wines. During the 2019 vintage, altitude was seen to influence the concentration of 1-hexanol, with wines from higher altitudes containing higher amounts of this compound. However, in the 2020 vintage, such differentiation among wines based on altitude was not observed.

Higher alcohols are crucial components in red wine, playing a significant quantitative role and being mainly produced as by-products of yeast amino acid metabolism during alcoholic fermentation. This group stands out as a vital category of Volatile Organic Compounds (VOCs) present in wine due to their high levels and potential to impact wine aroma directly and indirectly (Galan *et al.*, 2021). Particularly, the C6 alcohols, including 1-hexanol and 3-hexen-1-ol, are noteworthy, originating from the enzymatic oxidation of linolenic and linoleic acids in grape berries via the lipoxygenase pathway, contributing to herbaceous and green aromas. Galan *et al.* (2021) and Diez-Ozaeta *et al.* (2021) highlighted the importance of these compounds in shaping the aromatic profile of wines.

Esters, which are the primary group of volatile metabolites derived from yeast in wines, are synthesized specifically during the alcoholic fermentation. They include ethyl esters of fatty acids and acetate esters, which are crucial in the perception of fruity and floral aromas due to their low detection threshold, thus exerting a significant influence on the overall flavor profile of the wine, as reported by Ayestaran *et al.* (2019). Among these esters, ethyl octanoate and isoamyl acetate were identified as the predominant esters in all wines, with ethyl decanoate and hexyl acetate present in the lowest concentrations in the samples. Notably, ethyl octanoate, ethyl decanoate, and isoamyl acetate were detected at significantly higher levels in R2 and R3 wines in terms of quantity. It is also important mentioning that the wines produced exhibited relatively similar concentrations of esters, with R1 at 2.8 μg/L, R2 at 3.0 μg/L, and R3 at 2.7 μg/L during the 2019 vintage, and R1 at 2.8 μg/L, R2 at 2.9 μg/L, and R3 at 2.7 μg/L during the 2020 vintage, as showed in Table IV. The concentration of some esters is closely related to the seasonal climatic conditions (Song *et al.*, 2022).

Table IV

Mean concentration (± standard deviation,) of volatile compounds determined in ‘Agiorgitiko’ regional wine in two vintages at PDO Nemea

