AROMA POTENTIAL OF THREE AUTOCHTHONOUS GRAPEVINE VARIETIES FROM TUNISIA

I. SOUID[1], Z. HASSENE[1], E. SÁNCHEZ PALOMO[2],
M. S. PÉREZ-COELLO[2] and A. GHORBEL[1]

1: Institut of Biotechnology Laboratory of Grapevine Molecular Physiology
BP 901 Hammam Lif 2050 Tunisia
2: Area de Tecnología de los Alimentos, Facultad de Ciencias Químicas, Campus Universitario, 10, 13071 Ciudad Real, Spain

Abstract

Aims: The aim of the present work is to characterize the aromatic profiles of three Tunisian autochthonous grapevine (*Vitis vinifera* L.) varieties (Asli, Beldi and Ferrani).

Methods and results: The GC-MS analysis of the autochthonous grapevine varieties studied allowed identifying 38 volatile compounds in the free fraction and 36 glycosidically bound aroma compounds. Volatile C6 compounds were typical for the studied varieties especially for Asli variety. The analysis of the liberated aglycones through enzymatic hydrolysis show that α-terpineol and *cis* linalool oxide pyran that did not appear as free aroma compounds were found with significantly highest concentration in Asli grape juice. Also, 3-oxo-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol were found only under their glycosidically bound forms with significantly higher concentrations in Asli and Ferrani than in Beldi grape juices.

Conclusion: The results obtained in the present work show qualitative and quantitative differences in the aroma profiles among the studied varieties. However, non-terpenyl compounds were the most abundant aroma substances in the three studied varieties.

Significance and impact of the study: The present work characterize, for the first time, the aromatic profiles of three Tunisian autochthonous grapevine varieties (Asli, Beldi and Ferrani) and constitutes a major contribution to the aroma chemistry of these three grape varieties.

Key words: autochthonous grapevine, volatile aroma, glycosides, solid phase extraction, characterization

Résumé

Objectif : L'objectif de ce travail est de caractériser les profils aromatiques des jus de raisin de trois variétés autochtones de vignes cultivées en Tunisie. Il s'agit de Asli, Beldi et Ferrani (*Vitis vinifera* L.).

Méthodes et résultats : L'analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse des jus de raisin des variétés autochtones étudiées a permis d'identifier 38 composés volatils et 36 composés liés. Les composés libres en C6 ont caractérisé les jus de raisin des variétés étudiées, surtout pour la variété Asli. L'analyse des aglycones libérés suite à une attaque enzymatique a montré que les deux composés α-terpinéol et *cis* pyran oxyde de linalol, absents sous leurs formes libres, présentent des concentrations significativement plus importantes chez la variété Asli comparée à celles de Beldi et Ferrani. De plus, 3-oxo-α-ionol et 3-hydroxy-7,8-dihydro-β-ionol se présentent seulement sous leurs formes glycosylées avec des concentrations significativement plus importantes chez les variétés Asli et Ferrani comparées à celle de Beldi.

Conclusion : Les résultats trouvés dans le présent travail montrent des différences qualitatives et quantitatives des profils aromatiques des trois variétés étudiées. Cependant, leurs fractions volatiles se sont caractérisées par une dominance de composés non terpéniques.

Signification et impact de l'étude : Le présent travail caractérise, pour la première fois, les profils aromatiques des jus de raisin de trois variétés autochtones de vignes (Asli, Beldi et Ferrani) cultivées en Tunisie et constitue une contribution majeure dans la chimie des arômes de ces trois variétés.

Mots-clés : vigne autochtone, arômes libres, glycosides, extraction en phase solide, caractérisation

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*Corresponding author: imen_souid@yahoo.fr

INTRODUCTION

The typical flavour of the grape aroma is due to their volatile composition mainly to C6 aldehydes and alcohols (RIBÉREAU-GAYON and DUBOURDIEU, 2001), monoterpenes (VOIRIN *et al.*, 1992), methoxypyrazines (ALLEN and LACEY, 1999) and norisoprenoids (RAZUNGLES *et al.*, 1998). Grape aroma compounds are present as free volatiles and as flavourless non volatile bound to glycosides (GÜNATA *et al.*, 1985), S-cysteine conjugates (PEYROT des GACHONS *et al.*, 2000), polyhydroxilated compounds (SHOSEYOV and BRAVDO, 2001) or S-methionine precursors (SEGUREL *et al.*, 2005). Volatile compounds from glycosides can be released by acid or enzyme hydrolysis, representing a potential reservoir of flavour and enhancing the aromatic profile of juices and wines (SÁNCHEZ PALOMO *et al.*, 2005).

