Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds

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Abstract

A method for the simultaneous determination of major and minor volatiles composition in different types (dry, medium dry, sweet and medium sweet) of a young Tinta Negra Mole (TNM) monovarietal red wine from 2003 harvest has been validated. Wine samples preparation includes a dichloromethane liquid–liquid extraction followed by concentration under a nitrogen atmosphere. The extracted fraction was analysed by gas chromatography–mass spectrometry and give quantitative information for more than 86 analytes whose concentration range from few $\mu g\,l^{-1}$ to $259.1\,mg\,l^{-1}$. The method enables high recovery of volatile compounds in wine good linearity with (r^2) values higher than 0.980 and good sensitivity. The limits of detection range from 0.003 to $0.534\,mg\,l^{-1}$ and limits of quantification from 0.009 to $1.170\,mg\,l^{-1}$.

The method allows satisfactory determination of more than 80 compounds in the TNM red wines. These wines are characterized by a high content of higher alcohols, ethyl esters, fatty acids and lactones. The levels of sulphur compounds in Tinta Negra Mole medium sweet wines are very low, but they have the highest concentration of carbonyl compounds. Quantitative analysis of the main odorants followed by the determination of aroma index allow us elucidate the aroma of these varieties. On the basis of their odour description and odour threshold, the most powerful odorants of Tinta Negra Mole wines were tentatively established.
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1. Introduction

Tinta Negra Mole (TNM) is the main grape variety used in the production of Madeira wines. This variety represents 85–90% of the Madeira Island Vineyard, with a mean production of 42,000 hl. As far as we know, the aroma of this variety has not yet been characterized [1]. The Madeira wines alcoholic content lies between 17 and 22% (v/v) and is commercially available in different types: dry, medium dry, sweet, medium sweet, according to the sugar content [2].

Among the many factors that contributed to the typicity and quality of wine, aroma is probably the most important organoleptic characteristic and a key attribute for consumers. Several hundred chemically different flavour compounds such as: higher alcohols, aldehydes, ethyl esters of fatty acids, fatty acids, ketones, monoterpenes, volatile phenols, among others, have been found in wines [3]. They have quite different chemical and physical properties like polarity and volatility and their concentrations range from few $ng\,l^{-1}$ to more than $100\,mg\,l^{-1}$ [4].

Among the aromas that are released to the medium as secondary products of the metabolism of the yeasts, were the fusel alcohols: *iso*-butanol, *iso*-amyl alcohol, 2-phenylethanol and propanol. These compounds can be synthesised by yeast action through two mechanisms: anabolic pathway from glucose, or catabolic pathway from their corresponding amino acids (valine, leucine, *iso*-leucine and phenylalanine) [5]. Another compound related with the catabolic pathway and which was associated with this factor was methionol, formed from the amino acid methionine (Fig. 1). It should be remembered that the composition in amino acids depends on the variety of grape [6–8] and for that reason all these volatile compounds are related to the variety of grape used.

Over the last few decades wine aroma has been thoroughly studied, resulting in knowledge of about 800 compounds as con-

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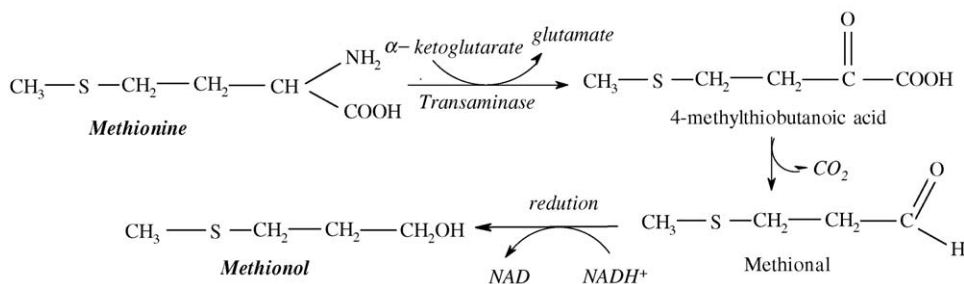*E-mail address:* jsc@uma.pt (J.S. Câmara).

Fig. 1. Suggested mechanism for methionol formation from methionine by yeasts.

stituents of the volatile fraction of the wine. Some components are present in high concentration (hundreds of $mg\,l^{-1}$), but most of them are found at the low $ng\,l^{-1}$ level [9–13]. Therefore some components need to be extracted and concentrated before analysis, while others can be analysed by GC with direct injection. Several classical analytical methods such as liquid–liquid extraction [14,15], simultaneous distillation–extraction [16], solid phase extraction [17], supercritical fluid extraction [18], microwaves extraction [19] and ultrasound extraction [20], among others, have been developed for the analysis of the minor volatile compounds in wines. Although it is a time-consuming technique liquid–liquid extraction is a widely used sample preparation method for the determination of wine volatiles which extract contains a wide spectrum of components [21]. Among the solvents that have been used for the enrichment of aroma substances, dichloromethane has been found to be well suited for extracting volatiles from a matrix with a high alcohol content such as TNM wines.

Identification of wine aroma components and the relationships between their relative content may be a useful tool in differentiating the wines from different varieties and establishing criteria of genuineness to improve the quality of the wines, prevent fraud and guarantee their origin. Volatile composition of TNM wines was investigated in this work. The pool of compounds analysed include: grape aroma compounds such as the terpene alcohols; pre-fermentative compounds which are the C_6 alcohols; a large group of secondary or fermentative volatile compounds in which are included: higher alcohols, ethyl esters of fatty acids, acetates of higher alcohols, fatty acids and lactones; and finally, post-fermentative compounds in which are included those extracted from wood such as vanillin, 2-furfural and its derivates. The aroma index values were determined to elucidate which compounds are considered as aroma contributing substances, in order to offer a means of evaluating the potential aroma of this variety.

2. Experimental

2.1. Reagents

All reagents used were of analytical quality. Absolute ethanol was purchased from Panreac (Barcelone, Spain), dichloromethane was HPLC grade quality was from LabScan and solid anhydrous sodium sulphate (analytical grade) was purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q purification system (Millipore). Solvents did not require additional destilation. The pure reference compounds used were from Sigma–Aldrich (Spain).

2.2. Standard solutions

Exact volumes of the chemical standard compounds were dissolved in absolute ethanol and made up to volume (50 ml). This standard solution was dissolved in ethanol at concentration three orders of magnitude higher than typically found in wines with approximately six calibration points for each standard. These solutions were then diluted with water and ethanol adjusting the final alcohol content to 18% (v/v) to prepare the calibration plots and to spike different wine samples. All the synthetic wines samples used in the calibration graphs were $6\,g\,l^{-1}$ of tartaric acid and pH 3.3–3.4 adjusted with 1 M NaOH (synthetic wine matrix). Octan-3-ol was employed as internal standard. All these solutions were stored at $4\,°C$.

