CHARACTERIZATION OF 'FETEASCĂ NEAGRĂ' RED WINES AGED WITH OAK STAVES: MAJOR VOLATILE COMPOUNDS, AROMATIC SERIES AND SENSORY ANALYSIS

Georgiana-Diana Dumitriu[1], Valeriu V. Cotea[2], Nieves López de Lerma[3] , Rafael A. Peinado[3] , Carmen Teodosiu[1] and Marius Niculaua[2]

[1]"Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection
RO-700050, Iasi, Dimitrie Mangeron 73
[2] University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iaşi, România, Faculty of Horticulture
RO-700490, Iasi, Mihail Sadoveanu Alley 3
[3] University of Córdoba, Agriculture Chemistry Department, Building Marie Curie, Campus de Rabanales, Agrifood Campus of International Excellence ceiA3,
ES-14014, Córdoba
E-Mail: dumitriu.diana22@yahoo.com

Aroma compounds represent an important part in the final wine quality; therefore wine scientists are continuously looking for alternative techniques to handle this aspect. Current techniques include the use of oak staves, which produce chemical and sensory properties similar to those of oak wood barrels. The effect of this technique on the major volatile composition, aromatic series and sensory characteristics of 'Feteasca neagră' autochthonous red wines from Iasi area, Romania, has been studied. Oak staves were added at the end of fermentation. A total of 10 major volatile compounds were quantified by Gas Chromatography-Flame Ionization Detection (GC-FID). Principal component analysis (PCA) of aroma compounds revealed a clear discrimination between wines aged 1.5 and 3 months. The aromatic profile of 'Feteasca neagră' wines included chemistry, fruity, buttery and floral series. Therefore, the aroma series showed that wines aged for 3 months have more complex characteristics than the wines aged for 1.5 months.
Keywords: alternative ageing, aroma compound, GC-FID, aromatic series, PCA

Charakterisierung von mit Eichenholzstäbchen (Staves) ausgebauten Rotweinen der Sorte 'Feteasca neagră': wichtigste flüchtige Verbindungen, Aromenreihen und sensorische Analyse. Aromastoffe sind ein wichtiger Bestandteil der endgültigen Weinqualität, daher suchen Oenologen ständig nach alternativen Methoden, um diesen Aspekt zu optimieren. Zu den gegenwärtig angewandten Methoden gehört die Verwendung von Eichenholzstäbchen, die chemische und sensorische Eigenschaften ähnlich denen von Eichenholzfässern erzeugen. Die Wirkung dieser Technik auf die Zusammensetzung der wichtigsten flüchtigen Substanzen, die Aromenreihen und die sensorischen Eigenschaften autochthoner Rotweine der Sorte 'Feteasca neagră' aus dem Gebiet Iasi, Rumänien, wurde untersucht. Eichenholzstäbchen wurden am Ende der Gärung hinzugefügt. Insgesamt wurden zehn flüchtige Hauptverbindungen durch GC-FID quantifiziert. Die PCA von Aromaverbindungen ergab eine klare Unterscheidung zwischen Weinen, die 1,5 bzw. 3 Monate ausgebaut wurden. Das Aromenprofil der Weine umfasste chemische, fruchtige, buttrige und blumige Reihen. Die Aromenreihen zeigten daher, dass Weine, die 3 Monate ausgebaut wurden, komplexere Eigenschaften aufweisen als Weine, die 1,5 Monate ausgebaut wurden.
Schlagwörter: alternativer Weinausbau, Aromaverbindung, GC-FID, Aromenreihe, PCA