The aromatic identity of a grape variety results on the combination of different concentrations of aroma compounds. Aromatic grape varieties, such as the Muscats, contain higher concentrations of terpenic compounds than neutral varieties, where the C13 norisoprenoids contribute more to their typical aroma (RIBÉREAU-GAYON *et al.*, 2001). For such neutral varieties, fermentation is important for the aroma enhancement. In fact, a large pool of odorant aroma compounds is formed from precursors by the action of yeasts belonging to different genera (LOSCOS *et al.*, 2007; HERNÁNDEZ-ORTE *et al.*, 2008; UGLIANO *et al.*, 2006). Furthermore, an eventual synergy between grape aroma composition and yeast metabolism determines the final concentration of aroma volatiles in the wine (UGLIANO and MOIO, 2008). Most of these compounds possess pleasant floral and fruity aromas and very low perception thresholds. Although the influence of each compound on the final aroma depends. Primarily its concentration is above its perception threshold (PEINADO *et al.*, 2004), it has been proved that the synergy between grape aroma compounds at subthreshold levels can be an active contributor to the formation of the varietal aroma (LOSCOS *et al.*, 2007).

Other factors which also contribute to the complexity of the juice aroma are geographical, viticultural (BUREAU *et al.*, 2000) and the grape ripening stage, since free and bound forms of varietal compounds are accumulated in the grape during ripening (SCHNEIDER *et al.*, 2002).

In Tunisian, vineyards are mainly located in the Northeast characterized by a semi-arid climate. The main cultivated *Vitis vinifera* wine grapes are Carignan, Syrah and Muscat of Alexandria; while, table grapes are essentially composed of Italia and Superior Seedless. Indigenous varieties are traditionally cultivated in specific narrow areas.

Asli, Beldi and Ferrani are three of the most important local Tunisian grapevine varieties of *Vitis vinifera* and have been the subject of investigation in the present paper.

As was described by BRANAS and TRUEL (1965), Beldi is the best known autochthones grapevine variety in Tunisia. It has big and long bunches as well as small fine-skinned white berries. On the other hand, Asli has been described as small white berries known for the high quality of its dry grapes. Since a long time, both Asli and Beldi varieties have been used for both table consumption and for the production of white wine. Ferrani, which has white berries, is cultivated on small areas and is used almost exclusively for the table consumption.

Consumers appreciate Asli, Beldi and Ferrani varieties for their high organoleptic quality and they usually consider them as non-floral varieties. To our knowledge, no previous research has been done on their berry and wine aroma composition.

The present work investigates for the first time the free and bound aroma fractions of the fresh juice of the three autochthonous grapevine varieties, in order to evaluate their flavour potential.

MATERIAL AND METHODS

1. Grape material

Twenty-year-old *Vitis vinifera* L. Beldi, Ferrani and Asli vines were cultivated in the vineyard of the Biotechnology Center of Borj Cédria in the North east of Tunisia, (10°20'E, 36°45'N) under a semi-arid climate. Vines were spaces 2.5 m between rows and 2 m within the row and cultivated according to the double-T training system.

From each variety, 6 Kg of clusters were randomly manually harvested at optimum maturity in the 2005 season. Yield parameters (healthy cluster and berry weights) were immediately measured.

2. General analyses

Brix degree was measured using a hand-held refractometer (OPTECH, Mod. RZT with ATC: 0-32 % Brix, Germany). Total phenols were determined by spectrometry, measuring UV absorption at 280 nm using an utropec 2000 spectrometer (Pharmacien Biotech, England). Titratable acidity (TA) was determined in the diluted juice by titration with 0,1N of NaOH to a pH 8,2 end point and expressed as g of tartaric acid/ litre. The pH of undiluted juice of each sample was determined using an equilibrated pH meter (EUTECH instruments, Cyberscan 500, Singapore).