2.3. Sample wines

The TNM red wines used in this study were made from the 2003 harvest grapes grown in the Portuguese RAM Appellation. Grapes of TNM were crushed, de-stemmed, racked and pressed. The musts were fermented in stainless-steel containers, with spontaneous yeast. Alcoholic fermentation was carried out at $22\,°C$ and stopped by addition of natural grape spirits according to the wine sugar content to obtain. The different TNM wines types produced were: dry (TNM-D), medium dry (TNM-MD), sweet (TMN-S) and medium sweet (TNM-MS). The wine samples, 12 of each wine type, were taken directly from the cellars in October 2004, and stored at $-28\,°C$ until analysis.

2.4. Sample extraction conditions

To 50 ml of each sample wines were added 25 μl of internal standard (octan-3-ol), in hydro alcoholic solution (1/1, v/v) at $422\,mg\,l^{-1}$ and 5 g of sodium sulphate was added to the samples which was extracted twice with 5 ml of dichloromethane. Both organic phases obtained were blended and dried over anhydrous sodium sulphate and concentrated in a roto-evaporator to a final volume of 2–3 ml and, finally, under a stream of pure N_2 to 500 μl. The extract was injected (1 μl) into the GC–MS. A total ion chromatogram of volatile compounds from TNM red wine samples is illustrated in Fig. 2. Identification was achieved by
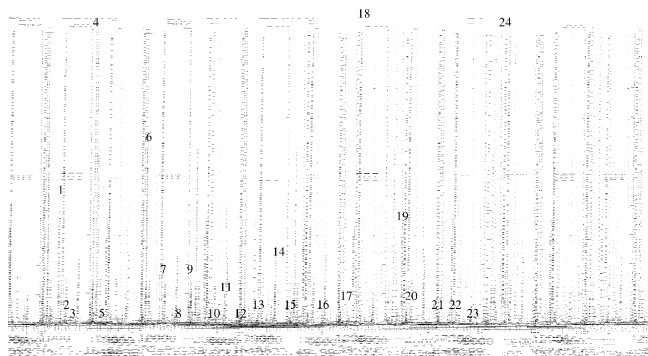
Fig. 2. TIC chromatogram of a TNM-MD red wine dichloromethane extract. Peak identification: (1) 2-methylpropan-1-ol; (2) isoamyl acetate; (3) butan-1-ol; (4) 3-methylbutan-1-ol; (5) ethyl hexanoate; (6) ethyl lactate; (7) octan-3-ol (internal standard); (8) ethyl octanoate; (9) acetic acid; (10) *cis*-dioxane; (11) (D,L)-butan-2,3-diol; (12) 2-methylpropanoic acid; (13) (R,S)-butan-2,3-diol; (14) ethyl decanoate; (15) diethyl succinate; (16) methionol; (17) methyl-2-ethylhexanoate; (18) β-phenylethanol; (19) ethyl 3-hydroxybutyrate; (20) octanoic acid; (21) γ-octalactone; (22) γ-nonalactone; (23) α-hydroxyphenylpropanoic acid; (24) ethyl succinate.

comparisons with mass spectra obtained from the sample with those from the pure standards injected in the same conditions by comparing the Kováts index and the mass spectra presents in the NIST MS library Database, or in the literature.

2.5. Gas chromatography–mass spectrometry (GC–MS) conditions

Extracts were analysed using a Varian Star 3400 Cx Series II gas chromatograph equipped with Varian Saturn III mass selective detector and Saturn GC–MS workstation software. The column used was DB-Waxetr (30 m × 0.25 mm i.d. × 0.25 μm film thickness) silica capillary column. Splitless injection was used. The carrier gas was helium at a flow rate of 1 ml min^{-1}. The oven temperature program was: 40 °C (for 1 min), then increased to 220 °C, at 2 °C min^{-1}, and held for 10 min. The ion trap detector was set as follows: transfer line temperature 220 °C; manifold and trap temperatures 180 °C. The mass range was m/z 30–300, the emission current 15 μA and the electron multiplier was set in the relative mode to the auto tune procedures. All mass spectra were acquired in the electron impact (EI) mode ($E_i = 70$ eV, source temperature, 180 °C).

2.6. Method validation

The validation parameters studied were, response linearity, the determination of repeatability (precision), evaluation of the recovery of known quantities of substances (accuracy) and the determination of limits of detection and quantification.

2.6.1. Study of linearity, repeatability and recovery

For each component five-point graphs were obtained in the range of concentrations showed in Table 1. Duplicate calibration graphs, were drawn by the least-squares linear regression method using the relative peak area as response versus concentration. The correlation coefficient was >0.98. Regression,

slope and origin intercept (Table 1) were calculated by linear least-squares regression.

Repeatability (precision) was evaluated by the relative standard deviation of six independent assays performed under the same analytical conditions in the shortest period of time. For each assay the mean values, standard deviation and coefficients of variation for all compounds were calculated.

Recovery was evaluated by addition of volatile compounds to wines. Samples were submitted to six successive extractions with dichloromethane, after concentration, each organic phase was injected twice into GC/MS. For each volatile compounds the recovery percentage was determined by the ratio $(C_1 - C_0/C_2) \times 100$, where C_0 is the concentration of the analyte in the wine, C_1 the concentration of the analyte in spiked wine sample and C_2 is the concentration of the analyte added to wine sample.

2.6.2. Limits of detection and quantification

The limits of detection (LOD) were estimated as the concentration of the analyte that produce a signal-to-noise ratio of three times the standard deviation of the y-residuals of the calibration graph, that is $3s_{y/x}/b$, where $s_{y/x}$ is the blank standard deviation and b is the slope of the line regression. The linear range experiments provide the necessary information to calculate the limits of detection, by extrapolating from the lowest concentration point on the linear calibration curve. The limit of quantification (LOQ) can also be estimated as the concentration of analyte producing a signal 10 times that of the noise.

2.7. Quantification

The quantification was carried out following the internal standard quantification method. Thus, octan-3-ol was chosen as internal standard [25 μl of a 0.422 mg l^{-1} solution in ethanol (1:1, v/v) of this internal standard was added to each standard and sample]. Quantitative data of the identified compounds were obtained by interpolation of the relative areas versus the internal standard area, in the calibration graphs built for pure reference compounds. The concentration of volatile compounds for which there was no pure reference available was obtained by using the same calibration graphs as one of the compounds with the most similar chemical structure. Since the repeatability of the chromatographic method was very good (with coefficients of variation lower than 4.0% in average), only two injection of each dichloromethane extract was carried out.