Volatile compounds are important factors that contribute to wine aroma. During the ageing period, multiple chemical interactions occur among volatile and other compounds, changing their concentration and composition and thus modify the sensory particularities of a wine. Commonly, red wines are aged in oak barrels for different periods to improve their quality, organoleptic characteristics and complexity. During this process, volatile and non-volatile compounds are transferred from wood into wine allowing also a simultaneous micro-oxygenation through the barrel (Oberholster et al., 2015). The practice of barrel maturation is costly, due to the price of oak barrels as well as their limited lifetime, the large space and maintenance required, the contamination risk by undesirable microorganisms and the loss of wine due to evaporation. Therefore winemakers use new inexpensive techniques that simplify the ageing process, while ensuring that the favorable volatile wood compounds are also present in the wine and generate sensory properties similar to wines aged in barrels. Some alternative types of woods that can be used in winemaking include staves, segments, chips, wood dust or oenological tannins. All the above-mentioned products are made from different kinds of oak wood (American, French, Romanian, Hungarian and Pyrenean) and are subjected to a variety of toasting methods (fire, hot air and infrared radiation) and degrees of toasting (light, medium, heavy levels or performed at specific temperatures) (Gomez García-Carpintero et al., 2014).

The Council Regulation EC No. 2165/2005 authorized the use of oak wood pieces in winemaking and consequently, the addition of oak chips has become a common phenomenon. Wines treated with oak chips need a shorter time for ageing than wines in barrels, and deeper colors and higher volatile oak extraction were observed (Arapitsas et al., 2004; Del Alamo-Sanza et al., 2004).

The effects produced by the addition of oak chips into wine depend on various factors, which describe the characteristics of the wine. These include the origin of the wood (Frangipane et al., 2007), the toasting process (Fernandez de Simon et al., 2010), the type of drying (Masson et al., 2000), the amount of pieces added to the wine (Fan et al., 2006), the contact time between wine and oak (Bautista-Ortin et al., 2008), and the size of the wooden pieces (Arapitsas et al., 2004).

Major volatiles in wine are most often determined by gas chromatography coupled to flame ionization detection (GC-FID) (Peinado et al., 2004; Palomero et al., 2009). In addition, they are important for quality control, classification and sensory evaluation of red wines. Therefore, direct injection of wine samples is advisable when quantifying polar volatile compounds, when dissolved in water and ethanol-water mixtures. Some examples of major compounds with particular sensory and biochemical properties are methanol, propanol, acetaldehyde, ethyl lactate and acetoin (Saenz and Cedron, 2000; Ribereau-Gayon et al., 2000).

The research presented here aimed at examining the changes in major volatile compounds and sensory parameters, which occur in 'Fetească neagră' wine with added oak staves and after ageing for 1.5 and 3 months, respectively. The outcomes of this research would be of great practical interest to winemakers since they could improve the quality of the produced wine and in the same time decrease the operational costs.

MATERIALS AND METHODS

WINEMAKING PROCESS

'Fetească neagră' red grapes (*Vitis vinifera*) from the North-East Romania winemaking region were harvested in their optimal ripening stage. The mash obtained after crushing the grapes was subjected to maceration-fermentation process at 10 to 12 °C for 7 days. After this, the mash was pressed and the wine obtained was transferred for alcoholic and malolactic fermentation. At the end of the fermentation process four oak mini staves were added to 5 l of red wine. The dimensions in centimeters for mini staves were 1 × 10 × 1 (width × length × thickness), they were obtained from heavy-toasted French oak (*Quercus petraea*). The wines were aged for two time periods, 1.5 and 3 months, respectively, in a room at 14 to 16 °C. The experiment was done in three replicates.

ANALYTICAL PARAMETERS

O.I.V. International Oenological Codex (O.I.V., 2016) was referred to for the analysis of pH, total and volatile acidity, alcoholic strength (%v/v), and total and free SO_2 in 'Fetească neagră' red wines. All analyses were performed in triplicate.

MAJOR AROMA COMPOUNDS

Major volatile compounds were quantified in a gas chromatograph HP 6890 Series II equipped with a capillary column with molten silica CP-WAX 57 CB (50 m in length, 0.25 mm in internal diameter and 0.4 μm in coating thickness) and a Flame ionization detector (FID) and split/splitless inlet (ratio 30:1) (all equipment: Agilent Technologies, Santa Clara, USA). The chromatographic conditions and sample preparation were described by Peinado et al. (2004). Identification and quantification of the major volatile compounds was performed using standards submitted to the same treatment as the analysed samples.