3. Extraction of the free and the bound fractions of the aroma

Pressed grape juice was centrifuged (30 min at 10000 rpm) at 4 °C. The extraction has been done in duplicate for each grape juice. To 100 mL of the clear juice, 40 µL of 4 nonanol (1,07 g/L in ethanol) (Merck) were added as internal standard, and were then passed through a 500 mg SPE cartridge LiChrolut EN resins (40-120 µm; 6 mL standard PP-Röhrchen, Merck) at about 2 mL/min.

Free and bound fractions of the aroma were eluted successively with 10 mL dichloromethane (Scharlau) and 50 mL of ethyl acetate (Scharlau). The dichloromethane extract was dried over glass wool and anhydrous sodium sulphate and concentrated to 100 µL under stream of nitrogen. The ethyl acetate extract was concentrated to dryness under vacuum (rotavapor) at 40 °C then dissolved in 1 mL of methanol (Scharlau). An aliquot of 500 µL was evaporated to dryness under a stream of nitrogen then re-dissolved in 200 µL of a citrate (0,1 M) phosphate (0,2 M) buffer pH 5. Enzymatic treatment with AR 2000 (Gist Brocades) (40 mg/mL) was conducted out at 40 °C for 18 hours. Released aglycones were recovered by liquid-liquid extraction with 5 x 2 mL of pentane-dichloromethane (2:1 V/V). 10 µL of 4 nonanol (1,07 g/L in ethanol) (Merck) were added to the organic extracts as internal standard. The mixture was concentrated under nitrogen stream then analysed by GC-MS.

4. GC and GC-MS analysis of free and bound compounds

An Agilent gas chromatograph model 6890 N coupled to a mass selective detector, model 5973 inert, was used. An amount of 1 µL of extract was injected in splitless mode on a BP-21 capillary column (60 m x 0, 32 mm i.d.; 0,25 µm film thickness). Oven temperature programme was as 70 °C (5 min) - 1 °C/min - 95 °C (10 min) - 2 °C/min- 190 °C (40 min). Injector and transfer line temperatures were 250 and 280 °C, respectively. Mass detector conditions were: electronic impact (EI) mode at 70 eV; source temperature: 178 °C; scanning rate: 1 scan/s; mass acquisition: 40 - 450 amu.

5. Identification and quantification

The identification was based on comparison of the GC retention times and mass spectra with authentic standards from Sigma-Aldrich. For quantification purposes, calibration curves were calculated when standards were available; otherwise semi-quantitative analyses were carried out assuming a response factor equal to one. The fragmentation pattern was compared in the mass spectra with those of the databases. Comparison of the aroma components with their linear retention indices (LRI), determined in relation to a homologous series of n-alkanes with those from pure standards has been also used for the identification. Concentration of the free fraction and aglycones enzymatically hydrolysed from glycosides were calculated using 4-nonanol as internal standard and expressed by average of two replicates in µg/L.

6. Statistical analysis

All data were analysed using EXCEL software to determine the average and the confidence limit for each parameter. RSD (Relative Standard Deviation) was calculated by dividing confidence limit by the average from independent complete analysis of two grape samples for each variety. A one-way analysis of variance (ANOVA) was also achieved using STATISTICA program (Stat Soft, France). Means were compared, using Duncan test at a significance level of $p \leq 0.05$ level, to compare the three studied varieties Ali, Beldi and Ferrani.

RESULTS AND DISCUSSION

1. General analysis

Cluster and berry weights, Brix degree, TA, pH, total polyphenols of the three studied varieties Asli, Beldi and Ferrani, are summarised in table 1.

Cluster weight was significantly the highest for Beldi grapevine variety; while, Asli variety presents significantly the lowest cluster and berry weights. Brix degree of juice was used as an index of the three studied varieties berry ripening. At harvest, there are small differences between Brix values, being significantly the highest for the Asli variety.

Both TA and pH are of great importance for juice stability and are parameters commonly used as an indicator of the wine quality. As can be shown in table 1, Beldi grapevine variety tended to produce juice with the highest TA however no significant difference have been proved through the studied varieties. In the contrast, the average pH recorded for Ferrani variety grape juice is the lowest.