3. Results and discussion

3.1. Method validation

The performance of the method in terms of linearity, precision and accuracy are shown in Tables 1 and 2. The method had to be assessed by estimating the linear range, limits of detection and quantification and percentage of recoveries and yields of extraction. The quantification was carried through construction of calibration curves at five levels, with the extracted standards, using the same analytical conditions that the samples

Table 1
Method linearity data for the compounds identified in different types TNM red wines

Kováts index	Compounds	Linear range (mg l^{-1})	r^2	Slope	Intercept	LOL (%)
Terpenes						
1501	β-Citronellal	0.4–103.2	0.997	0.921 ± 0003	−2.178	99.0
1641	Linalool	0.4–115.2	0.997	0.473 ± 0.031	−0.562	99.0
1725	α-Terpineol	0.24–45.4	0.998	1.358 ± 0.004	−0.389	99.7
1827	Nerol	0.5–41.7	0.999	0.550 ± 0.046	−0.449	99.0
1845	β-Damascenone	0.5–112.1	0.983	0.636 ± 0.057	−1.088	99.0
1877	Geraniol	0.4–105.4	0.994	0.579 ± 0.017	−1.104	99.0
2007	(E)-Nerolidol	0.4–105.0	0.997	0.548 ± 0.061	−0.399	99.0
Higher alcohols						
1165	Butan-1-ol	2.5–689.8	0.993	0.228 ± 0.0009	−0.566	99.6
1227	3-Methylbutan-1-ol	6.7–2825.6	0.982	0.082 ± 0.0004	−0.046	99.6
1241	Hexan-2-ol	4.0–996.3	0.992	0.157 ± 0.001	−1.464	99.3
1378	Hexan-1-ol	1.2–1023.9	0.995	0.025 ± 0.0001	−0.020	99.4
1410	(E)-3-hexen-1-ol	0.7–50.7	0.998	0.292 ± 0.004	−0.129	98.5
1434	(Z)-2-hexen-1-ol	0.4–50.0	0.999	0.250 ± 0.002	−0.089	99.3
1515	2-Ethyl hexan-1-ol	1.2–349.6	0.987	0.179 ± 0.001	−0.101	99.4
1606	(R,S)-Butan-2,3-diol	17.0–1406.5	0.992	0.002 ± 0.001	−0.030	100.0
1689	Propan-2-ol	5.6–235.6	0.987	0.006 ± 0.0001	−0.032	97.4
1938	2-Phenylethanol	14.9–744.3	0.993	0.750 ± 0.002	−6.649	99.8
2107	2-Phenoxyethanol	19.8–2980	0.992	0.158 ± 0.002	−2.117	98.8
Acetates						
1141	Phenylethyl acetate	2.2–540.7	0.997	0.007 ± 0.0001	−0.014	100.0
1845	Isoamly acetate	0.4–302. 8	0.991	0.2536 ± 0.008	−0.120	96.5
Ethyl esters						
1066	Ethyl butyrate	0.9–259.2	0.987	0.275 ± 0.013	−0.338	95.2
1148	Ethyl pentanoate	1.0–100.9	0.998	0.547 ± 0.028	−0.588	95.0
1254	Ethyl hexanoate	1.8–259.5	0.988	0.177 ± 0.007	−0.223	95.8
1371	Ethyl lactate	2.0–480.4	0.999	0.113 ± 0.002	−0.121	98.1
1456	Ethyl octanoate	19.5–994.8	1.000	0.002 ± 0.000	−0.053	96.9
1546	Ethyl 3-hydroxybutanoate	1.5–102.0	0.990	0.290 ± 0.010	−0.482	96.6
1659	Ethyl decanoate	0.1–211.4	0.996	0.599 ± 0.000	−0.077	100.0
1673	Ethyl benzoate	1.2–303.1	0.985	0.165 ± 0.000	−0.770	100.0
1697	Ethyl 2-furoate	2.1–256.2	0.994	0.010 ± 0.000	−0.018	97.1
1706	Ethyl succinate	27.6–2158.7	0.987	0.136 ± 0.001	−3.582	99.2
1837	Ethyl salicylate	5.2–1297.2	0.987	0.120 ± 0.003	−4.724	97.8
1865	Ethyl dodecanoate	4.6–205.6	0.994	0.012 ± 0.000	−0.014	100.0
2104	Ethyl cinnamate	4.8–1202.9	0.994	0.061 ± 0.003	−0.674	95.6
Fatty acids						
1485	Acetic acid	10.1–853.1	0.990	0.052 ± 0.0014	−0.370	97.3
1600	3-Methylbutanoic acid	0.5–1036.9	0.994	0.059 ± 0.000	−0.032	100.0
1882	Hexanoic acid	1.5–363.1	0.999	0.477 ± 0.000	−0.684	100.0
2059	Octanoic acid	5.6–1037.2	0.981	0.042 ± 0.000	−0.216	99.7
2196	Decanoic acid	0.5–37.8	0.985	0.053 ± 0.000	−0.005	100.0
2280	Benzoic acid	2.4–360	0.986	0.007 ± 0.000	−0.041	97.9
2345	Phenylacetic acid	7.0–60.2	0.996	0.041 ± 0.006	−0.035	84.9
Carbonyl compounds						
1101	Hexanal	1.2–300	0.992	0.209 ± 0.007	−0.161	96.6
1556	(Z)-2-nonanal	1.2–124.3	0.999	0.060 ± 0.000	−0.088	100.0
1549	Benzaldehyde	0.6–381.4	0.995	0.033 ± 0.000	−0.006	99.4
1674	Phenylethanal	1.3–586.7	0.989	0.118 ± 0.000	−0.151	100.0
Furan compounds						
1494	2-Furfural	2.8–310.6	0.987	0.179 ± 0.002	−0.512	98.7
1599	5-Methyl-2-furfural	1.1–286	0.992	0.128 ± 0.003	−0.319	97.7
Sulphur compounds						
1748	Methionol	1.2–297.2	0.983	0.865 ± 0.004	−1.183	99.6
Volatile phenols						
1894	2-methoxyphenol	5.6–1400	0.983	0.090 ± 0.001	−1.428	99.1
2023	Phenol	1.1–38.4	0.986	0.033 ± 0.002	−0.039	95.2
2118	Eugenol	0.5–127.2	0.991	0.028 ± 0.036	−0.424	86.9
2143	4-Ethylphenol	2.4–360	0.982	0.005 ± 0.000	−0.015	100.0
2276	Vanillin	6.2–59.5	0.996	0.031 ± 0.004	−0.070	87.5

Table 2
Performance characteristics of the liquid–liquid extraction method. Detection (LOD) and quantification (LOQ) limits, recoveries and yield extraction data