AROMATIC SERIES

In order to assess the major volatile compound contribution wine aroma was analysed and the odour activity value (OAV) was calculated. The calculation was performed by dividing the amounts of an individual major volatile compound to the perception threshold described in literature (Etievant, 1991; Ferreira et al., 2000; Moreno, 2005; Czerny et al., 2008; Lopez de Lerma and Peinado, 2011). Furthermore, odorant series were formed by grouping compounds with similar aroma descriptors and calculating specific OAVs by adding the OAVs of each compound that was in that group.

SENSORY ANALYSIS

For evaluating the effect of ageing on the organoleptic characteristics, the wine samples underwent a sensory analysis. Sample preparation used the norm UNE 87-020-93 (1997) as a reference. In addition, the samples were stored in cold and transferred at room temperature (20 °C) two hours before the sensory test. Nine wide and well-defined tasting places were set up. A tasting panel of 15 tasters was used. The team leader explained all objectives and methodology. The tests consisted of evaluating the organoleptic properties aroma and flavor of the wines by means of the utilization of an ordinal scale, following the indications of the international norm ISO 4121:1987, consisting of three quality degrees, undesirable, acceptable and desirable, designating three points for each degree. Means of the absolute frequencies and the medians were used for analysis of the information, as is recommended by the norm UNE 87-020-93.

STATISTICAL ANALYSIS

The effect of the aged wines on major volatile compounds and general composition was examined via homogeneous group analysis. Principal component and cluster analyses were made to establish differences between wines due to ageing time. Statistical data analyses were performed using Statgraphics Centurion XVI of StatPoint Technologies Inc. (Warrenton, Virginia, USA).

RESULTS AND DISCUSSION

GENERAL COMPOSITION OF WINES

The general composition of 'Fetească neagră' wines treated with oak staves for 1.5 and 3 months are shown in Table 1. Wine analysis results are consistent with usual parameter determinations and were as expected for 'Fetească neagră' red wines from the North-East Romania region. Total acidity, volatile acidity and pH were similar to previous researches done in wines that used the same grape variety (Dumitriu et al., 2016) and present two homogenous groups. The volatile acidity of the wines aged for 3 months increased around 0.03 g/l in relation to the wines aged for 1.5 months. This slight variation did not affect the organoleptic quality of the wine in a negative manner. All wines had a relevant alcoholic grade for this type of red wine. Total SO_2 concentration was below the legal limits between 115 mg/l and 139 mg/l.

Table 1: Conventional parameters of aged 'Fetească neagră' wines

Parameters	Oak staves for 1.5 months					Oak staves for 3 months				
	Average	SD[a]	Min	Max	HG[b]	Average	SD[a]	Min	Max	HG[b]
pH	3.65	0.01	3.65	3.66	A	3.69	0.01	3.68	3.70	B
Total acidity (tartaric acid g/l)	5.67	0.05	5.62	5.70	A	5.40	0.03	5.37	5.43	B
Volatile acidity (acetic acid g/l)	0.53	0.02	0.51	0.54	A	0.56	0.02	0.54	0.57	B
Ethanol (%v/v)	14.95	0.05	14.90	14.99	A	14.93	0.05	14.89	14.98	A
Reducing sugars (g/l)	2.35	0.01	2.34	2.36	A	2.35	0.01	2.35	2.36	A
Free SO_2 (mg/l)	40.80	0.03	50.76	50.82	A	37.44	0.70	46.64	47.92	B
Total SO_2 (mg/)	138.45	0.98	147.32	149.11	A	114.87	0.78	124.2 3	125.7 3	B

Note: SD[a], Standard deviation; HG[b], Homogeneous Groups; Different letters indicate significant differences at $p < 0.05$ level according to the LSD test.

MAJOR AROMA COMPOUNDS AND PRINCIPAL COMPONENT ANALYSIS

In order to quantify major volatiles, we used GC-FID to analyze 'Fetească neagră' wines in contact with oak staves for 1.5 and 3 months. The ten compounds identified and quantified included alcohols, aldehydes and esters. In Table 2 the specific values are presented.