2. Free volatile compounds

Aromatic compounds content is shown in table 2. The GC-MS analysis allowed to identify in the free fraction 38 volatile compounds including 6 C6 alcohols and aldehydes, 2 higher alcohols, 11 benzene compounds, 2 monoterpenols, 11 acids, 4 norisoprenoids, 1 lactone and 1 C9 aldehyde.

Table 1 - Morphological and juice chemical analyses of Ferrani, Asli and Beldi grapevine varieties.

	Ferrani	Asli	Beldi
Cluster weight	376.80±44.96 b	208.60±32.46 c	518,00±72.32 a
100 berries weight	505.66±63.47 a	209.33±30.81 c	322.66±26.69 b
TA (g tartric acid/L)	4.01±0.11 a	4.26±0.28 a	4.30±0.56 a
Brix degree	21.83±0.06 b	22.06±0.06 a	21.13±0.06 c
OD280	13.88±1.21 b	21.3±2.01 a	13.63±0.75 b
pH	3.87±0.03 b	4.03±0.06 a	4.10±0.10 a

Means indicated by different letters are significantly different at ≤ 0.05, Duncan's multiple range test.

As was reported by LÓPEZ-TAMAMES *et al.* (1997) alcohols and aldehydes of six atoms of carbons have greasy odor and their concentrations depend on the grape variety. The C6 compounds (hexanal, (E)-2-hexenal, 1-hexanol, (Z)-3-hexen-1-ol, (E)-3-hexen-1-ol and (E)-2-hexen-1-ol) were more abundant in Asli variety than the otherstudied varieties (table 2) and contribute to 42,89 % of its total volatile aroma. The C6 aldehydes (hexanal and (E)-2-hexenal), formed from fatty acid precursors in the grape (TRESSEL and DRAWET, 1973), are likely relevant contributors to the final aroma of Asli and Beldi grape juices since their concentrations reached their respective perception threshold (table 3). The (E) isomer of 2-hexenal exceeds its perception threshold also in Ferrani grape juice.

Among the benzene compounds, benzaldehyde which has cherry and fruity notes (MIRANDA-LÓPEZ *et al.*, 1992), was found in the three studied varieties at concentrations lower than its perception threshold (table 3). Guaiacol and 4-vinylguaiacol have both smoky notes (FISCHETTI, 1994; ROWE, 2000) and while only traces of guaiacol have been identified in Ferrani grape juice. 4-vinylguaiacol was found in the three varieties with a significantly highest concentration in Asli grape juice.

Vanillin was present in Asli, Beldi and Ferrani varieties with significantly different concentrations. However, it reached its perception threshold only in Asli grape juice.

The concentrations of benzyl alcohol were much lower than its perception threshold; which suggests that its sensory importance in the three varieties of grape juice is just secondary. Whereas, 2-phenylethanol concentration exceeded its perception threshold only for Asli variety (table 3). Eugenol which has a clove note (McGORRIN, 2002) was identified only in the Beldi grape juice. Acetovanillone has a pleasant aroma with spicy, caramel and vanilla attributes (DARRIET, 1996), it was detected as significantly highest concentration in Asli grape juice while was not detected in Ferrani variety.

Monoterpenes were not very abundant in the free fraction of the studied autochthonous varieties. In fact, only trace amounts of geraniol could be detected in Asli, Beldi and Ferrani grape juices. Linalool, the major compound in floral varieties (BAUMES *et al.*, 1994) was found at significantly highest concentrations in Asli grape juice. The low levels of monoterpenes in the three studied varieties (Asli, Beldi and Ferrani), compared to those detected in Muscats and Gewürztraminer (VOIRIN *et al.*, 1992) constitute their neutral nature.

C13 norisoprenoids are grape-derived compounds but they are usually not present in the free form and arise in juice and wines by hydrolytic degradation of precursor substances (STRAUSS *et al.*, 1987). 3-hydroxy-β-damascone and 4-oxo-isophorone, dehydrovomifoliol and vomifoliol were the four C13 norisoprenoids identified in the three autochthonous grapes. 3-hydroxy-β-damascone has tea and tobacco aroma notes (RIBÉREAU-GAYON *et al.*, 2001; DARRIET, 1996) and it was found on traces only in Beldi grape juices. This same variety presented the significantly highest concentration of vomifoliol, while, Ferrani grape juice had the significantly highest levels of dehydrovomifoliol. The highest norisoprenoid concentrations identified in Ferrani variety may contribute to the pleasant floral and fruity aroma of its grape juices.