Compounds	LOD (mg l^{-1})	LOQ (mg l^{-1})	%Recovery	R.S.D. (%)	%Yield extraction
Terpenes					
β-Citronellal	0.008	0.028	43.5	7.2	99.9
Linalool	0.008	0.028	51.6	4.6	91.1
α-Terpineol	0.003	0.011	73.9	5.3	100.7
Nerol	0.002	0.007	45.1	2.9	99.9
β-Damascenone	0.022	0.072	60.3	2.1	103.7
Geraniol	0.011	0.038	54.2	3.5	102.1
(E)-Nerolidol	0.021	0.011	49.9	6.2	106.4
Higher alcohols					
Butan-1-ol	0.087	0.290	56.6	5.1	103.9
3-Methylbutan-1-ol	0.534	1.780	73.9	0.7	103.6
Hexan-2-ol	0.127	0.374	68.6	3.2	102.4
Hexan-1-ol	0.104	0.348	112.8	1.1	101.5
(E)-3-hexen-1-ol	0.003	0.010	80.5	4.3	101.2
(Z)-2-hexen-1-ol	0.003	0.009	81.2	10.6	101.0
2-Ethyl hexan-1-ol	0.057	0.190	75.2	4.9	104.5
(R,S)-Butan-2,3-diol	0.196	0.653	42.9	6.7	95.8
Propan-2-ol	0.041	0.135	55.6	2.7	98.4
2-Phenylethanol	0.095	0.317	76.3	0.9	97.4
2-Phenoxyethanol	0.414	1.378	52.7	2.9	101.8
Acetates					
Phenylethyl acetate	0.047	0.157	76.9	1.4	100.6
Isoamly acetate	0.038	0.126	81.8	3.8	102.7
Ethyl esters					
Ethyl butyrate	0.038	0.128	84.7	9.1	102.7
Ethyl pentanoate	0.006	0.015	71.8	4.9	100.6
Ethyl hexanoate	0.037	0.123	108.7	8.5	103.0
Ethyl lactate	0.029	0.096	86.8	4.6	100.5
Ethyl octanoate	0.084	0.281	109.3	1.3	100.1
Ethyl 3-hydroxybutanoate	0.013	0.042	55.1	5.1	103.0
Ethyl decanoate	0.017	0.056	75.0	1.9	101.7
Ethyl benzoate	0.082	0.287	52.1	7.2	105.2
Ethyl 2-furoate	0.030	0.101	28.9	6.1	96.6
Ethyl succinate	0.351	1.170	93.7	7.9	103.1
Ethyl salicylate	0.215	0.682	43.4	9.4	103.4
Ethyl dodecanoate	0.024	0.081	111.6	5.3	104.0
Ethyl cinnamate	0.131	0.548	56.8	8.3	114.9
Fatty acids					
Acetic acid	0.126	0.420	54.7	2.0	103.9
3-Methylbutanoic acid	0.106	0.352	88.8	4.9	93.8
Hexanoic acid	0.014	0.048	88.9	1.8	100.7
Octanoic acid	0.204	0.679	65.8	1.1	103.6
Decanoic acid	0.007	0.022	81.4	8.5	106.2
Phenylacetic acid	0.005	0.018	59.4	3.4	100.5
Benzoic acid	0.066	0.221	49.5	1.5	105.6
Carbonyl compounds					
Hexanal	0.040	0.123	15.6	7.2	102.5
(Z)-2-nonanal	0.006	0.021	70.6	6.4	100.5
Benzaldehyde	0.039	0.131	67.1	1.9	119.0
Phenylethanal	0.081	0.269	103.8	4.7	98.7
Furan compounds					
2-Furfural	0.051	0.171	80.3	1.3	103.1
5-Methyl-2-furfural	0.037	0.149	54.5	4.6	102.4
Sulphur compounds					
Methionol	0.056	0.188	13.4	1.0	100.7
Volatile phenols					
2-Methoxyphenol	0.265	0.837	48.0	6.0	103.6
Phenol	0.007	0.022	57.7	9.2	81.8
Eugenol	0.017	0.056	59.3	7.1	116.3
4-Ethylphenol	0.075	0.251	34.5	9.4	84.9
Vanillin	0.074	0.245	33.8	8.8	99.6

were tested in duplicate. The (volatile compound/internal standard) peak area ratio was used for each compound. Calibration solutions, in the range specified in Table 1, were prepared by suitable dilution of the global solution. To calculate this calibration graphs, linear least-square regression was used. For most of the compounds studied, the resulting calibration curves obtained by plotting the GC–MS response versus analyte concentration were found to have good linearity in the range of concentrations studied, with regression coefficient (r^2) values ranging between 0.981 (octanoic acid) and 1.000 (ethyl octanoate). It was also corroborated by "on-line linearity" (LOL), with values higher than 85%.

The method sensitivity given by the slope of the straight calibration graphs depends on extraction efficiency and detector response for each compound. With this procedure, high sensitivities were obtained for α-terpineol, β-citronellal, methionol and 2-phenylethanol.

The recovery percentage of the studied compounds added to TNM wine were calculated and are shown in Table 2. As expected, the range of recoveries is very wide. The results show that the compounds highly soluble in water, such as acetic acid, methionol and ethyl lactate, are poorly extracted.

The repeatability of the method was estimated by the relative standard deviation (R.S.D.) of the concentrations for six consecutive extractions of a synthetic wine. The values obtained for this parameter ranged from 0.7% for 3-methylbutan-1-ol to 10.6% for (Z)-2-hexen-1-ol, with an average of about 4.0% for all analytes considered. The limits of detection (LOD) were estimated from the area corresponding to three-fold the system noise. As presented in Table 2 the obtained values ranged from 2.0 μg l^{-1} for nerol to 0.53 mg l^{-1} for 3-methylbutan-1-ol. For the limits of quantification (LOQ) the values ranged from 9.0 μg l^{-1} for (Z)-2-hexen-1-ol to 1.78 mg l^{-1} for ethyl 3-hydroxybutanoate (Table 2).

3.2. Identification of volatile components

The analytical method proposed allowed the correct identification and quantification over than 90 compounds in the volatile fraction of TNM red wines, the majority being higher alcohols (mainly isoamyl alcohols and (R,S)-butan-2,3-diol), ethyl esters of medium-chain fatty acids (hexanoic and octanoic acids), fatty acids, carbonyl compounds and acetates from higher alcohols. Amongst the other components present were detected several furan derivatives (5), seven lactones, the isomers of dioxanes and dioxolanes (4) and some volatile phenols. Only three sulphur-containing compounds were identified: 2-(methylthio)-ethanol, methionol and 5-ethoxythiazole.

Free volatile compounds isolated from TMN-D red wine accounted for 569.7 mg l^{-1}), a value much higher than that obtained for the others TMN wine types, which present contents of 247.5, 203.1 and 205.8 mg l^{-1}, for TMN-MD; TMN-S and TMN-MD, respectively. The results are consistent with the vinification process. The average values (mean ± standard deviation) determined for the volatile compounds in TNM-D, TNM-MD, TNM-S and TNM-MS red wines from the 2003 harvest, are present in Table 3.

3.2.1. Higher alcohols

Are quantitatively the largest group of the volatile compounds in TNM red wines. This volatile fraction is composed mainly by n-alcohols of C_6 chain length (related to the lipoxygenase activity of the grape) and aromatic compounds such as benzyl alcohol and 2-phenylethanol. The presence of these two compounds may cause a "flowery" and "sweet" notes which could be considered as a positive characteristic for TNM wines variety. The total concentration of this family of compounds calculated in the different TNM wine types analysed are shown in Fig. 3.