			Higher Alcohols					Esters				Fatty Acids			
Vintage	**Region**	**ID**	**Isoamyl Alcohol**	**3-(Methylthio) Ethanol**	**2-Phenylethanol**	**1-Hexanol**	**(Z)-3-Hexanol**	**Isoamyl Acetate**	**Hexyl Acetate**	**Ethyl Octanoate**	**Ethyl Decanoate**	**Hexanoic Acid**	**Isobutyric Acid**	**Butyric Acid**	**Isovaleric Acid**
2019	**R1**	7	290±2ab	1.70±0.01f	41.3±0.3ef	5.5±0.2f	0.15±0.01abc	0.33±0.01f	0.26±0.01cde	1.20±0.01e	0.44±0.03f	1.3±0.1f	1.90±0.01d	1.50±0.01a	1.5±0.1ef
		45	222±1e	1.90±0.01e	50.1±0.6ab	5.6±0.1f	0.15±0.01abc	0.60±0.01d	0.34±0.01a	1.50±0.01a	0.65±0.01e	2.1±0.1 c	2.30±0.01c	0.82±0.01f	2.6±0.3a
	R2	33	209±4f	2.30±0.01a	44.8±0.5d	7.2±0.1c	0.13±0.01cd	0.86±0.01c	0.24±0.01ef	1.30±0.01d	0.90±0.02b	2.1±0.1c	2.30±0.10c	1.07±0.01de	1.7±0.0def
		28	244±1d	2.10±0.01c	47.7±0.5c	6.6±0.1d	0.17±0.00a	0.60±0.01d	0.28±0.01c	1.30±0.01d	0.73±0.01d	1.7±0.1e	1.90±0.01d	1.11±0.03d	1.9±0.0cde
		40	271±11c	2.00±0.01d	48.9±0.5bc	5.8±0.3ef	0.14±0.02bc	0.61±0.01d	0.25±0.01de	1.40±0.01b	0.91±0.01b	2.2±0.1ab	1.70±0.01e	0.80±0.00fg	1.5±0.1f
		24	228±1e	1.50±0.01h	41.0±0.3f	4.5±0.1g	0.16±0.00ab	0.97±0.02a	0.27±0.01c	1.40±0.01b	0.44±0.02f	2.0±0.1c	2.50±0.01b	0.77±0.00g	2.2±0.1bc
	R3	68	263±2c	2.20±0.01b	37.0±0.4g	9.3±0.1a	0.16±0.02ab	0.33±0.01f	0.27±0.01cd	1.40±0.01bc	0.75±0.03d	2.2±0.1b	3.30±0.01a	1.16±0.03c	2.0±0.01bcd
		74	286±2b	2.10±0.01c	50.6±0.5a	6.5±0.2d	0.15±0.01abc	0.41±0.01e	0.31±0.01b	1.40±0.01b	0.97±0.02a	2.3±0.1a	1.90±0.01d	1.05±0.02e	1.5±0.1f
		17	299±1a	1.60±0.01g	49.1±0.2b	8.6±0.2b	0.15±0.00abc	0.95±0.02ab	0.25±0.01de	1.30±0.01c	0.84±0.01c	1.9±0.1d	1.90±0.01d	1.20±0.01c	1.5±0.1ef
		47	225±1e	1.90±0.01d	42.5±0.4e	6.3±0.2de	0.11±0.01d	0.93±0.01b	0.22±0.02f	1.40±0.01c	0.32±0.01g	1.7±0.1e	2.40±0.01b	1.25±0.01b	2.3±0.2ab
2020	**R1**	7	294±5a	1.80±0.01c	52.9±0.4c	3.6±0.1ab	0.13±0.01de	0.40±0.01i	0.22±0.01b	1.40±0.01ab	0.62±0.01c	1.2±0.1f	1.60±0.01c	0.66±0.01d	2.1±0.0f
		45	255±1e	2.70±0.01 a	43.1±0.4f	2.6±0.1f	0.12±0.01e	0.30±0.01j	0.29±0.01a	1.20±0.01abc	0.61±0.02c	1.5±0.1e	1.70±0.01b	1.04±0.01a	2.0±0.1g
	R2	33	260±1de	2.00±0.01b	48.2±0.4d	3.2±0.1cd	0.24±0.01bc	1.37±0.01a	0.11±0.01d	1.20±0.01abc	0.83±0.01ab	1.7±0.1d	1.50±0.01d	0.73±0.01c	2.6±0.1e
		28	275±1c	1.20±0.01f	58.2±0.4a	3.8±0.1a	0.34±0.01a	0.67±0.01e	0.21±0.01b	1.20±0.01abc	0.45±0.01e	1.2±0.1f	1.90±0.01a	1.03±0.01a	1.9±0.1h
		40	286±2b	1.50±0.01e	41.1±0.4g	3.4±0.2bc	0.14±0.01de	0.52±0.01g	0.11±0.01d	1.20±0.01abc	0.51±0.01d	1.9±0.1c	1.60±0.01c	0.74±0.01bc	2.7±0.1d
		24	294±1a	1.70±0.01c	41.5±0.4fg	2.4±0.1g	0.15±0.01d	0.85±0.01d	0.14±0.01cd	0.90±0.50c	0.83±0.01b	1.9±0.1c	1.80±0.01a	0.59±0.01ef	1.5±0.1i
	R3	68	261±1d	2.70±0.01a	55.1±0.4b	3.3±0.1c	0.26±0.01b	0.65±0.01f	0.32±0.03a	1.60±0.01a	0.59±0.02c	2.3±0.1a	1.50±0.01d	0.54±0.01f	3.8±0.1a
		74	272±2c	2.70±0.01a	53.4±0.4bc	3.3±0.1cd	0.15±0.02d	0.49±0.01h	0.21±0.01b	1.40±0.01ab	0.88±0.01a	2.2±0.1b	1.90±0.01a	1.01±0.01a	2.0±0.1g
		17	285±1b	1.70±0.01c	45.2±0.4e	3.1±0.1de	0.15±0.02d	1.31±0.00b	0.15±0.02c	1.00±0.01bc	0.60±0.05c	1.1±0.1g	1.60±0.01c	0.63±0.01de	3.0±0.1b
		47	211±1f	1.60±0.01d	54.5±0.4bc	2.8±0.1ef	0.23±0.01c	0.94±0.01c	0.29±0.01a	1.20±0.01abc	0.59±0.01c	1.5±0.1e	1.70±0.01b	0.78±0.01b	2.8±0.1c

*Values followed by different letters are significantly different according to the post-hoc test ($p<0.05$).

In addition to esters, another group of volatile compounds identified in wines are fatty acids, which are produced through yeast's lipid metabolism during fermentation, and serve as precursors for the formation of ethyl esters (Ayestaran *et al*., 2019). The 2019 vintage showed a higher concentration of fatty acids in the R2 wines, with R1 at 6.2, R2 at 7.7, and R3 at 6.3 mg/L, while no differences were observed in 2020, with R1 at 6.9, R2 at 6.8, and R3 at 7.0 mg/L, as indicated in Table IV. Four fatty acids were consistently measured in all wines produced during the two vintages, being the isobutyric acid the most abundant, varying between 1.7 and 3.3 mg/L and displaying significant differences among the three subzones. Hexanoic acid and isovaleric acid exhibited similar concentrations, whereas butyric acid was detected in the lowest level among all volatile fatty acids. The availability of nitrogen in the vineyard and grapes is a significant factor that influences the disposal of fatty acids (Lola *et al*., 2022).

