THE EFFECT OF TERROIR ZONING ON POMOLOGICAL, CHEMICAL AND AROMATIC COMPOSITION OF MUSCAT D'ALEXANDRIE GRAPEVINE VARIETY CULTIVATED IN TUNISIA

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Key words
Muscat d'Alexandrie, grape juice, aroma, terroir, Tunisia

Abstract

The effect of terroir zoning on the pomological, chemical and aromatic composition has been studied on the Muscat d'Alexandrie grapevine variety over two years 2001 and 2002. This variety was cultivated in three terroirs (RafRaf, Baddar and Kelibia) in the North-East of Tunisia.
Muscat d'Alexandrie from each terroir was randomly harvested at commercial maturity, in 2001 and 2002. Pomological parameters (bunch and berry mean weights) and chemical characteristics (total acidity, pH, density, Brix degree and total polyphenol index) have been immediately measured. The aroma free and bound fractions were analyzed using CPG equipped by FID detector.
The results showed that the pomological and chemical parameters were the less affected by the terroir zoning. Nevertheless, zoning affected mainly the aromatic composition of the berry. Although, the global value MT of the free monoterpenols (linalool+nerol+geraniol) was included in the Muscat aroma perception interval, the distribution of the concentration of each changed from region to another. Indeed, linalool and geraniol compounds were the most sensitive to environmental changes and consequently terroirs.
During 2001 and 2002 and according to the terroir, the glycosidically bound fraction has been increased 4 to 6 times.

Résumé
La composition du raisin de la variété Muscat d'Alexandrie a été étudiée dans trois terroirs différents au Nord-Est de la Tunisie (RafRaf, Baddar et Kelibia).
Des échantillons de raisins ont été récoltés à maturité industrielle durant les saisons 2001 et 2002 dans les trois régions citées. Les paramètres pomologiques (poids moyen de la grappe et de la baie) et physico-chimiques (acidité totale, pH, densité, degré Brix et indice des polyphénols totaux) ont été immédiatement mesurés. Les composés libres et liés de l'arôme ont été analysés par chromatographie en phase gazeuse (C.P.G.) équipée d'un Détecteur à Flamme d'Ionisation (FID).
Les caractéristiques pomologiques et physico-chimiques n'ont pas subi une modification importante dans les différentes régions étudiées. Cependant, l'effet significatif du terroir se reflète essentiellement sur la composition de la baie en arôme. Bien que la somme des trois monoterpénols (MT; linalol+nérol+géraniol) a toujours été comprise dans le seuil de perception de la note muscatée, une nette différence au niveau de leur distribution a été constatée. Linalol et geraniol sont les composés d'arôme les plus sensibles aux changements des conditions du milieu.
Selon l'année (2001 et 2002) et le terroir, la fraction liée des composés d'arôme est de 4 à 6 fois plus importante que la fraction libre.
Mots clés : Muscat d'Alexandrie, jus de raisin, arôme, terroir, Tunisie.

Introduction

The effect of environmental factors on the grape quality at harvest has been the subject of several investigations (Reynolds et *al*., 1994; 1996; Bureau et *al*., 1998a, 2000; Dirninger et *al*., 1998; Bravdo 2001). As was reported by Douglas et *al*., (2001), the concept of "terroir" is used to describe all aspects of environment, geography and cultural practices that influence grape production. The effect of vine environment modification contributes significantly to a great variability in berry composition. This is often reported, particularly on the ripeness date (Reynolds et *al*., 1995) and consequently on sugar and acidity amounts.

Much attention has been focused on the effect of grapevine microclimate on the fruit composition of Muscat grapes which are known for their strong aromatic properties (Razungles et *al*., 1996, Bureau et *al*., 1998b). It was proved that the high levels of terpenoids in the Muscat berry, juice and wine are responsible of their floral and fruity flavour (Bayonove et Cordonnier 1982; Günata et *al*.,1985; Etiévant 1992). However, their concentrations can be modified by geographical, seasonal and viticultural factors (Castro Vázquez et *al*., 2002). In fact, as was proved by Belancic et *al*., (1997), the bunch microclimate seems to modify the free and bound monoterpene levels in grape and wine. However, it did not decrease the bound phenols and C13-norisoprenoïds (Bureau et *al*., 2000).

Muscat d'Alexandrie is one of the most popular grapevine cultivar in Tunisia. It's known for its high pomological and organoleptic qualities. Thus, it has a non negligible importance in terms of national economy. It is cultivated in several geographically different areas which impart distinctive qualities to the grapevine. In each location, the geographical and geological properties of the site based not only on the climatological data, but also on the soil structure and texture, can be described as "basic terroir".