TNM-S and TNM-MS are the wine types that show by far the lowest content of higher alcohols. The alcohol fraction of TNM-D is significantly different at the 95% level from the other types of TNM red wines studied. 3-Methylbutan-1-ol and (R,S)-butan-2,3-diol were markedly the most abundant higher alcohols, being present at levels higher than its perception threshold (30 mg l^{-1} for 3-methylbutan-1-ol), thus its sensorial contribution with "banana" and "alcohol, fusel" odour, is expected. Identical values of 3-methylbutan-1-ol were observed in the analysed wines from the TNM-S and TNM-MS (29.7 ± 0.3 mg l^{-1}), while in those from TNM-MD these values were slightly higher 61.9 ± 0.03 mg l^{-1}). The highest contents of 3-methylbutan-1-ol (significantly different at the 95% level) were determined in TNM-D (259.1 ± 0.9 mg l^{-1}). The high contents of 3-methylbutan-1ol could be justified by the higher content of the amino acid precursors of this alcohol, leucine and isoleucine [22]. The contents of 2-phenylethanol (18.0 ± 0.9 mg l^{-1}) were notably higher in the TNM-D wine and the other varietal alcohol, benzyl alcohol appeared in low concentrations but were similar in all the TNM wine types (Fig. 4).

The next most abundant higher alcohol in the TMN red wines is hexan-1-ol, that contributed with "herbaceous" and "vegetal" odour when its concentration surpass 8 mg l^{-1}. The average concentration of hexan-1-ol in TNM red wines studied (8.4 ± 0.5 mg l^{-1}), is higher than the perception threshold thus its sensorial contribution is expected. The highest concentration of this compound was present in TNM-D wines. The obtained values were similar to those reported by Falqué et al. [22].

3.2.2. Ethyl esters and acetates

One of the most important groups of aroma compounds in wine are the ethyl esters of fatty acids that are produced enzy-
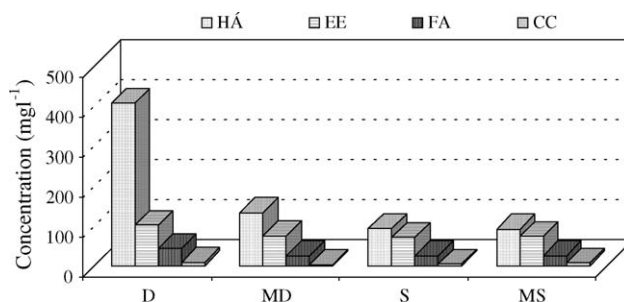

Fig. 3. Total concentration of the main chemical classes of volatile compounds (HA: higher alcohols; EE: ethyl esters; FA: fatty acids; CC: carbonyl compounds) determined in TNM red wines (D: dry; MD: medium dry; S: sweet; MS: medium sweet).

Table 3
Average concentration (mean ± standard deviation) of volatile composition of TNM red wines from 2003 harvest

Compounds	Concentration (mg l^{-1})[a]			
	TNM-D	TNM-MD	TNM-S	TNM-MS
Terpenes				
α-Terpineol	0.288 ± 0.733	0.288 ± 0.733	0.287 ± 0.733	0.287 ± 0.733
Subtotal (mg l^{-1})	0.29	0.29	0.29	0.29
Subtotal (%)	0.05	0.12	0.14	0.14
Higher alcohols				
2-Methypropan-1-ol[b]	3.056 ± 0.199	0.723 ± 0.035	0.516 ± 0.034	0.601 ± 0.084
Butan-1-ol	2.585 ± 0.198	2.513 ± 0.199	2.678 ± 0.198	2.518 ± 0.199
3-Methylbutan-1-ol	259.09 ± 0.881	61.974 ± 0.028	29.476 ± 0.112	29.951 ± 0.110
3-Methylpentano-1,5-diol[b]	0.070 ± 0.002	0.007 ± 0.000	0.002 ± 0.000	0.006 ± 0.002
α-Phenylbenzenemethanol[b]	0.014 ± 0.002	0.006 ± 0.000	0.014 ± 0.001	0.021 ± 0.006
2-Acethoxypropan-1-ol[b]	ND	ND	0.003 ± 0.000	0.005 ± 0.000
3-Methylpentan-1ol[b]	0.010 ± 0.001	0.002 ± 0.000	0.000 ± 0.000	0.002 ± 0.000
Heptan-1-ol[b]	0.006 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.006 ± 0.000
Hexan-1-ol	8.779 ± 0.466	7.604 ± 0.473	8.366 ± 0.468	8.685 ± 0.467
(Z)-3-Hexen-1-ol[b]	0.005 ± 0.001	0.008 ± 0.000	0.007 ± 0.001	0.007 ± 0.001
3-Etoxypropan-1-ol[b]	0.031 ± 0.000	0.013 ± 0.001	0.008 ± 0.001	0.016 ± 0.000
(E)-3-hexen-1-ol	0.488 ± 0.093	0.505 ± 0.093	0.523 ± 0.094	0.518 ± 0.115
(Z)-2-hexen-1-ol	ND	0.397 ± 0.115	0.397 ± 0.115	0.444 ± 0.115
2-Ethylhexan-1-ol	0.574 ± 0.216	0.574 ± 0.216	0.575 ± 0.216	0.574 ± 0.216
(D,L)-Butan-2,3-diol[b]	1.264 ± 0.201	0.327 ± 0.009	0.232 ± 0.021	0.231 ± 0.033
(R,S)-Butan-2,3-diol	104.102 ± 3.134	40.209 ± 3.134	34.646 ± 3.134	38.076 ± 3.134
Propan-2-ol	8.983 ± 0.348	7.045 ± 0.397	6.358 ± 0.415	ND
2,2-Dimethylpentan-3-ol[b]	0.030 ± 0.002	0.003 ± 0.000	0.001 ± 0.000	ND
Benzyl alcohol[b]	0.111 ± 0.003	0.224 ± 0.007	0.106 ± 0.008	0.107 ± 0.008
2-Phenylethanol	18.005 ± 0.998	11.881 ± 1.012	9.706 ± 1.017	9.8 ± 1.017
Subtotal (mg l^{-1})	407.20	134.02	93.62	91.57
Subtotal (%)	7147	54.14	46.09	44.58
Acetates				
Isoamly acetate	0.654 ± 0.339	0.515 ± 0.344	0.508 ± 0.344	0.491 ± 0.345
Hexyl acetate[b]	0.002 ± 0.000	0.036 ± 0.003	0.032 ± 0.001	0.003 ± 0.000
2-Phenylethyl acetate	5.09 ± 0.113	3.182 ± 0.113	2.993 ± 0.113	3.07 ± 0.113
Subtotal (mg l^{-1})	5.75	3.73	3.53	3.56
Subtotal (%)	1.01	1.51	1.74	1.73
Ethyl esters				
Ethyl butyrate	ND	ND	1.372 ± 0.444	ND
Ethyl hexanoate	2.271 ± 0.383	1.73 ± 0.406	1.608 ± 0.411	1.544 ± 0.414
Ethyl pyruvate[b]	0.006 ± 0.001	0.032 ± 0.001	0.017 ± 0.003	0.046 ± 0.001
Ethyl lactate	7.206 ± 0.135	6.855 ± 0.142	4.051 ± 0.196	3.088 ± 0.215
Ethyl octanoate	54.823 ± 7.160	32.696 ± 6.464	30.437 ± 6.383	29.069 ± 6.350
Ethyl 3-hydroxybutanoate	1.706 ± 0.228	1.674 ± 0.229	1.659 ± 0.230	1.66 ± 0.223
Ethyl decanoate	0.38 ± 0.014	0.238 ± 0.014	0.192 ± 0.014	0.181 ± 0.014
Diethyl succinate[b]	1.049 ± 0.065	0.702 ± 0.030	0.184 ± 0.030	0.329 ± 0.029
Ethyl pentanedioate[b]	0.007 ± 0.001	0.006 ± 0.001	0.001 ± 0.000	0.003 ± 0.000
Ethyl benzeneacetate[b]	0.016 ± 0.003	0.006 ± 0.000	0.003 ± 0.000	0.005 ± 0.000
Methyl 2-ethylhexanoate[b]	0.284 ± 0.019	0.193 ± 0.030	0.073 ± 0.014	0.060 ± 0.001
Ethyl dodecanoate	1.318 ± 0.116	1.236 ± 0.116	ND	ND
Ethyl 3-hydroxyhexanoate[b]	1.786 ± 0.103	1.325 ± 0.121	1.761 ± 0.324	3.613 ± 0.546
Ethyl 2-furoate	ND	ND	2.525 ± 0.129	6.535 ± 0.244
Ethyl succinate	30.079 ± 1.659	28.867 ± 1.678	27.056 ± 1.683	27.562 ± 1.679
Subtotal (mg l^{-1})	100.93	75.56	70.94	73.70
Subtotal (%)	17.72	30.52	34.93	35.88
Fatty acids				
Acetic acid	27.101 ± 2.673	14.388 ± 3.017	14.995 ± 3.000	14.492 ± 3.014
Propanoic acid[b]	ND	0.031 ± 0.001	0.002 ± 0.000	ND
2-Methylpropanoic acid[b]	0.160 ± 0.019	0.045 ± 0.002	0.043 ± 0.003	0.822 ± 0.014
Butanoic acid[b]	0.040 ± 0.001	0.022 ± 0.002	0.022 ± 0.007	0.019 ± 0.001
3-Methylbutanoic acid	1.83 ± 0.121	0.902 ± 0.121	0.884 ± 0.121	0.763 ± 0.121