Acid fractions represent 35.27, 21.16 and 31.82 % in Asli, Beldi and Ferrani varieties, respectively. The highest acid concentrations were found in Asli variety (table 2) and the lowest concentrations were those of Beldi. Hexanoic and octanoic acids were present in the three studied varieties. ETIEVANT (1991) describe their odors as buttery, cheesy and sweaty like. Their contribution to the aroma of Asli, Beldi and Ferrani varieties cannot

Table 2 - Concentrations (µg/L) of free volatile compounds from Asli, Beldi and Ferrani grape juices.

Compound	RT.	LRI	Asli		Beldi		Ferrani	
			Mean (n=2)	RSD(%)	Mean (n=2)	RSD(%)	Mean (n=2)	RSD(%)
C6 compounds								
Hexanal	6.29	1021	955.45 a	1.06	116.19 b	21.08	nd c	
(E)-2-hexenal	10.33	1144	243.41 a	6.13	152.70 a	3.19	221.16 a	25.23
1-hexanol	16.81	1266	672.95 a	5.78	216.71 b	13.52	234.90 c	43.60
(E)-3-hexen-1-ol	17.43	1275	nd b		10.95 **a**	4.70	7.89 ab	5.32
(Z)-3-hexen-1-ol	18.90	1296	145.12 a	13.15	18.87 **b**	24.59	18.03 b	1.03
(E)-2-hexen-1-ol	20.59	1315	428.00 a	0	161.29 **b**	4.93	250.02 b	14.81
Total			**2444.93**		**676.71**		**732.00**	
Higher alcohols								
2-penten-1-ol	14.68	1236	75.36 a	20.24	11.46 b	11.36	nd c	
3,7-octadiene-2,6-dimethyl-2,6-diol	67.76	1800	30.92 a	3.43	3.65 ab	22.72	52.97 b	14.60
Total			**106.28**		**15.11**		**52.97**	
Benzene compounds								
Benzaldehyde	30.61	1416	36.04 a	40.54	3.32 b	15.52	5.35 b	0
Benzeneacetaldehyde	44.25	1527	18.88 a	10.76	13.94 a	28.40	nd b	
Guaiacol	61.99	1713	nd **b**		nd b		tr a	
Benzyl Alcohol	63.02	1728	279.80 a	32.08	8.72 b	22.52	6.03 b	41.87
2-Phenylethanol	65.12	1760	165.84 a	37.87	24.85 b	10.80	11.94 b	24.27
Eugenol	79.04	2005	nd b		10.63 a	25.85	nd b	
4-vinylguaiacol	80.24	2029	246.93 a	4.94	15.72 b	33.05	8.42 b	29.86
Vanillin	96.02	2365	274.84 a	32.08	11.45 b	16.24	tr c	
Methyl vanillate	98.10	2406	20.24 a	8.47	nd b		nd b	
Acetovanillone	99.93	2436	31.56 a	5.82	2.49 b	19.78	nd b	
Methylvanillyl ether	115.09	x	nd b		9.18 a	28.41	nd b	
Total			**1074.13**		**100.30**		**31.74**	
Monoterpenols								
Linalool	34.01	1443	10.05 a		2.76 c		7.30 b	
Geraniol	62.80	1725	tr a		tr a		tr a	
Total			**10.05**		**2.76**		**7.30**	
Acids								
Butanoic acid	53.19	1607	6.19 a	15.20	tr b		nd c	
Hexanoic acid	61.03	1698	277.81 a	12.62	59.04 b	1.15	18.18 c	41.15
(*E*)-2-hexenoic acid	68.34	1810	22.53 a	22.89	nd **b**		nd b	
Octanoic acid	73.45	1898	100.78 a	17.97	10.06 b	16.23	9.22 b	13.80
Nonanoic acid	78.76	1999	121.70 a	24.04	12.76 b	0.64	11.58 b	32.44
Decanoic acid	83.71	2100	143.33 a	30.72	nd b		nd b	
Benzoic acid	90.10	2239	55.28 a	14.94	7.31 b	28.04	6.70 b	15.40
Dodecanoic acid	92.83	2300	nd b		4.66 a	16.44	nd b	
Tetradecanoic acid	104.20	2504	176.86 b	16.01	3.31 c	20.21	255.85 a	18.21
Pentadecanoic acid	112.76	x	118.36 a	39.37	nd b		nd b	
Hexadecanoic acid	124.38	x	987.65 a	35.86	118.07 b	25.02	93.79 b	5.10
Total			**2010.49**		**215.12**		**395.32**	
Norisoprenoids								
4-oxo-isophorone	49.20	1570	tr a		nd b		nd b	
3-hydroxy-β-damascone	94.80	2340	nd b		tr a		nd b	
Dehydroyvomifoliol	96.20	2369	nd c		1.91 b	5.86	22.96 a	12.89
Vomifoliol	110.10	2578	nd b		3.68 a	2.54	nd b	
Total			**tr**		**5.59**		**22.96**	
C9 aldehyde								
2,4-nonadienal	50.88	1585	39.63 a	0	0.74 b	32.52	nd c	
Total			**39.63**		**0.74**		**nd**	
Lactone								
Pantolactone	71.72	1868	14.77 a	24.55	nd b		nd b	
Total			**14.77**		**nd**		**nd**	
TOTAL			**5700.28**		**1016.33**		**1242.29**	