Alcohols are the major group, according to their quantities in the volatile compounds present in red wine. In wine samples aged 3 months, we found slightly higher contents in methanol, isobutanol, 2-methyl-1-butanol, propanol and 2-phenylethanol than in wine aged for 1.5 months, perhaps because of hydrolysis of the esters (Bayonove et al., 2000). During yeast fermentation of sugars and yeast metabolism of amino acids mainly alcohols are produced. Recently, Cameleyre et al. (2015) have suggested that higher alcohols have an impact on the fruity aromatic expression in model wines, such as 2-phenylethanol, which attributes to wine descriptors like rosé and perfume (Falqué et al., 2001). Furthermore, 2-methyl-1-butanol was the compound that showed the biggest concentration in all wine variants, ranging from 526.79 mg/l to 549.74 mg/l. Moreover, the single compound that could differentiate between wines in contact with oak staves aged for 1.5 and 3 months was methanol, as seen in Table 2.

Acetaldehydes, an important sensory carbonyl, are compounds formed during alcoholic fermentation and that offer herbaceous and oxidative notes in wines (Liu and Pilone, 2000). In Table 2 it can be observed that acetaldehydes values range from 31.36 mg/l to 51.37 mg/l, and acetoin values from 34.87 mg/l to 100.66 mg/l. This increase is in accordance with the ageing time. Acetoin concentration arose from the presence of acetaldehyde, which is a precursor for this compound (Romano and Suzzi, 1996).

Ethyl esters of acetic and lactic acids appear in wines during the fermentation processes (alcoholic or malolactic) as a product of yeast and malolactic bacteria. Nevertheless, during ageing there is a formation of diethyl succinate because of chemical esterification. Esters are compounds known to play a positive role in wines by the generation of a higher quality aroma, especially the fruity aromas (Perestrelo et al., 2006). Higher concentrations of ester compounds are present in wines in contact with oak staves after 3 months. Therefore, ethyl lactate increased after 3 months of ageing from 58.51 mg/l to 162.35 mg/l. During malolactic fermentation, ethyl lactate formation is a common phenomenon, but we should not rule out the existence of bacterial esterase. Therefore, the formation of the lactate ester could also be a strictly chemical process.

Wines aged for 3 months showed differences in major volatile compounds in relation to the wines aged 1.5 months. Ethyl acetate, 2-methyl-1-butanol, 2-phenylethanol and isobutanol registered higher OAV values for the analysed samples. Typically, higher values of major compounds were in wines aged with oak staves for 3 months, which may contribute to various changes in

aroma composition.

Principal component analysis (PCA) is a frequently used tool for high-dimensional data analysis, and it represents a popular multivariate statistical technique used in various scientific disciplines (Zhou et al., 2017). PCA datasets that contain observations described by several dependent variables are, in general, intercorrelated. One important aim is to extract the essential information from datasets and to present this information in orthogonal variables, also called principal components.

In Figure 1 we present the principal component analysis (PCA) results. PCA offers a visual image of the relationship between the red wines according to their major volatile compounds. With regard to the red wines (Fig. 1), the two principal components (PC1 and PC2) accounted for 98.45 % of the variance (83.13 % and 15.32 %, respectively). The first component (PC1) was characterized by a concentration of diethyl succinate, ethyl lactate and methanol in the positive loading. For the second principal component (PC2), the volatile compounds of alcohols (propanol, isobutanol and 2-methyl-1-butanol) showed positive loading.

A good separation between the two wine groups can be observed in the PCA results. The first one contains the wines aged for 1.5 months and located on the negative side of PC1 and PC2. The second group includes the wines aged for 3 months, positioned on the positive side of PC1 and PC2. Most major compounds presented a similar weight in the first principal component (Table 3) and we consider that all these compounds participate in an identical manner to discriminate wines according to ageing time.