Table 3 (*Continued*)

Compounds	Concentration (mg l^{-1})[a]			
	TNM-D	TNM-MD	TNM-S	TNM-MS
Hexanoic acid	1.625 ± 0.208	1.535 ± 0.208	1.502 ± 0.208	1.486 ± 0.208
(*E*)-Hex-2-enoic acid	0.001 ± 0.000	0.003 ± 0.000	0.010 ± 0.000	0.006 ± 0.002
Octanoic acid	9.076 ± 0.543	6.654 ± 0.551	5.911 ± 0.551	5.843 ± 0.554
Decanoic acid	1.607 ± 0.080	0.098 ± 0.080	0.507 ± 0.080	0.565 ± 0.080
α-Hydroxyphenylpropanoic acid[b]	0.200 ± 0.008	0.064 ± 0.002	0.021 ± 0.003	0.031 ± 0.009
2-Furancarboxilic acid[b]	0.022 ± 0.002	0.021 ± 0.001	0.023 ± 0.004	ND
Dodecanoic acid[b]	0.021 ± 0.002	0.030 ± 0.001	ND	ND
Phenylacetic acid	1.188 ± 0.538	1.161 ± 0.539	1.033 ± 0.554	1.046 ± 0.542
Subtotal (mg l^{-1})	42.87	24.92	24.95	25.07
Subtotal (%)	7.53	10.07	12.29	12.21
Carbonyl compounds				
P-aldehyde[b]	0.038 ± 0.009	0.097 ± 0.016	0.228 ± 0.012	0.701 ± 0.020
Benzaldehyde	6.649 ± 0.104	1.35 ± 0.138	2.742 ± 0.129	4.07 ± 0.120
Phenylethanal	1.611 ± 0.039	1.28 ± 0.039	1.358 ± 0.039	1.573 ± 0.039
5-Methylheptan-2-one[b]	0.331 ± 0.012	0.010 ± 0.000	0.230 ± 0.073	0.567 ± 0.070
3-Penten-2-one[b]	0.000 ± 0.000	ND	0.007 ± 0.002	0.004 ± 0.000
5-Methoxypentan-2-one[b]	0.002 ± 0.000	0.003 ± 0.000	0.041 ± 0.003	0.011 ± 0.002
2-Hydroxypentan-3-one[b]	0.010 ± 0.003	0.008 ± 0.001	0.009 ± 0.001	0.018 ± 0.005
5-Acethoxypentan-3-one[b]	0.008 ± 0.001	0.066 ± 0.002	0.041 ± 0.003	0.032 ± 0.002
Cyclopentanone[b]	0.015 ± 0.003	0.045 ± 0.017	0.066 ± 0.012	0.119 ± 0.031
N-ethyl acetamide[b]	0.004 ± 0.000	0.002 ± 0.000	ND	ND
N-(3-Methylbutyl) acetamide[b]	0.083 ± 0.007	0.059 ± 0.001	0.009 ± 0.001	0.024 ± 0.003
(N,N)-ethylphenyl acetamide[b]	0.020 ± 0.006	0.030 ± 0.001	ND	ND
Subtotal (mg l^{-1})	8.77	2.95	4.73	7.12
Subtotal (%)	1.54	1.19	2.33	3.46
Lactones[b]				
Butalactone	0.001 ± 0.000	0.003 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Pantolactone	0.032 ± 0.002	0.017 ± 0.001	0.012 ± 0.003	0.019 ± 0.004
γ-Hexalactone	0.010 ± 0.002	0.001 ± 0.0030	0.000 ± 0.000	0.001 ± 0.000
γ-Octalactone	0.511 ± 0.116	0.098 ± 0.010	0.065 ± 0.018	0.102 ± 0.021
γ-Nonalactone	0.317 ± 0.087	0.144 ± 0.007	0.063 ± 0.010	0.084 ± 0.023
γ-Decalactone	0.001 ± 0.000	0.001 ± 0.000	ND	0.001 ± 0.000
THMP	0.001 ± 0.000	0.000 ± 0.000	0.005 ± 0.001	0.009 ± 0.000
Subtotal (mg l^{-1})	0.87	0.26	0.15	0.21
Subtotal (%)	0.15	0.11	0.07	0.10
Acetals[b]				
cis-Dioxane	0.040 ± 0.000	0.021 ± 0.002	0.031 ± 0.002	0.048 ± 0.008
1,1-Diethoxyethane	0.004 ± 0.001	0.003 ± 0.000	0.006 ± 0.001	0.003 ± 0.000
cis-Dioxolane	0.017 ± 0.001	0.034 ± 0.001	0.029 ± 0.006	0.034 ± 0.004
trans-Dioxane	0.014 ± 0.001	0.008 ± 0.001	0.013 ± 0.001	0.023 ± 0.005
Subtotal (mg l^{-1})	0.08	0.07	0.08	0.11
Subtotal (%)	0.01	0.03	0.05	0.05
Furan compounds				
2-Furfural	2.89 ± 0.346	2.92 ± 0.345	2.961 ± 0.345	3.015 ± 0.344
5-Ethyl hydro-2(3H)-furanone[b]	ND	0.005 ± 0.000	ND	0.006 ± 0.002
5-Ethoxydihydro-2(3H)-furanone[b]	ND	0.010 ± 0.001	0.010 ± 0.001	0.127 ± 0.024
5-Methyl-2-furancarboxaldehyde[b]	ND	ND	0.001 ± 0.000	0.006 ± 0.001
HMF	ND	0.064 ± 0.004	0.065 ± 0.013	0.127 ± 0.024
Subtotal (mg l^{-1})	2.89	3.00	3.04	3.28
Subtotal (%)	0.51	1.21	1.49	1.59
Sulfur compounds				
2-(Methylthio)-ethanol[b]	0.005 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Methionol	1.623 ± 0.306	1.439 ± 0.306	1.385 ± 0.307	1.384 ± 0.307
5-Ethoxythiazole[b]	0.103 ± 0.005	0.010 ± 0.001	0.007 ± 0.001	0.015 ± 0.001
Subtotal (mg l^{-1})	1.73	1.45	1.39	1.40
Subtotal (%)	0.30	0.58	0.68	0.68