Fatty acid ethyl esters represent the predominant category within ethyl esters, playing a vital role in imparting fruity aromas; ethyl hexanoate, octanoate, and decanoate are responsible for them. The formation of these specific compounds is facilitated by the anaerobic conditions present during fermentation; however, in the production of red wine, oxygen is often introduced at various stages of vinification, as pointed out by Tarko *et al*. (2020). This deliberate introduction of oxygen is aimed at influencing the generation of fatty acid ethyl esters, thereby impacting the aromatic characteristics of the final wine product.

The impact of altitude on the chemical composition of grape berries has only been recognized and highlighted in a limited number of recent academic articles (Barreto de Oliveira *et al*., 2019; Gutiérrez-Gamboa *et al*., 2021; Rienth *et al*., 2020); thus, to date, the effects of altitude on the chemical composition of grapes and wine remain inadequately explored. It is crucial to emphasize that climate change may result in modifications of wine geography, with projections indicating an upward migration of viticultural regions (Pomarici and Seccia, 2016). Indeed, the cultivation of vines at elevated altitudes represents one of the most efficacious novel viticultural approaches to alleviate the adverse effects of climate change on the quality of grapes and wine, particularly as it postpones the ripening of grapes (Gutiérrez-Gamboa *et al*., 2020). The viability of growing wine grapes in high-altitude regions, characterized by cooler climates, in the context of predicted higher temperatures, merits further investigation.

The generation of high-quality grapes is contingent upon the daily thermal amplitude engendered by lower nighttime temperatures typically linked to high-altitude vineyards (Gutiérrez-Gamboa *et al*., 2020). Grapevines cultivated in vineyards characterized by reduced nighttime temperatures possess an enhanced capacity for accumulating color and volatile compounds. In the domain of cool-climate viticulture, the optimal effect of *terroir* is obtained when the precocity of the grapevine variety facilitates the ripening of its fruits towards the conclusion of the growing season (van Leeuwen and Seguin, 2006), when the grapes exhibit balanced levels of soluble solids, acidity, phenolic compounds, nitrogenous substances, and aromatic constituents. The wines derived from high-altitude locations are typically characterized by more fresh aroma, elevated acidity, superior aromatic characteristics, and a lower alcohol content (Gutiérrez-Gamboa *et al*., 2020).

Multivariate analysis

The possibility to examine traits associated with the significant influence of production of volatile and phenolic aspects of the wine is provided by the huge dataset derived from wines produced by the three separate zones (R1, R2, and R3) in Nemea area, which is defined by 19 quantitative variables. As a result, when using a traditional "one-at-a-time-approach" to analyze the zone's impact on many attributes of a complex matrix like wine, conventional statistics is unable to show how similar the samples are under the influence of all factors studied. The interpretation of the experimental data is easier by multivariate analysis, which finds comparable interfaces where numerous features are reduced to lower variables. Principal Component Analysis (PCA) is a type of multivariate analysis that finds patterns in multidimensional data arrays that are similar, making it possible to select fewer conclusions with comparable characteristics. To study the influence of three different zones in a PDO on volatile and phenolic traits, as a first step, PCA was carried out in order to visualize eventual grouping of samples (Figure 1 and 2). PCA was conducted to analyze the discrimination of volatile and phenolics compounds of the experimental wines during two consecutive vintages (2019 and 2020) (Figure 1 and 2).

Concerning 2019 vintage, as it is presented in Figure 1, PC1 (25.85% variance explained) and PC2 (18.98% variance explained) did not allow perfect discrimination between wines produced in different Nemea sub zones. However, the PCA showed that the color intensity, BSA, and Total Phenolic Index) scattered in the first quadrant, delimited by the positive axis of PC1 and PC2, indicating the positive correlation with wines produced from the R3 sub zone, and more specifically the 68 and 47 vineyards from the R3 sub zone. In contrast, the two other vineyards, 17 and 74, form the R3 subzone were clustered at the opposite part of the PCA compared to the 68 and 47. In contrast, wines produced from the 17 and 74 vineyards, were clustered in the second quadrant (positive PC1-negative PC2), and were characterized by higher values of cis-3-hexanol and ethyl decanoate compared to wines produced from the 68 and 47 vineyards. Furthermore, wines produced by the R1, R2 were clustered in the left part of the PCA plot with not such a clear discrimination. It should be noticed that most wines produced by R2 subzone were clustered in the center of the PCA (Figure 1).