In the Tunisian vineyard, several names have been attributed to Muscat d'Alexandrie. Each one referred to the "basic terroir" in which this variety is cultivated and in which it has acquired a typical taste.

Muscat d'Alexandrie, when cultivated in the well defined geographical area of Kelibia (North-East of Tunisia), brings a particular aromatic properties to the most appreciated A.O.C wine in the country. Thus, since many decades, Muscat d'Alexandrie has been called Muscat Kelibia when cultivated in this location, the same for Muscat RafRaf, Muscat Ghézala and others.

Although several efforts were made for the protection of the Tunisian Muscat terroirs and the conservation of the genetic resources, the aroma compounds of Muscat cultivars have not yet been studied.

The objective of this study is to examine the effect of the vineyard site on the pomological, physico-chemical and aromatic content of grape juice from Muscat d'Alexandrie cultivated in three terroirs (Baddar, RafRaf and Kelibia)

Material and methods

Twenty years old grapevine from Muscat d'Alexandrie variety were cultivated in three different viticultural regions. Geographical and pedo-climatic data of the three vineyards in 2001 and 2002, are given in the table 1.

In all cases, the vines were not irrigated and cultivated according to the traditional "cup system".

30 Muscat d'Alexandrie bunches from each terroir were randomly harvested at commercial maturity. Berries were manually pressed. Soluble solids were measured using a hand-held refractometer (OPTECH 0-32% Brix/ ATC). pH was measured by means of a standard pH meter (EUTECH instruments, Cyberscan 500). The total acidity was determined following the titration of 10 ml of fresh juice in 25 ml distilled water, to pH 7. The results are given in g of tartric acid per litre of fresh grape juice.

The aroma free and bound fractions were separated by adsorption/ desorption on Amberlite-XAD-2 resin (copolymeric polystyrene and divinylbenzene, 50-80 mesh) (Fluka), according to the method of Günata *et al.*, (1985) as modified by Razungles *et al.,* (1993). The pressed juice was clarified by centrifugation at 4°C for 25 min (10000 ×*g*). Exactly 10.0 µl of the internal standard (2-octanol 0.1% in ethanol) were added to 50 ml of grape juice. The clear juice was passed through a (35 cm × 1 cm ID) glass column containing 7g of resin with a flow-rate of 1.5-2 $ml.min^{-1}$.

The free volatiles were carried out with 50 ml of the azoetrope mixture (pentane–dichloromethane) (2:1 V:V), dried with anhydrous sodium sulphate, concentrated at 40°C to a final volume of 400 µl then stocked at –20°C for a subsequent chromatographic analyses.

The bound fraction was eluted from the column with 50 ml of ethyl acetate. The eluent was dried by filtering through anhydrous sodium sulphate and concentrated, in the first step up to 1 ml under vacuum (rotary evaporator) at 40°C and in the second step at 45°C using a stream of nitrogen. To release the bound aroma, 200 µl of 0.1M citrate phosphate buffer (pH5) were added and washed four times with 200 µl of pentane–dichloromethane (2:1 V:V) in order to remove the remaining free compounds.

14 mg of AR-2000 enzyme (Gist-Brocades) were added (Oliviera *et al.,* 2000). The mixture was incubated at 40°C for 16 h then extracted 5 times with 200 µl of pentane–dichloromethane (2:1 V:V). After addition of 10.0 µl of 4-nonanol (3.2 $mg.ml^{-1}$), as internal standard, the solvent was eliminated by rectification using a nitrogen stream at 45°C.

Gas Chromatography analysis of aroma compounds

The volatile compounds of each fraction were separated through a Hewlett-Packard 5890 gas chromatograph, equipped with a flame ionization detector (250°C) and a Carbowax 20 M column (30 m x 0.25 mm with 0.25 µm film thickness J&W). The samples were injected in split mode (split ratio 80:1; sample size 1µL). The oven temperature was held at 60°C for 3 min, then was gradually increased (3°C.min^{-1}) up to 245°C then 250°C for 20 min. The carrier gas was Hydrogen. Linalool, nerol, geraniol, α-terpineol and citronellol were identified by comparing their retention time with the corresponding authentic standards. The analysis of grape juice free and bound aromatic compounds from Muscat d'Alexandrie in the three terroir was performed in triplicate.