RT : Retention Time, LRI : Linear Retention Index calculated on DB capillary column, Tr: concentrations <0.05 µg/L, RSD: Relative standard Deviation, nd: not detected. Means indicated by different letters are significantly different at pd0.05, Duncan's multiple range test.

Table 3 - Odor threshold, sensory description and odor activity values of Asli, Beldi and Ferrani grape juice.

Compounds	Odor threshol value*	Sensory description	Odor Activity Values**		
			Asli	Beldi	Ferrani
Hexanal	20[(a)]	Green. grassy[(i)]	47.77	5.80	-
(E)-2-hexenal	40[(b)]	Green. alomnd-like[(i)]	6.08	3.81	5.27
1-hexanol	8000[(c)]	Green. floral[(i)]	<0.10	<0.10	<0.10
(Z)-3-hexen-1-ol	70[(d)]	Green [(e)]	2.07	0.26	0.25
Benzaldehyde	300[(e)]	Almond[(j)]	0.12	<0.10	<0.10
Benzyl alcohol	620[(a)]	Cloudberry-like[(a)]	0.45	<0.10	<0.10
2-phenylethanol	86[(a)]	Slightly rose-like[(a)]	1.92	0.28	0.13
Vanillin	200[(f)]	Sweet. Vanilla[(k)]	1.37	<0.10	<0.10
Methyl vanillate	3000[(f)]	-	<0.10	-	-
Linalool	6[(a)]	Floral[(a)]	1.67	0.46	1.21
Geraniol	130[(g)]	Floral[(g)]	<0.10	<0.10	<0.10
Hexanoic acid	420[(c)]	Sweaty[(i)]	0.66	0.14	<0.10
Octanoic acid	10000[(h)]	Fatty. rancid[(h)]	<0.10	<0.10	<0.10
Decanoic acid	1000[(c)]	Fatty. rancid[(h)]	0.14	-	-
2,4-nonadienal	0.05 [(e)]	Fatty [(e)]	792.60	14.80	-

*Odor perception threshold values (µg/L) in water according to :(a) LATRASSE 1991 (b) VANDERLIN, 1995 (c) FERREIRA *et al.*, 2000 (d) BUTTERY *et al.*, 1971 (e) DARRIET *et al.*, 2002 (f) LÓPEZ *et al.*, 2002 (g) DARRIET, 1996 (h) PEINADO *et al.*, 2004 (i) GROSH and SCHIEBERLE, 1991 (j) FISCHETTI, 1994 (k) SCHIEBERLE and PFNUER, 1999.

be considered important because their concentration were much lower then their perception thresholds (table 3).

Pantolactone, the only lactone identified, was detected in the Asli grape juice (table 2).

3. Bound fraction

Concentrations of the aroma compounds released by enzymatic hydrolysis of the glycosides in the three studied autochthonous grapevine varieties are shown in table 4. A total of 36 bound volatile compounds were identified by GC-MS including 3 C6 compounds, 5 higher alcohols, 16 benzene compounds, 4 terpenes, 6 norisoprenoids and 2 lactones.