Table 2: Concentrations of major volatile compounds (mg/l) in 'Fetească neagră' aged wines with the addition of oak staves during 1.5 and 3 months

Compounds	Oak staves for 1.5 months					Oak staves for 3 months					OS[c]	Odour threshold (mg/l)
	Average	SD[a]	Min	Max	HG[b]	Average	SD[a]	Min	Max	HG[b]		
Alcohols	884.54	6.13	878.77	890.97	A	936.85	30.46	901.75	956.31	B		
Methanol	210.90	3.09	207.80	213.98	A	233.38	9.13	223.00	240.17	B	1	500
Propanol	36.35	0.55	35.71	36.69	A	37.51	1.57	35.71	38.63	A	1	800
Isobutanol	46.18	0.57	45.84	46.84	A	48.24	2.13	45.85	49.92	A	1	40
2-Methyl-1-butanol	526.79	5.49	520.91	531.80	A	549.74	17.61	529.42	560.37	A	1	30
2-Phenylethanol	64.32	3.49	60.80	67.77	A	67.98	0.88	67.22	68.94	A	4	10
Carbonylic compounds	66.22	4.18	63.67	71.04	A	152.04	2.02	150.55	154.34	B		
Acetaldehyde	31.36	2.21	29.39	33.75	A	51.37	1.29	50.59	52.86	B	1,2	110
Acetoin	34.87	2.19	33.03	37.29	A	100.66	0.79	99.89	101.47	B	3	150
Esters	112.92	5.20	108.53	118.66	A	225.00	1.48	223.45	226.39	B		
Ethyl acetate	33.56	0.84	32.93	34.52	A	33.77	0.51	33.25	34.27	A	2	7.5
Ethyl lactate	58.51	4.62	54.92	63.72	A	162.35	1.12	161.08	163.21	B	3	150
Diethyl succinate	20.84	0.78	20.15	21.70	A	28.88	0.88	28.11	29.84	B	2	100

Note: SD[a], Standard deviation; HG[b], Homogeneous Groups; Different letters indicate significant differences at $p < 0.05$ level according to the LSD test; OS[c], Odorant series: 1 Chemical; 2 Fruity; 3 Buttery and 4 Floral

Table 3: Weight of the major volatile compounds used as classifying variables in principal component analysis

Compounds	PCA 1 (83.13%)	PCA 2 (15.32%)
Methanol	0.3825	0.0314
2-Methyl-1-butanol	0.3554	0.3222
Acetaldehyde	0.3662	-0.296
Propanol	0.2949	0.5619
Isobutanol	0.3325	0.4554
Acetoin	0.3632	-0.3071
Ethyl lactate	0.3618	-0.3174
Diethyl succinate	0.3646	-0.2992