Statistical analysis:

A one-way analysis of variance (ANOVA), was achieved during the whole period, using STATISTICA program. Means were compared, using F test Duncan test at $p<0.05$ level, to determine significance between the three locations for each season and each cultivar.

Results and discussion

The difference in pomological components (cluster and berry mean weights) of the three cultivars was not statistically proved during 2001 and 2002 (table 2). In this study, these two parameters do not seem to be influenced by terroir conditions. Rainfall means, which are slightly different in the three sites (table 1), seem to impose the pomological components.

The results indicate that the effect of terroir on the grape juice Brix degree was proved over the two years (Figures 1a and 1b). In both years, Muscat Kelibia had the highest soluble solids concentration. This fact could be essentially due to the temperature and the global radiation which are different from one site to another. According to Dirninger et *al*., (1998), the site origin strongly affects the titrable acidity. As can be observed in figures 1a and 1b, Muscat RafRaf cultivar presented the highest acidity level.

The total polyphenol index, estimated by the optical density at λ=280 (OD_{280}), was the most sensible to terroir variation. The highest OD_{280} was found in Muscat variety when cultivated in Baddar during 2001 and 2002.

Free Fraction

Linalool, nerol and geraniol are the most important terpenols determining the Muscat typicity (Boidron and Torres, 1982). According to Baumes et *al*., (1994), the strongest aromatic character is obtained at around 1000 $\mu g.l^{-1}$ when total (linalool+nerol+geraniol) concentration (MT) is considered. While a concentration above 1400 $\mu g.l^{-1}$ results in a loss of Muscat character, probably due to the aroma denaturation. The results given in table 3 indicate that each variety achieved the Muscat typicity in all terroir conditions. MT allowed to differentiate between the three sites, only in 2001. In fact, Muscat d'Alexandrie cultivated in RafRaf produced a grape juice with a significantly high level of MT when compared to those of Baddar and Kelibia.

Linalool, a major compound in the floral varieties, was statistically the less abundant in Muscat Baddar in 2001 (table 3). However, in 2002, its level was not significantly different in the grape juices from Muscat varieties cultivated in the three locations. These levels are higher than its odor perception threshold (Ribéreau Gayon et *al*., 1975). The high proportion of linalool with regard to MT seems to be more dependent on the season than on the cultivation site (figures 4a and 4b).

As was reported by Etiévant (1992), it is obvious that linalool and geraniol contribute essentially to the Muscat aroma. Bayonove et *al*., (1976) proved that the strongest muscat aroma is reached when the ratio [linalool+ geraniol / linalool+nerol+geraniol] (L+G/MT) is above 75%. According to figures 2a and 2b, this ratio is extremely useful in order to examine the terroir effect on Muscat d'Alexandrie since it is more discriminating than linalool and geraniol concentrations (table 3). These results seem to be essentially due to the mesoclimate of the considered vineyard. In fact, Bayonove and Cordonnier (1982) suggested that linalool and geraniol are the most sensible to the climatic conditions.

Although, nerol concentration increased for all cultivars from 2001 to 2002, its level was very low when compared to linalool and geraniol. Moreover, nerol concentration was much lower than its odor perception threshold (Ribéreau Gayon et *al*., 1975).

Muscat RafRaf grape juice was the richest in α-terpineol in both years. Citronellol amounts, however, were affected by the terroir.

Bound fraction

The concentrations of bound compounds determined as their glycosidic forms are presented in table 4. The high proportion of bound terpenols compared to the free ones is of considerable interest. In fact, it is more discriminating since it distinguished between the three Muscat d'Alexandrie grape juices according to their terroir, in both years 2001 and 2002. López-Tamames et *al*., (1997) indicated that the glycoside composition was highly variable and depended on the geographical origin and/ or year.

As proved by Günata et *al*., (1985), linalool and geraniol are among the most abundant bound monoterpenols. This was not the case of Muscat Kelibia in 2001, since nerol concentration was higher than geraniol.

The ratio of bound MT/ free MT was extremely greater than 1 in all varieties. As presented in figures 3a and 3b, Muscat Baddar had, during both years, the highest ratio, which easily reached 5.46 in 2001 and 4.07 in 2002.

Conclusion

This research backs up the appropriateness of the different appellations attributed, since many decades, to Muscat d'Alexandrie when cultivated in three different Tunisian locations.