Only the aglycons of 1-hexanol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol were identified in the bound fraction of the autochthonous grapevine varieties. The same bound C6 aglycones were found in different autochthonous Portuguese grapevine cultivars (OLIVEIRA *et al.*, 2000; CABRITA *et al.*, 2006). The enzymatically released C6 alcohols were quantitatively less important than the free fraction, as occur in other grape varieties.

Benzene compounds were very abundant as bound fraction and they represent 88.86, 64.44 and 65.77 % of the total bound fraction of Asli, Beldi and Ferrani aroma, respectively. Moreover, some benzene compounds, such as benzaldehyde,2-hydroxy; phenol; phenol, 4-methyl; phenol, 3-methyl; benzenemethanol, 3,5-dimethyl; isoeugenol; 2,3-deshydrobenzofuran and phenol,3, 4-dimethoxy; were not detected in the free fraction. Benzyl alcohol and 2-phenyl ethanol were identified in the three studied varieties and presented the highest concentrations in Asli grape juice. Voirin *et al.* (1992) indicated that the varieties richest on this non-terpene glycosides are neutral cultivars.

The terpene pattern was essentially composed by aglycones of linalool, α-terpineol, cis linalool oxide pyran and geraniol. The distribution of terpene aglycones was not the same within the different varieties. The biggest difference was found between Beldi and Asli. In fact, the 4 glycosidically bound terpenes were present in Asli grape juice. While, α-terpineol and cis linalool oxide pyran were not even identified in Beldi grape juice. These two alcohols are important aroma compounds in Muscat varieties. On the other hand, Ferrani showed significantly the highest concentrations of both linalool and geraniol. These precursors play an important role in the elaboration of aromatic wines (BAUMES *et al.*, 1994; DARRIET, 1996).

Among bound norisoprenoids, 3-oxo-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol were identified only under their bound forms. The related aglycone of the 3-hydroxy-7,8-dihydro-β-ionol was present with the significantly highest concentration in Ferrani grape juice however only traces were identified in Asli variety. In the contrary, the significantly highest concentration of the two oxygenated C13 norisoprenoids 3-oxo-α-ionol and 3-hydroxy-β-damascenone in Asli grape juice may be very important, as it has been proved that these two norisoprenoidic glycoconjugates are precursors of potent odorants such as β-damascenone (WINTERHALTER, 1993).

Table 4 - Concentrations (µg/L) of bound volatile compound from Asli, Beldi and Ferrani grape juice.