As illustrated in Figure 2, Principal Component 1 (PC1), which accounts for 31.55% of the variance, and Principal Component 2 (PC2), which accounts for 15.78% of the variance, exhibit a similar trend that was also identified in the 2020 vintage assessment conducted. (Figure 1). More especially, all wines from R3 Nemea subzone were clustered in the lower part of the PCA (Figure 2). However, most of the wines (47, 68 and 74) were clustered in the fourth quadrant (PC1 positive and PC2 negative) and wine 17 was clustered in the third quadrant (PC1 negative and PC2 negative). The 47, 68 and 74 wines are positive correlated to total anthocyanins, MCP, Total Phenolic index, color intensity. In addition, wines produced by the R1 Nemea subzone were clustered in the middle of the PCA plot (Figure 2).

Differences in physicochemical composition were found in red wines from different Nemea zones (R1, R2 and R3). Some volatile compounds and absorbance values associated with TPI, MCP, and Total Anthocyanins, allowed clustering of wines from different Nemea zones. It was possible to separate the wines mainly from the higher altitudes (R3), and more especially 68 and 47 vineyards; the phenolic determinations, such as TPI, MCP, and Total Anthocyanins, were the most important variables for this differentiation. Other variables such as isoamyl alcohol and isoamyl acetate were relatively important in this differentiation, which were mainly associated with the wines produced by the vineyard of R2.

In order to further investigate and elucidate the complexities of the dataset we have gathered, PCA was used as a statistical technique to extract and characterize the most influential and significant factors that have impacted the characteristics of ‘Agiorgitiko’ wines that are produced in the three distinct geographical zones designated as R1, R2, and R3. The results of this analysis are visually represented in the form of a Biplot for the two vintages examined, wherein the projection of the first principal component (PC1) in relation to the second principal component (PC2) accounts for a proportion of 44.8% for the 2019 vintage and a slightly higher proportion of 47.33% for the 2020 vintage, collectively representing a substantial portion of the total variance observed. Moreover, it is worth noting that the plot reflects a relatively minor amount of variance explained by the first two principal components in both vintages, indicating that only limited trends can be discerned from the data presented. However, when one evaluates the overarching trends of the results in their entirety, it becomes feasible to gain a generalized understanding of the effects associated with the cultivation of the ‘Agiorgitiko’ grapevine variety at different altitudes. This outcome was somewhat anticipated, particularly in light of the fact that abiotic factors have been shown to exert a considerable influence on the biosynthesis of secondary metabolites in the plants, as supported by the findings of Gao *et al*. (2019) and Pavarini *et al*. (2012).

Since PCA is an unsupervised algorithm, red wine samples have differences in the ‘Agiorgitiko’ grapevine variety and could be affected by differences in geographical origins and the techniques used by wineries and viticulturists. Therefore, these factors may lead to significant intravarietal differences, and if the intra group differences are large enough, they can affect the inter group differences, resulting in the overlapping phenomenon of wine samples from different zones. The PCA plots, as depicted in Figures 1 and 2, provide a comprehensive visualization of the distribution of wine samples among the various subgroups, and upon careful examination, it becomes evident that there exists a notable degree of overlap among these samples within the graphical representations. Consequently, this observed phenomenon of minor overlapping among the plotted points serves to suggest that the subgroups in question exhibit a certain degree of similarity in their respective data characteristics, thereby leading to the conclusion that, in extreme instances, it may encounter challenges in effectively distinguishing between the different groups based on the available data.