The pomological components of the three cultivars were slightly the same during the two seasons of study. They were, however, differentiated by profiles of their free and bound compounds. The global value of the free monoterpenols linalool+nerol+geraniol (MT) was included in the Muscat aroma perception interval. The distribution of the concentration of each monoterpenol changed from one region to another. Indeed, linalool and geraniol compounds were the most sensitive to environmental changes and consequently terroirs. Moreover, the geographical area influenced their bound rather than their free aroma.

Thus, Muscat d'Alexandrie acquired different aromatic characters when it is cultivated in Baddar, RafRaf and Kelibia. The new phenotypes, significantly different, may be imposed by the micro and mesoclimatic conditions of each site.

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Table 1. Geographical and pedo-climatic data in Baddar, RafRaf and Kelibia in Tunisia.

			Baddar		**RafRaf**		**Kelibia**	
Localisation		Longitude	10° 30'N		10° 15'N		11°07'N	
		Latitude	36°43'E		37° 12'E		36°51'E	
		Altitude	30m		50m		40m	
Distance from the sea (Km)			5		0.5		1	
Soil texture			Sandy-clayey		Sandy-clayey		Sandy-clayey	
			2001	**2002**	**2001**	**2002**	**2001**	**2002**
Mean Temperature (°C)	Winter	T°min	7.4	7.1	6.8	6.3	7.9	7.5
		T°max	14.4	14.2	13.7	13.6	15.3	14.9
	Summer	T°min	20	20.8	19.5	19.3	20.6	20.8
		T°max	33	32.5	32.1	31.6	30.7	29.5
Global radiation (cal. cm^{-2})		January	6510	6528	6389	6382	6630	6632
		July	19830	19785	19251	19243	19985	19800
Relative Humidity (%)		January	75	77	79	78	82	85
		July	60	59	65	66	66	60
Mean rainfall (mm/year)			380	435	365	448	355	453

Table 2. Pomological and chemical composition of Muscat d'Alexandrie fresh grape juice cultivated in three terroirs, 2001 and 2002.

	terroirs	**Cluster mean weight (g)**	**Berry mean weight (g)**	**Density**	**pH**	**OD_{280}**
2001	**Baddar**	423.8**a**	5.31**a**	1.087**a**	3.85**b**	16.53**a**
	RafRaf	414**a**	5.15**a**	1.086**a**	3.94**a**	11.75**c**
	Kelibia	394**a**	5.22**a**	1.085**a**	3.93**a**	13.36**b**
2002	**Baddar**	322.3**a**	3.11**a**	1.092**c**	3.8**b**	17.71**a**
	RafRaf	327.8**a**	3.05**a**	1.095**b**	3.89**a**	11.25**c**
	Kelibia	340.9**a**	3.2**a**	1.097**a**	3.92**a**	15.43**b**

Mean of three replications of the same sample. Means indicated by different letters are significantly different at $p \leq 0.05$, Duncan's multiple range test.

Table 3. Free monoterpenes in grape juice of Muscat d'Alexandrie (µg.l^{-1}) cultivated in three terroirs, 2001 and 2002.

	Terroirs	linalool	nerol	Geraniol	MT	α-terpineol	citronellol
2001	Baddar	904**b**	61.6**a**	168**b**	1134**b**	22.3**b**	102.3**a**
	RafRaf	1092**a**	35.3**b**	254.6**a**	1382**a**	65.3**a**	13**b**
	Kelibia	1071.3**a**	7.6**c**	73.3**c**	1152.3**b**	66.6**a**	0**c**
2002	Baddar	813.3**a**	43**b**	512.6**a**	1369**a**	23.6**c**	92.6**b**
	RafRaf	772.2**a**	209.7**a**	341**b**	1323**a**	208.7**a**	108.2**a**
	Kelibia	801**a**	187**a**	281**c**	1269**a**	130**b**	112**a**

Mean of three replications of the same sample. Means indicated by different letters are significantly different at $p \leq 0.05$, Duncan's multiple range test.

Table 4: Glycosidically bound monoterpenols in grape juice of Muscat d'Alexandrie ($\mu g.l^{-1}$) cultivated in three terroirs, 2001 and 2002.

	Terroirs	linalool	nerol	Geraniol	MT	α-terpineol	citronellol
2001	Baddar	4852.3**a**	509**a**	824.6**b**	6186**a**	319.3**b**	272.3**a**
	RafRaf	4155.3**b**	447.3**b**	380**c**	4982.6**b**	110.6**c**	225.3**b**
	Kelibia	3431.3**c**	223**c**	873**a**	4527**c**	364**a**	96.6**c**
2002	Baddar	3602.3**a**	856**a**	1119.6**a**	5581**a**	147.6**c**	191.3**b**
	RafRaf	3587.6**a**	668**b**	789.6**c**	5045.3**b**	359**a**	281.6**a**
	Kelibia	3316.6**b**	357**c**	1036.3**b**	4710**c**	267**b**	166**c**

Mean of three replications of the same sample. Means indicated by different letters are significantly different at $p \leq 0.05$, Duncan's multiple range test.