Table 3 (*Continued*)

Compounds	Concentration (mg l^{-1})^a			
	TNM-D	TNM-MD	TNM-S	TNM-MS
Volatile phenols				
Phenol	1.254 ± 0.145	1.269 ± 0.144	1.237 ± 0.145	ND
Vanillin	ND	3.008 ± 1.015	2.19 ± 1.117	2.356 ± 1.096
Acetovanillin^b	0.005 ± 0.002	0.015 ± 0.000	0.001 ± 0.000	ND
Subtotal (mg l^{-1})	1.26	4.29	3.43	2.36
Subtotal (%)	0.22	1.73	1.68	1.15
Total (mg l^{-1})	569.75	247.54	203.10	205.38

ND: not detected; THMP: tetrahydro-4-hydroxy-4-methyl-2-H-pyran-2-one; HMF: 5-hydroxymethyl-2-furfural.

^{a} Mean of 12 extraction replicates.

^{b} Concentration determined by the equation $C_x = n \times C_{is}$, where C_x is the concentration of x compound, n the relative peak area and C_{is} is the internal standard concentration in the sample.

matically during yeast fermentation and from ethanolysis of acylCoA that is formed during fatty acids synthesis or degradation. Their concentration is dependent on several factors mainly: yeast strain, fermentation temperature, aeration degree and sugar contents. These compounds make a positive contribution to the general quality of wine being responsible for their "fruity" and "floral" sensory properties. The TNM-S wines showed the lowest concentration of ethyl esters of fatty acids (6–10 carbon atoms). The maximum values for all the ethyl esters were found for TNM-D and TNM-MD, being higher than the respective TNM-MS. With exception of the TNM-D wines, the concentration of ethyl esters in the different types of studied wines was reasonably constant and the differences were not significant. Between the ethylic esters that it has been possible to identify, ethyl esters of C_6 and C_8 fatty acids, which are responsible for the "fruity" and "wine-like" aroma, hexanoate and octanoate, were those found at the highest concentrations in the TNM analysed wines. They have similar concentrations with exception for ethyl octanoate determined in TNM-D wines (significantly different at the 95% level), and are present at concentrations exceeding their flavour threshold (Table 4). From the ethyl esters of diprotic acids, the concentration of diethyl succinate (28.4 ± 1.3 mg l^{-1}) is much higher than that found for ethyl lactate (Table 3). Identical concentrations of diethyl succinate were observed in all the analysed TNM wines (Table 3). The concentration of ethyl lactate in TNM-D and TNM-MD was higher than that determined
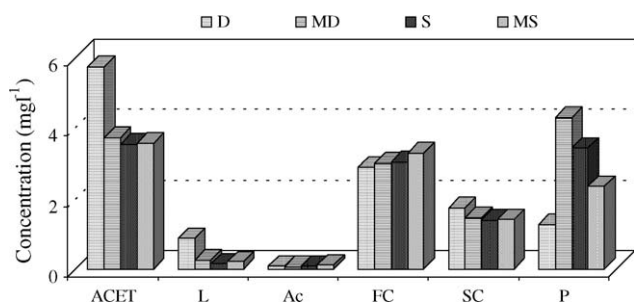

Fig. 4. Total concentration of the minor chemical classes of volatile composition (ACET: acetates from higher alcohols; L: lactones; Ac: acetals; FC: furan compounds; SC: sulphur compounds; P: volatile phenols) determined in TNM red wines (D: dry; MD: medium dry; S: sweet; MS: medium sweet).

in the TNM-S and TNM-MS red wines. Fig. 3 compare the total concentration of the ethyl esters of fatty acids in the different TNM wine types analysed.

3.2.3. Acetates

Are the result of the reaction of acetylCoA with higher alcohols that are formed from degradation of amino acids or carbohydrates. Isoamyl acetate with a characteristic odour of "banana", was found at similar values in different TNM wine types and above its perception threshold (30 µg l^{-1}) in all the samples with an average content of about 0.54 ± 0.08 mg l^{-1}. The concentration of 2-phenylethyl acetate determined in TNM-D, which give "roses, flowery, honey" nuances to the wine, was significantly different at the 95% level, from the determined in TNM-MD, TNM-S and TNM-MS wines.

3.2.4. Fatty acids

Within the family of fatty acids (Table 3), acetic, hexanoic and octanoic acids were notable for their higher concentrations. Acetic acid was markedly the most abundant acid, being present at levels lower than its perception threshold (200 mg l^{-1}). The TNM-D wine, present the highest concentration of these compounds (42.9 mg l^{-1}). The fatty acids concentration in the TNM-MD, TNM-S and TNM-MS red wines were not significantly different at the 95% level. The contents of hexanoic, octanoic and decanoic acids although high, showing values of 1.5, 6.9 and 0.7 mg l^{-1}, respectively, were in agreement with those found for other wine varieties. Fig. 3 shows the total concentration of these compounds in the different TNM wine types analysed.