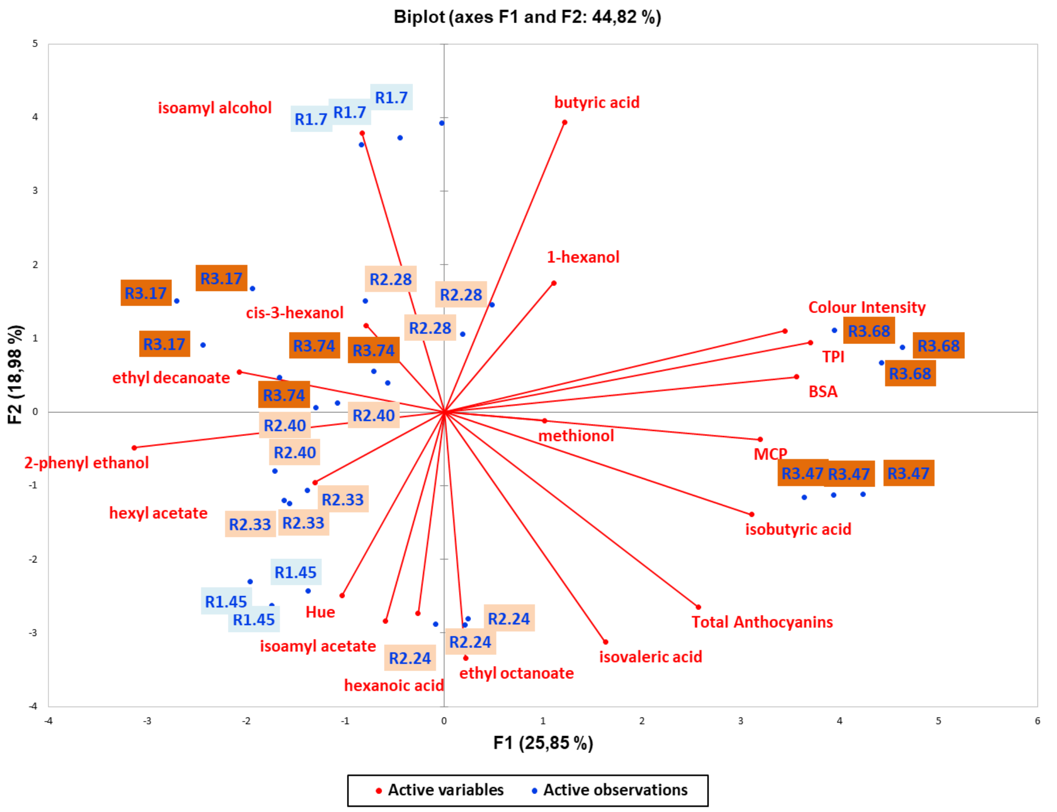

Figure 1. Unsupervised classification using a Principal Component Analysis on volatile compound and spectrophotometric assays for 2019 vintage.

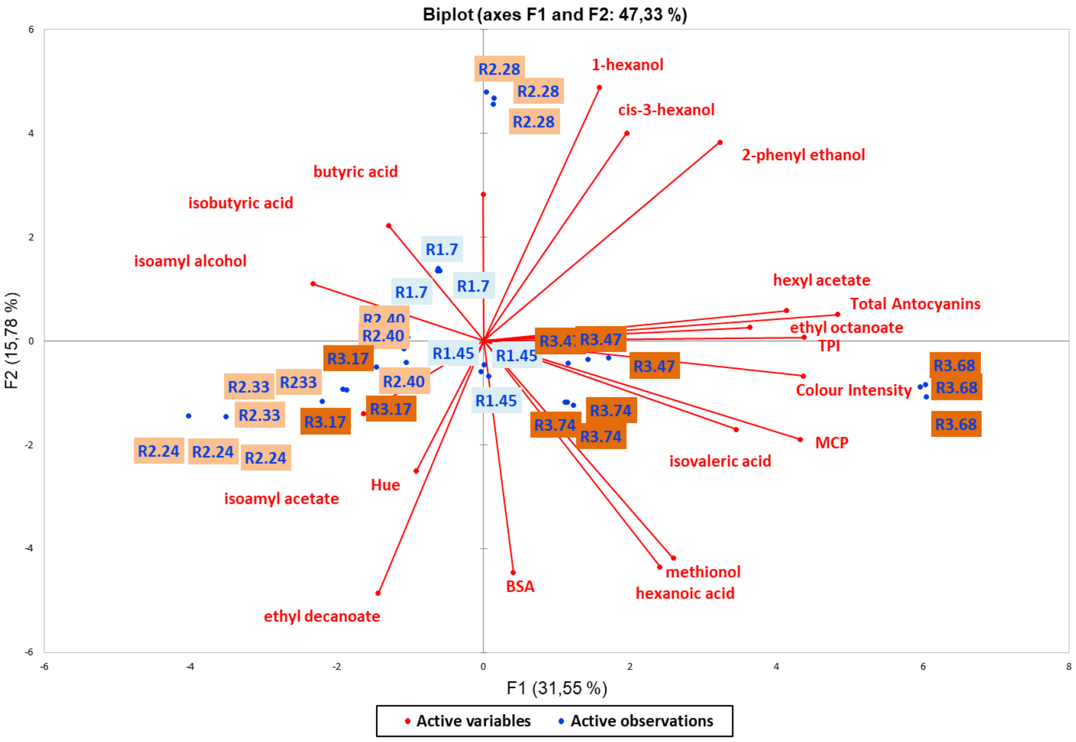

Figure 2. Unsupervised classification using a Principal Component Analysis on volatile compound and photometric assays for 2020 vintage.