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Figure 1a: Brix degree and total acidity (g of tartaric acid. l^{-1}) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2001

Figure 1b: Brix degree and total acidity (g of tartaric acid. l^{-1}) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2002

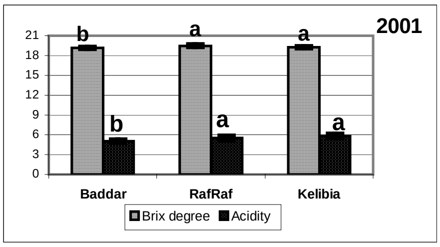


Figure 1a

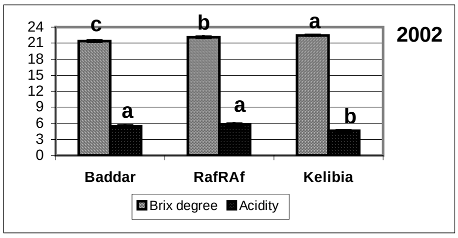


Figure 1b

Figure 2a: Concentration of linalool+geraniol (% of MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2001

Figure 2b: Concentration of linalool+geraniol (% of MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2002

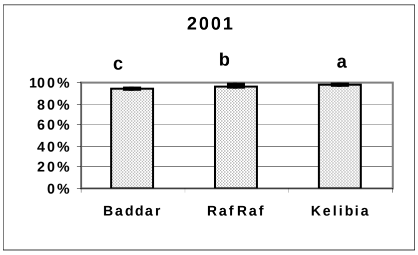


Figure2a

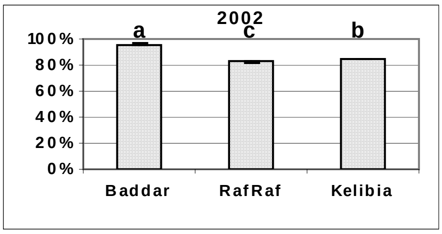
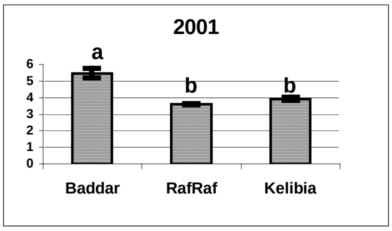


Figure 2b

Figure 3a: Bound MT/ Free MT (MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2001

Figure 3b: Bound MT/ Free MT (MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2002



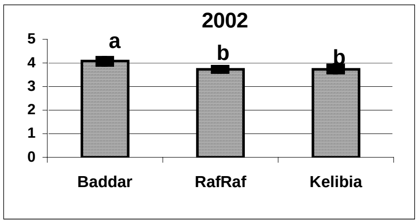
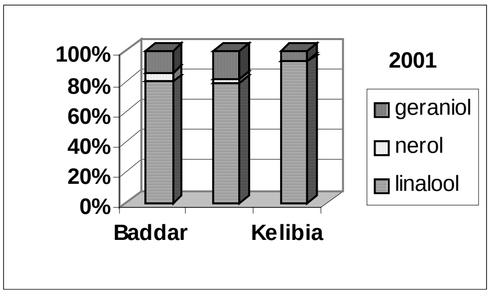


Figure 3a **Figure3b**

Figure 4a: Concentration of linalool, nerol and geraniol (% of MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2001

Figure 4b: Concentration of linalool, nerol and geraniol (% of MT; linalool+geraniol+nerol concentrations) in Muscat d'Alexandrie grown at three locations (Baddar, RafRaf and Kelibia) in Tunisia, 2002



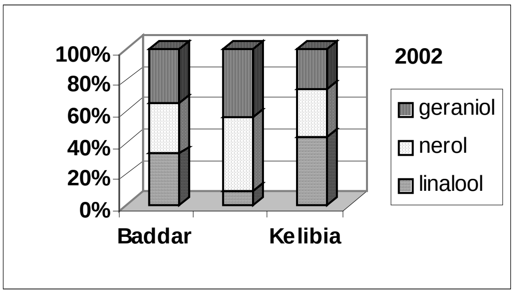


Figure 4a **Figure4b**