Compound	R.T.	LRI	Asli		Beldi		Ferrani	
			Mean (n=2)	RSD (%)	Mean (n=2)	RSD (%)	Mean (n=2)	RSD (%)
C6 compounds								
1-hexanol	16.78	1266	42.09 b	34.08	90.04 a	6.44	48.74 b	15.38
(Z)-3-hexen-1-ol	18.90	1296	9.33 b	6.03	41.29 a	26.33	14.68 b	70.42
(E)-2-hexen-1ol	20.59	1315	nd b		nd b		12.32 a	4.50
Total			**51.42**		**131.33**		**75.74**	
Higher alcohols								
Butan-1-ol, 2-methyl	9.18	1109	nd b		nd b		20.92 a	2.10
3-buten-1-ol, 3-methyl	9.20	1110	26.46 a	10.91	47.71 a	1.88	19.19 a	3.52
2-buten-1-ol, 3-methyl	14.75	1237	55.21 a	11.89	34.78 a	0.39	31.70 b	7.52
2-furanmethanol	46.54	1547	39.90 a	11.26	38.15 a	2.44	5.26 b	16.51
3,7-octadiene-2,6-diol, 2,6-dimethyl	67.76	1800	11.66 b	48.70	nd c		97.41 a	0
Total			**133.23**		**120.64**		**174.48**	
Benzene compounds								
Benzaldehyde	30.61	1416	39.58 b	37.75	90.02 a	15.35	169.15 a	133.01
Benzaldehyde, 2-hydroxy	47.54	1556	9.40 a	6.88	nd c		2.71 b	0
Benzyl alcohol	63.01	1728	1677.40 a	11.95	599.50 b	11.08	482.82 b	11.44
2-Phenylethanol	65.12	1760	571.65 a	8.84	363.00 b	9.69	234.15 c	13.57
Phenol	70.51	1847	107.62 a	6.05	38.06 c	8.82	69.36 b	11.04
Phenol, 4-methyl	74.59	1920	22.87 a	34.96	nd b		9.83 ab	48.84
Phenol, 3-methyl	74.76	1923	9.14 a	3.98	nd b		9.62 a	31.34
Benzenemethanol, 3,5-dimethyl	76.64	1959	nd b		nd b		7.32 a	4.69
Eugenol	79.04	2005	75.40 a	0	nd b		nd b	
4-vinylguaiacol	80.49	2029	1146.10 a	2.93	nd c		44.98 b	14.23
Isoeugenol	87.13	2173	34.71 a	0	nd b		nd b	
2,3-dihydrobenzofuran	88.87	2211	218.41 a	3.97	nd b		nd b	
Vanillin	96.04	2365	31.44 a	28.65	10.42 b	36.45	34.60 a	13.90
Acetovanillone	99.93	2435	56.27 a	2.98	2.66 b	12.55	1.10 b	16.60
Phenol, 3,4-dimethoxy	109.50	2570	nd b		nd b		29.02 a	11.82
Methyl vanillyl ether	115.08	x	71.93 b	2.58	229.40 a	2.25	nd c	
Total			**4071.92**		**1333.06**		**1094.66**	
Terpenes								
Linalool	33.66	1443	19.97 a	7.54	19.76 a	21.74	13.19 a	22.31
α-terpineol	50.39	1580	19.78 a	2.93	nd c		5.72 b	0
cis linalool oxide pyran	53.50	1610	8.85 a	0	nd b		nd b	
Geraniol	62.80	1725	3.62 c	0	6.83 b	0	31.82 a	0
Total			**52.22**		**26.59**		**50.73**	
Norisoprenoids								
4-oxo-isophorone	49.20	1570	4.58 a	0	nd b		5.44 a	0
3-hydroxy-βdamascenone	94.80	2340	138.59 a	10.33	140.90 a	6.04	159.65 a	1.49
3-oxo-α-ionol	99.94	2436	71.33 a	4.36	2.21 b	8.34	3.16 b	18.08
3-hydroxy-7,8-dihydro-β-ionol	107.89	2550	tr b		nd c		66.49 a	9.19
Dehydrovomifoliol	96.20	2369	nd c		8.64 a	1.98	tr b	
Vomifoliol	110.10	2578	6.39 b	2.73	305.16 a	7.87	19.36 b	5.03
Total			**220.89**		**456.91**		**254.10**	
Lactones								
2(5H) furanone, 3-methyl	51.63	1585	20.59 a	20.49	nd b		nd b	
Pantolactone	71.72	1868	31.84 a	5.16	nd c		14.56 b	20.34
Total			**52.43**		**nd**		**14.56**	
TOTAL			**4582.11**		**2068.53**		**1664.28**	

RT : Retention Time, LRI : Linear Retention Index calculated on DB capillary column, Tr: concentrations < 0.05 µg/L, RSD: Relative Standard Deviation, nd: not detected. Means indicated by different letters are significantly different at ≤ 0.05, Duncan's multiple range test.

The glycosidically bound dehydrovomifoliol and vomifoliol were found at significantly highest concentrations in Beldi grape juice.

Among the identified lactones, 2(5H) furanone, 3-methyl, was found only in Asli variety.

CONCLUSION

In the present work the aromatic profiles of three Tunisian autochthonous grapevine varieties (Asli, Beldi and Ferrani) have been characterized. 38 volatile compounds in the free fraction and 36 glycosidically bound aroma compounds were identified in the three autochthonous grapes. Free aroma compounds of the studied varieties were significantly different. In fact, Asli variety had higher number and concentrations of the identified volatile compounds than Beldi and Ferrani. Non-terpenyl compounds were the most abundant aroma substances in the three studied varieties. OAVs values proved that grassy notes contributed mostly to Beldi and

Asli varieties rather than to Ferrani flavors. The total amounts of the detected aroma bound forms is always higher than those of the free forms. Enzymatic hydrolysis released higher number of valuable compounds such as monoterpenol and C13 noisoprenoid aglycones in Ferrani and Asli grape juices than in Beldi. This reveals a potent aroma potential of these two varieties.

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