Several tools, like Linear discriminant analysis (LDA), PCA, canonical analysis, HJ-biplot, and Artificial Neural Networks (ANN), have been applied to processing data and facilitating the categorization of wines based on their grapevine varieties and regions of origin, as evidenced by several studies (Diaz *et al*., 2003; Setkova *et al*., 2007; Marquez *et al*., 2008; Römisch *et al*., 2009; Saurina, 2010; Ziółkowska *et al*., 2016). These tools used in the analysis of wine, together with creation of wine profiles encompassing both chemical and sensorial aspects, and the application of multivariate procedures for data processing, play an indispensable and pivotal role in the intricate process of the multifaceted properties of wine, offering a more recent perspective for the objective annotation of various wine attributes such as grape variety, production year, and vineyard of origin. Building upon these insights, the establishment of meticulously annotated, variety-specific databases containing the profiles of numerous wine samples could serve as an initial stride towards the systematic classification of wine samples, extending beyond different subzones and other wines with Protected Designation of Origin (PDO). The application of multivariate methods such as PCA, and more especially performing 3D PCA, leads to discriminating the experimental wines, which allows them to be better classified.

By generating a PCA, the initial two components are utilized to decrease dimensionality. Consequently, to enhance the visualization of patterns like clusters of comparable expression profiles that may not be discernible in a two-dimensional PCA biplot, a three-dimensional PCA was created to more distinctly illustrate the differentiation. Therefore a 3D PCA (Figure 3 and 4) was generated by SIMCA software and shows clearer the clustering among the different Nemea subzones. A study of the data structure by 3D PCA was carried out to aid the interpretation of the obtained data and to establish whether the wines from different viticulture zones in the wider Nemea PDO zone constitute distinctive, well-defined groups. PCA was performed for all wines and variables (spectrophotometric assays and volatile compounds) to determine Nemea subzones had influenced the chemical wine profile. When the 3D Score plot is defined, a clearer grouping can be observed (Figure 3 and 4).

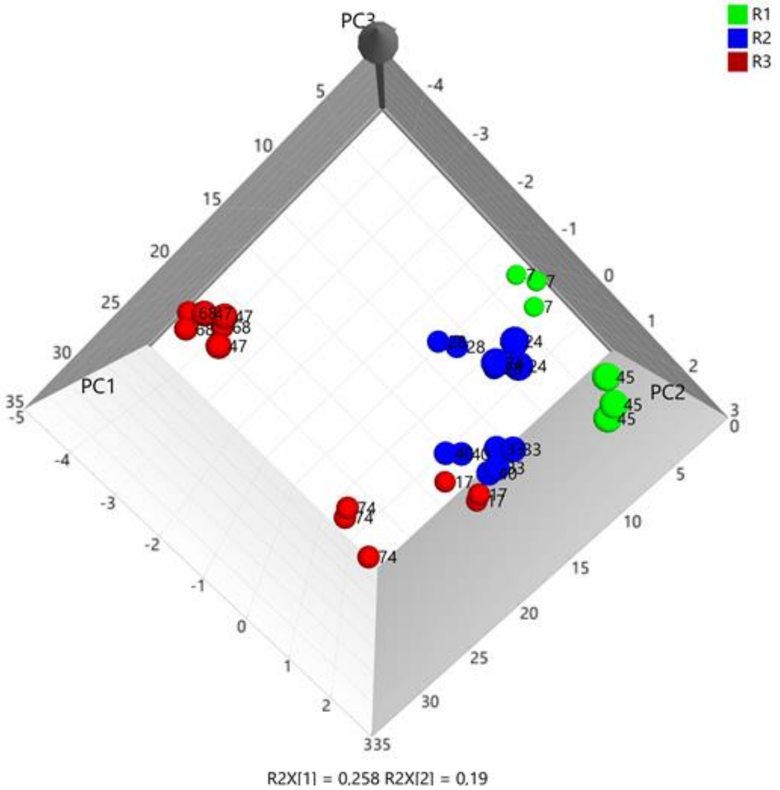

Figure 3. 3D Score plot created by 10 distinctive vineyards in PDO Nemea Zone during the 2019 vintage (3 replications of each vineyard).