3.2.5. Carbonyl compounds

This group includes aldehydes and ketones. The former compounds, namely C_6 aldehydes, are formed from unsaturated fatty acids, such as linoleic and linolenic acids. Also, can be considered as products of lipoxygenase catalysis. Only few aldehydes have been detected among the wine aroma constituents, probably because they can be reduced to the corresponding alcohols during the course of fermentation. Benzaldehyde and phenylethanal present the highest levels of this group of compounds. They have similar concentrations in TNM-MD; TNM-S and TNM-

Table 4
Odour descriptor, odour threshold (LOP) and aroma index (*I*) of the main odorants found in TNM red wines

Compounds	Odour descriptor[a]	LOP (mg l⁻¹)[a]	Aroma index (*I*)			
			TNM-D	TNM-MD	TNM-S	TNM-MS
Ethyl butyrate	Fruity, apple	0.02	ND	ND	68.6	ND
2-Methylpropan-1-ol	Bitter, green, harsh	0.2	15.3	3.6	2.6	3.0
Phenylethyl acetate	Roses, flowery	0.25	20.4	12.7	12.0	12.3
3-Methylbutan-1-ol	Alcohol, fusel	30.0	8.6	2.1	1.0	1.0
Ethyl hexanoate	Green apple, anise	0.014	162.2	123.5	114.9	110.3
Hexan-1-ol	Green, grass	8.0	1.1	1.0	1.0	1.1
Ethyl octanoate	Sweet, fruity, fresh	0.005	10964.6	6539.1	6087.5	5813.8
Benzaldehyde	Bitter, cherry	2.0	3.3	0.7	1.4	2.0
3-Methylbutanoic acid	Cheese, fatty, rancid	0.0334	54.8	27.0	26.5	22.8
Ethyl decanoate	Pleasant, soap	0.2	1.9	1.2	1.0	0.9
Phenylethanal	Flowery, rose, honey	0.005	322.1	256.0	271.6	314.6
Ethyl succinate	Wine	6.0	5.0	4.8	4.5	4.6
α-Terpineol	Piney, iris, teil	0.11	2.6	2.6	2.6	2.6
Methionol	Baked cabbage	1.0	1.6	1.4	1.4	1.4
Isoamly acetate	Banana	0.03	21.8	17.2	16.9	16.4
Hexanoic acid	Fatty acid, cheese	0.42	3.9	3.7	3.6	3.5
Octanoic acid	Fatty acid, rancid	0.5	18.2	13.3	11.8	11.7
2-Phenylethanol	Roses, sweet	14.0	1.3	0.8	0.7	0.7
γ-Nonalactone	Coconut	0.03	10.6	4.8	2.1	2.8
Vanillin	Vanilla, candy	0.2	ND	15.0	10.9	11.8
Decanoic acid	Fatty, rancid, soap	1.0	1.6	0.1	0.5	0.6
Phenylacetic acid	Honey, pollen, flowery	2.5	1.2	1.2	1.0	1.0

ND: not detected.

[a] Odour descriptor and odour threshold reported in the literature [14–22].

MS wines, with exception for benzaldehyde in TNM-D wines whose concentration is significantly different at the 95% level. The ketones can be formed by condensation of activated fatty acids. The most important in the TNM analysed wines are cyclopentanone.

3.2.6. Furan compounds

Another group of aroma compound that have been studied were the furanic compounds, formed by degradation of carbohydrates. The major components in this group were 2-furfural and 5-hydroxymethyl-2-furfural (HMF). Furfural was the most abundant compound (Table 4) but is present at levels lower than its perception threshold. The average values for the different TNM wine types are similar (not significantly different at the 95% level).

3.2.7. Lactones

Seven lactones were identified. These compounds are among the most important to the sensory characteristics of wines namely when aged in oak wood. These compounds are formed by cyclization of the corresponding γ-hydroxycarboxylic acids. The odour of these lactones depends on the chemical structure, functional groups and the length of side chains. The odour of these compounds are described as being "fruity" and in some cases as "coconut-like; fruity" (γ-hexalactone); "coconut-like" (γ-octalactone); "peach-like, milky" (γ-decalactone) and "fruity, sweet floral" (γ-dodecalactone). γ-Octalactone is the most abundant lactone in TNM-D (58.5%), TNM-S (44.8%), TNM-MS (49.3%) red wines and γ-nonalactone is the most representative of TNM-MD (54.5%) wine.

3.2.8. Sulphur compounds

Comprise a structurally diverse class of molecules that a whole range of aromatic notes, generally considered detrimental to wine quality [23]. Most of the sulphur compounds identified in wines are usually found at level below their threshold values. The main sulphur compound identified in TNM red wines was 3-(methylthio)-propan-1-ol (methionol). This is usually found at levels above its olfactive perception threshold value. The analysis of TNM red wines (Table 3) showed that the highest concentration of sulphur compounds was observed for methionol in TNM-D wine.

3.2.9. Volatile phenols

These compounds detected in different wine samples, can originate from *p*-coumaric and ferulic acids by decarboxylation. Within the family of volatile phenols (Table 3) vanillin was notable for their higher concentrations. Its content exceed their flavour threshold contributing with "vanilla" and "candy" odours to the TNM wines (Table 4).

3.3. Identification of the main odorants

As a preliminary step to achieve the identification of the potentially most important wine odorants of TNM wine, the aroma index (*I*), the ratio between the concentration of each volatile compound (*c*) with the corresponding odour threshold (*s*), was assessed using the equation *I* = *c*/*s* (Table 4). On the basis of their odour description and threshold, the most powerful odorants of TNM wines were tentatively established. As shown in Table 4, at least 22 components were present at concen-

trations higher than their corresponding odour thresholds. Thus these compounds exhibit an aroma index value higher than the unity were considered to contribute individually to the TNM wine aroma.

There was a great similarity among the intensities of these odorants in the different of TNM red wine samples. According to the results presented in Table 4, the five most potent aromas of each wine type are practically the same. The highest *I* values were obtained for several well-known by-products of yeast metabolism such as ethyl octanoate, ethyl hexanoate and their corresponding fatty acids; isoamyl acetate; 2-phenylethyl acetate, phenylethanal; 3-methylbutanoic acid; the higher alcohols 2-methylpropan-1-ol and 3-methylbutan-1-ol; metionol and γ-nonalactone and seems to be important odorants of this wine. Diethyl succinate, phenylacetic acid, 2-phenylathanol and hexan-1-ol were also at concentrations higher than their corresponding threshold. The relevant content of vanillin, associated with "vanilla" and "chocolate" odour descriptors, with *I* values higher than 10 for TNM-MD, TNM-S and TNM-MS wines, should be considered to have a sensorial contribution for these red wines.

4. Conclusions

TNM red wines are characterized by the presence of higher levels of higher alcohols, ethyl esters and acetates, fatty acids, carbonyl compounds and sulphur compounds. The TNM-MD shows the highest values of volatile phenols. In contrast, they have the lowest contents of acetals. The highest values of furan compounds and acetals were determined in TNM-S wines. These wines present the lowest concentrations of higher alcohols. TNM-MS shows the lowest values of acetates, lactones and sulphur compounds.

Quantitatively, the higher alcohols (aliphatic and aromatic) are the largest group of the volatile composition in the TNM red wine. The ethyl esters and fatty acids formed enzymatically during the fermentation process constitute important groups of aroma compounds that contribute with "fruity" and "cheese/fatty" notes to wine sensory properties, respectively. The dominating esters are the ethyl esters of fatty acids and acetates of higher alcohols. Ethyl octanoate and ethyl hexanoate predominated in TNM red wines analysed.

From all compound identified in TNM red wines, ethyl octanoate, phenylethanal, ethyl hexanoate, isoamyl acetate, octanoic acid and 2-phenylethyl acetate are the most powerfull

odorants. The proposed methodology seems to be adequate to establish the potentially most important wine odorants of TNM wines.

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