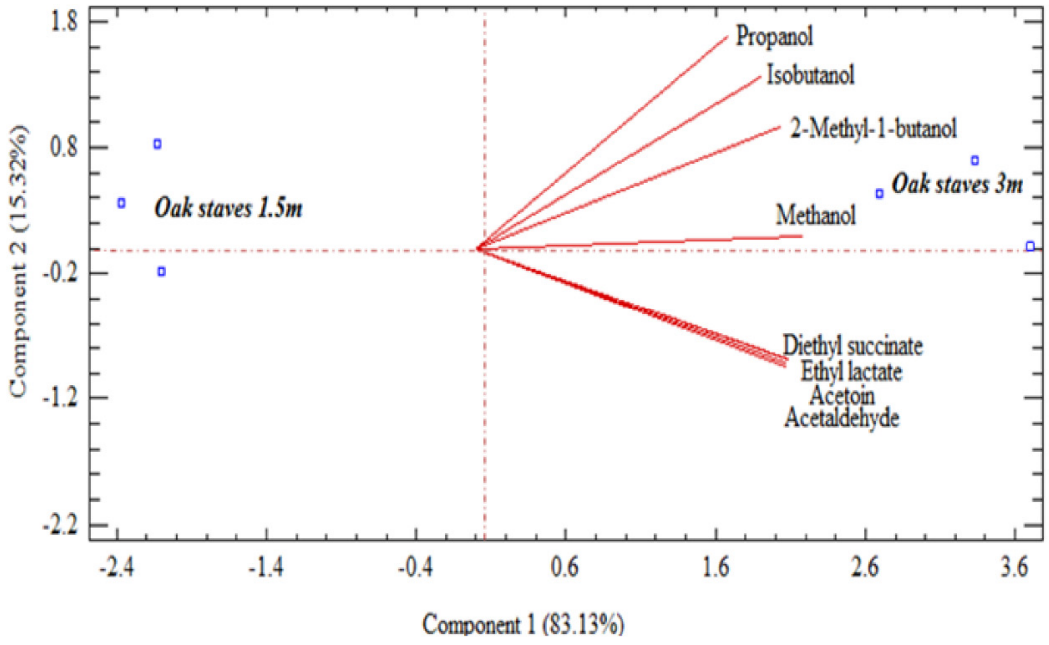

Fig. 1: Principal component analysis of wines aged for 1.5 and 3 months with oak staves

AROMATIC SERIES AND CLUSTER ANALYSIS

Aromatic series contain compounds in groups with similar odour descriptors and these represent the main constituents for 'Fetească neagră' wines, namely chemistry, fruity, buttery and floral series. We calculated the total intensities for aromatic series as the sum of the OAVs for each of the compounds assigned to a given series. Results are presented in Figure 2.

Methanol, propanol, isobutanol and 2-methyl-1-butanol presented a chemical, medicinal, nail polish odor descriptor and we grouped them in the chemistry odorant series. Particularly, 2-phenylethanol presents a rose flower descriptor. The acetaldehyde has a ripe fruit and pungent descriptor and can belong to the same group with ethyl acetate and the diethyl succinate on the fruity odorant series. Finally, acetoin and ethyl lactate constitute the buttery series (Table 2). The highest aroma contributions in the 'Fetească neagră' wines aged with oak staves were from the chemistry, floral, fruity series followed in the end by the buttery series. Wines aged for 3 months showed slightly higher values for all series than wines aged for 1.5 months.

In order to better visualize the relative distribution of the aroma series a cluster analysis was performed (Fig. 3) that separated the wine samples into clusters in terms of their nearness or similarity. The squared Euclidean distance distinguished between series similarity measurement. A smaller distance indicates the highest degree of relationship, therefore, those samples grouped to the same aromatic series. Autochthonous red wines aged with oak staves formed two clusters, thus differentiating the wines according to the ageing time. Therefore, data obtained suggest that the ageing time is the factor with a high influence on the major volatile compounds.