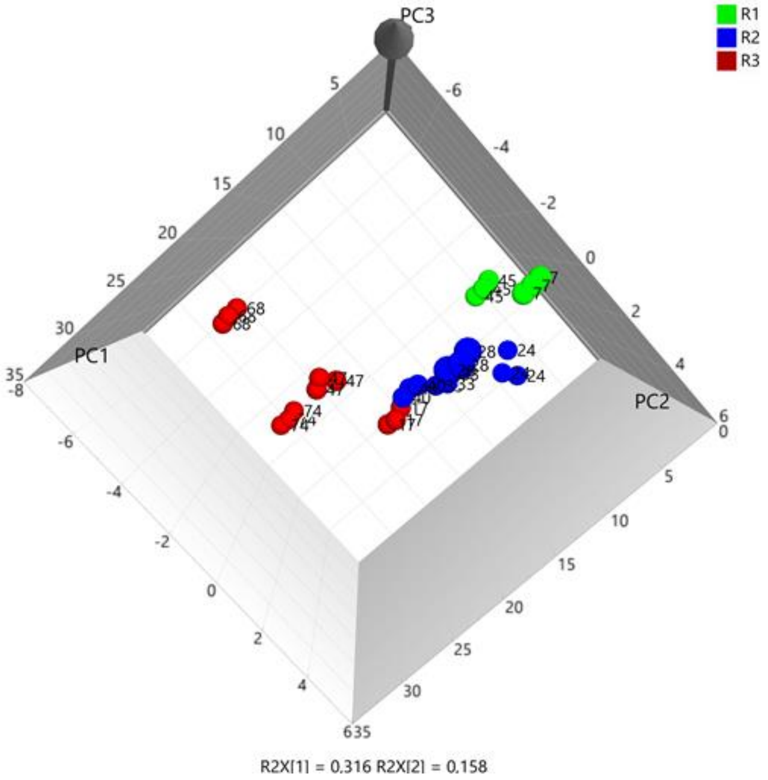

Figure 4. 3D Score plot created by 10 distinctive vineyards in PDO Nemea Zone during the 2020 vintage (3 replicantions of each vineyard).

3D score plot of PCA for the experimental 'Agiorgitiko' wine samples is showing the projection of the cases (according to the Nemea sub zones R1, R2 and R3) on the factor planes (Figure 3 and 4); wines from the R1 and R2 Nemea sub zones and wines produced by the 17 vineyard from R3, in both vintages (2019 and 2020), are positioned on the right side of the PC3, while wines produced from the R3 Nemea subzone are positioned on the left side. More specifically, wines produced from the R1 subzone were clustered on the right part. Therefore, an interesting observation from the 3D score plot, is the clear separation between the wines that were produced in higher altitude (R3) and in lower altitude (R1) in both vintages.

Sensory evaluation

A trained sensory panel described the aroma characteristics of the six 'Agiorgitiko' wines produced in this study (Figure 5). Panellists identified 2 visual, 7 aroma and 2 flavor attributes that defined the sensory properties of the 'Agiorgitiko' wines. Figure 5 shows the first two principal components. which accounted for 54.33% of the variation. PC2 separated samples that were earthy and vegetative with bitter and astringent taste from those that exhibited red fruit character with high acidity. Samples were also differentiated along PC1 between the absence and lack of olfactory attributes, aroma intensity, spices and black fruits, the visual attributes color intensity and color hue and the global mark (Overall Quality). A positive correlation between black fruit and aroma intensity was observed, which indicated the large contribution of fruity character to the overall sensory expression of all wines. Global mark (Overall Quality) was highly correlated with color intensity and hue. This fact suggests the great importance of the color characteristics to the wine quality. The vintage effect proved to be stronger than the different sub-regions. However, 'Agiorgitiko' wines belonging to R1 region were grouped in both vintages. R1 wine samples vinified in 2019 vintage were characterized by red fruit aroma, high acidity and lack of earthy and vegetative notes, while the wine samples vinified in 2020 vintage were correlated with fruity, spicy, earthy and vegetal attributes and bitter and astringent mouthfeel. On the contrary, there is no clear grouping of the 'Agiorgitiko' wines from R2 and R3 regions according to the sensory attributes analyzed in this work.

The chemical and sensory characterization of red wines produced from 'Agiorgitiko' grapes originating from various sub regions within the Nemea appellation has contributed significantly to understanding the influence of *terroir* on the sensory and aromatic qualities of wines from proximate geographical areas. Moreover, it sheds light on the distinctive aromatic and sensory profile of red wines. The influence of divergent climatic conditions is notably evident in the outcomes. The findings facilitated the discrimination among wines from different sub-regions: wines from vineyards at lower and medium altitudes exhibited similarities, whereas those from higher altitudes displayed different characteristics. Such correlations are often challenging to establish, underscoring the advantage of collaborating with a proficient sensory panel in this domain. Through the subdivision of the appellation into three sub-regions, the acquired results can provide winegrowers with specific details that can be incorporated into their wine descriptions, thereby strengthening their brand identity among consumers. The unique regional characteristics of wines pinpointed in this investigation will be further scrutinized in subsequent research endeavors, with a particular emphasis on the impact of specific winemaking techniques in defining the typical attributes of a sub-region.

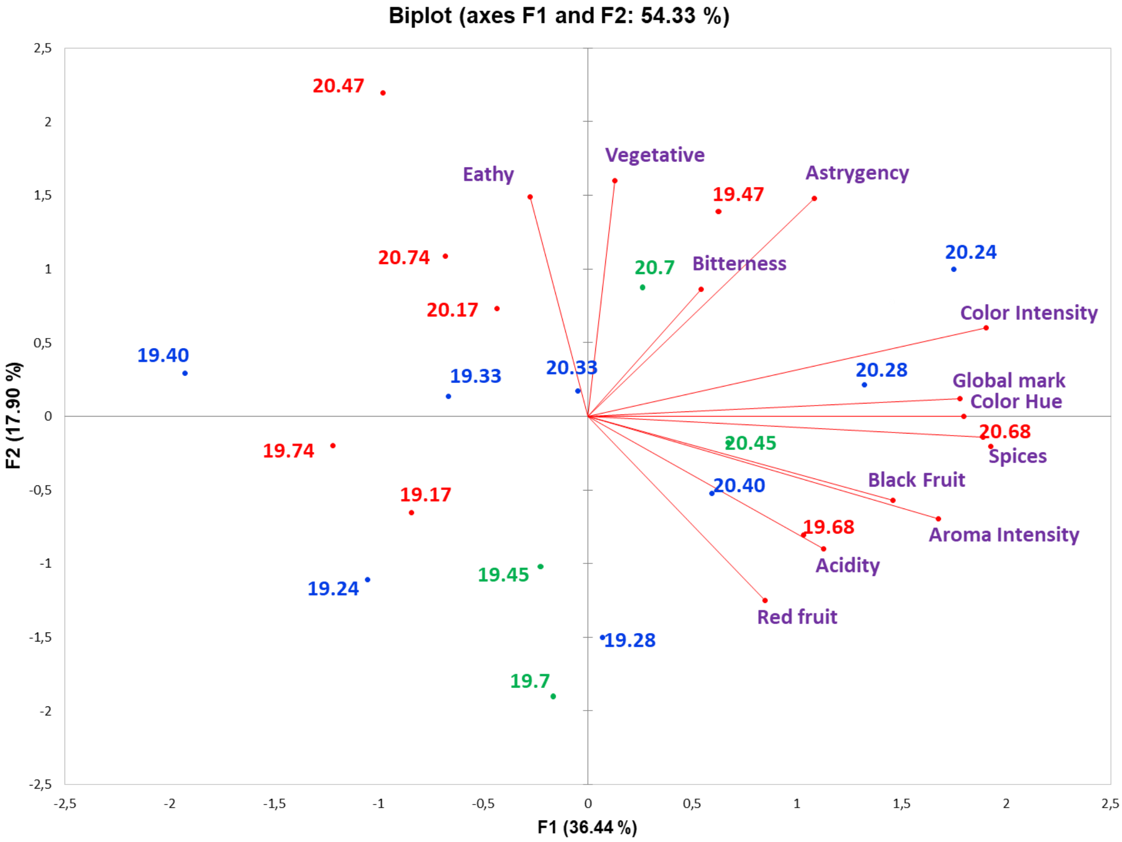

Figure 5. Principal component analysis (PCA) biplot of the sensory attributes of regional ‘Agiorgitiko’ wines at PDO Nemea. Wines colored in green belong to the R1 region, in blue to the R2 region and in red to the R3 region. Numbers 19 and 20 above the ID number referred to the 2019 and 2020 vintage, respectively.

CONCLUSIONS

It is worth emphasizing that this study represents the first successful attempt to compare and differentiate ‘Agiorgitiko’ wines, produced from different area inside the PDO Nemea zone, an important step in determination of its typicity and uniqueness of this nobble red Greek grapevine variety. Although the differences among wines produced from different vineyards in three different zones, the viticultural techniques used by each winegrower, and even by each winery, could also have a significant effect.

It can be shown that wine made from the ‘Agiorgitiko’ grape variety is an attractive and viable substitute for the more recognized international varieties, thereby increasing the portfolio available to consumers and promoting the diversification of Greek wines within both the domestic and global market. The findings acquired hold significance for the oeno-viticultural industry, offering valuable insights for the identification and distinction of Greek and Nemean single-varietal wines. These outcomes serve as practical recommendations for enhancing the characterization and differentiation processes of Greek wines, contributing to enrich the oenological landscape on various fronts. Further studies should be carried out in order to analyze more physicochemical variables that could differentiate the red wines according to their origin, such as more volatile compounds, and sensory attributes.

More studies on different Greek varieties in different *terroirs* are needed in order to increase the knowledge concerning the impacts of altitude on grape and wine quality in relation to climate and the biosynthesis of phenolic and aromatic compounds. Furthermore, the recent interest in considering the incorporation of altitude within methodologies aimed at postponing grape maturation and facilitating this phase at reduced thermal conditions can be attributed to the significance of this critical viticultural variable in mitigating the adverse impacts of climate change.

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