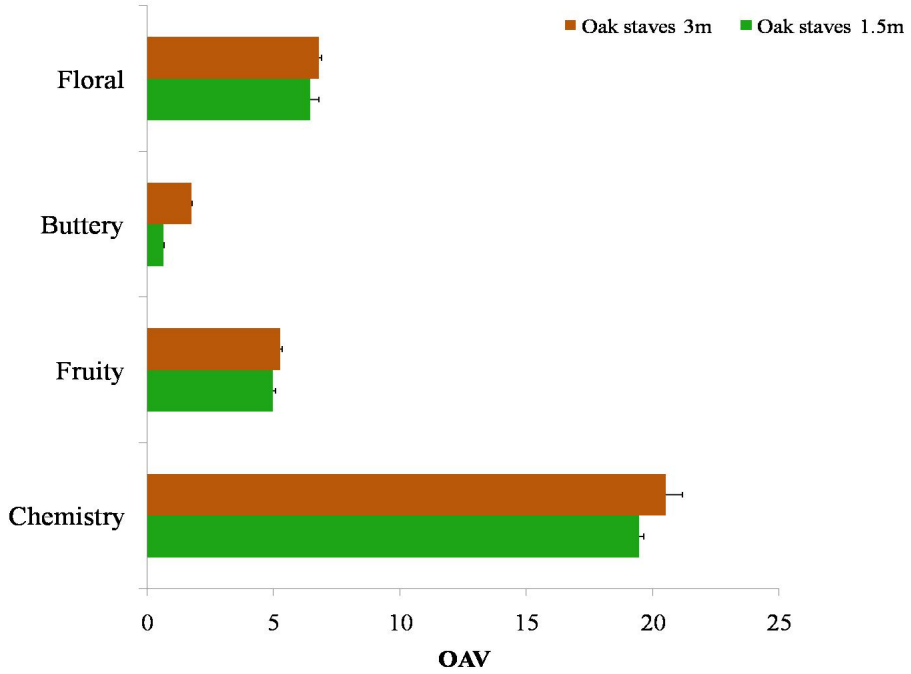

Fig. 2: Aromatic series of 'Fetească neagră' red wines aged for 1.5 months (green) and 3 months (orange)

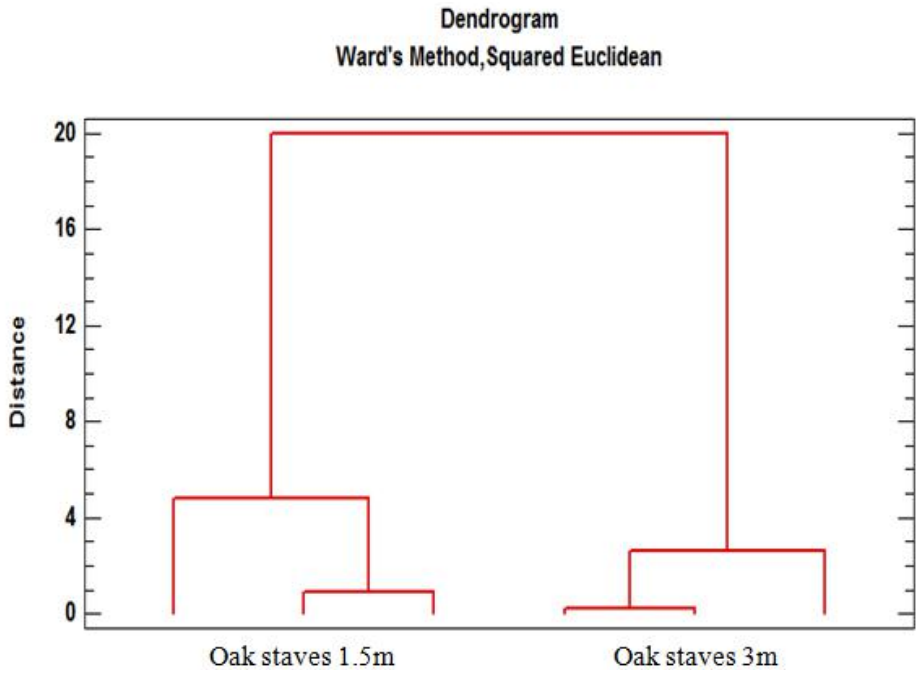

Fig. 3: Cluster analysis according to the Ward's method of aromatic series

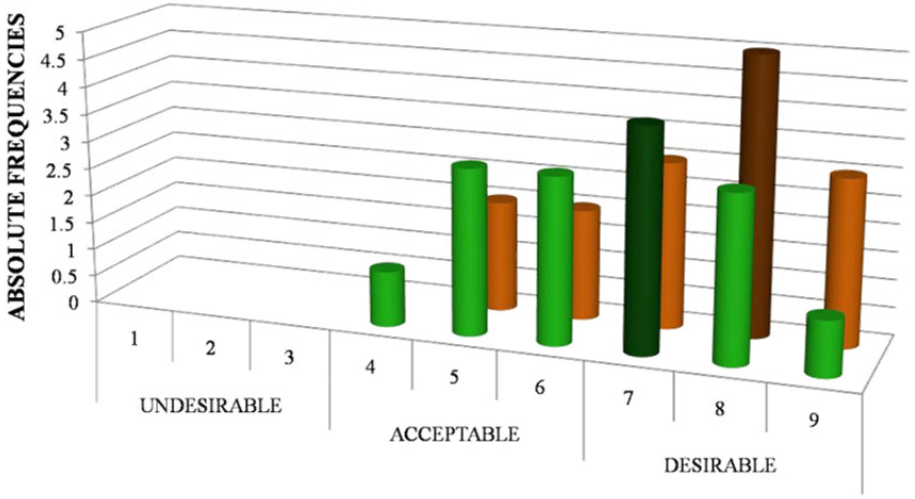

Fig.4A:

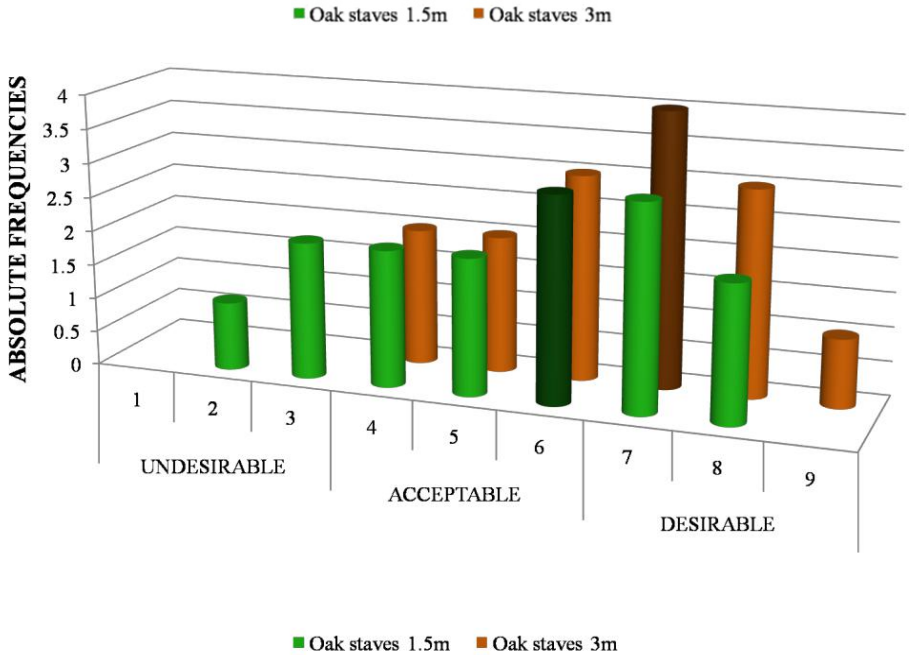

Fig.4B:

Fig. 4: Distribution of absolute frequencies and medians (darker bar) obtained for the aroma (A) and the flavor (B) of wine aged for 1.5 months (first bars) and 3 months (second bars), resp., by a tasting panel

SENSORY ANALYSIS

Samples have undergone sensory analyses in order to assess the effect on ageing on their aroma and flavor and then classed as undesirable, acceptable and desirable.
In Figure 4 (A, B) the distribution of frequencies obtained for the wine aroma and flavor by the tasting panel and its medians (darker bar) can be seen. The median values for aroma (Fig. 4A) of wines aged with oak staves for 1.5 and 3 months are located in the desirable area. The similar behavior was observed for flavor of autochthonous red wines. These results could explain the increased complexity of the aroma with ageing time.

CONCLUSIONS

Major volatile compounds and sensory attributes pre-

sented differences in red wines aged with oak staves over 1.5 and 3 months, respectively. Volatile compounds profile in analyzed samples showed an increase in the concentration of alcohols, aldehydes and esters with ageing time. The ageing technology used influenced the aroma profiles of the aged wines. Principal component analysis differentiated the two periods of ageing considered in this study. Generally, for odorant series wines aged for 3 months cause a raise, due to the contact with oak staves. Therefore, the use of oak staves is a promising option because it ensures a high wine quality accompanied by a lower cost and an environmental friendly winemaking. Moreover, oak staves provide a viable alternative to the methods of traditional production and could thus lead to a diversification of the offer for the consumers. Thus, more studies are required aimed at identification and quantification of minor aroma compounds and the effect of alternative ageing on these compounds.

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