RED GRENACHE VARIETY IN RHÔNE VALLEY : IMPACT OF "TERROIR" AND VINTAGES ON THE AROMATIC POTENTIAL OF THE GRAPES.

LE GRENACHE EN VALLEE DU RHONE : INCIDENCE DU TERROIR ET DES MILLESIMES SUR LA COMPOSITION AROMATIQUE DES RAISINS

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RESUME

Le cépage Grenache noir, de par son originalité et sa représentativité, participe très directement à la qualité et à la typicité des vins de la vallée du Rhône. Il est généralement apprécié pour sa palette aromatique variée et pour les caractères de rondeur et de souplesse qu'il confère aux vins. Depuis 1995, l'Institut Rhodanien a mis en place un réseau de parcelle de référence représentative des différents type de terroir présent en zone méridionale de l'Appellation Côtes du Rhône (TRUC, 1997; VAUDOUR *et al.*, 1996). Les publications sur la composition aromatique des raisins et des vins est très abondante, mais seulement quelques articles sont parus sur le cépage grenache (BAUMES *et al.*, 1986).Comme quelques autres cépages référencés comme « neutres », ce cépage engendre des vins très typés, dont la qualité aromatique est reconnu à travers le monde entier. Une première étape nous a permis d'étudier d'une manière systématique l'ensemble des composés aromatiques présents à l'état libre ou sous forme glycosylée (précurseurs d'arômes) au cours de 3 millésimes consécutifs. Les résultats présentés mettent en évidence la partie importante des précurseurs d'arôme (jusqu'à 70% du potentiel aromatique total) et l'impact du millésime, mais surtout du terroir sur ces composés. Cette constatation nous a conduit à mettre au point une technique de vieillissement accéléré permettant une révélation rapide du potentiel aromatique lié, afin de pouvoir étudier rapidement son impact sensoriel. Cette étude est complétée par des analyses sensorielle dont les résultats mettent en évidence l'intérêt et l'importance de ce potentiel aromatique dans les caractéristiques olfactives finales du vins après son évolution optimale.

INTRODUCTION

The red Grenache cultivar is the most grown in the Côtes du Rhône (South east of France). Since 1995, the Institut Rhôdanien has established a vineyard network including all different soil types found in the Southern part of Côtes du Rhône (TRUC, 1997; VAUDOUR *et al.*, 1996). As reviewed by DUBOIS (1993-1994a,b) or ETIEVANT (1991), the literature on the aroma composition of grapes and wines is abundant. but few papers on Grenache grapes and wines have been published (BAUMES *et al.*, 1986). As some other neutral grape varieties, this cultivar produces typical wines known for their aromatic quality all over the world. The typical aroma of these wines could be partly related to grape non-volatile, odourless glycoconjugates which could give rise to odorant compounds during the different steps leading from grape to the aged wine (ORMIERES *et al.*, 1997b), as it was demonstrated for other grape cultivars (FRANCIS *et al.*, 1994; FRANCIS *et al.*, 1992; SEFTON *et al.,*1994; Strauss *et al.*, 1987). Thus we undertook a detailed study; first to identify in Grenache grapes the free volatiles and the enzymaticaly released volatile aglycones of the glycoconjugates, secondly to elucidate the origin of varietal aromas of Grenache wines. This paper reports the result of the first part of this study obtained on red Grenache batches of the 1995-1997 vintages from the network of 19 vineyards selected in the Southern part of Côtes du Rhône by the Institut Rhôdanien.

MATERIAL AND METHODS

Plant materials

Grenache grapes (2 kg) of the 1995-1997 vintages, from 19 vineyards selected by the Institut Rhôdanien as representatives of the different soil types found in the southern part of Côtes du Rhône, were hand-harvested at the same technological maturity. The vines were established on 110R rootstocks, clone 70, bilateral cordon de Royat pruning, 15-20 years old, 3600-4000-vine stock/Ha. In each vineyard, 100 vine stocks on 4 lines were identified. 15 groups of 3 successive vine stocks were selected to control and sampling. Two kilograms of berries were frozen and stored at low temperature (-25°C) until analysis.

Preparation of samples and analysis

Grenache juice samples were obtained from the 19 vineyards of the 1995-1997 vintages. Berries were defrosted (+4°C), crushed in a blender for 20 s, the pulp filtered through gauze and centrifuged (4°C for 20 min at 8000 rd/min). The clear juice was refiltered through glass wool and immediately extracted.

Extraction of the free and bound fractions from 0.1L of juice was performed on Amberlite XAD2 (FLUKA) as described previously (GÜNATA *et al., 1985 a, b*). Selective solvents were used for the elution of the free and bound fractions. The free fraction was eluted with pentane / dichloromethane azeotrope (2/1 v/v).

The bound fraction was eluted with 50ml of methanol / water (60/40 v/v). The aglycons from the bound fraction were released by enzymic hydrolysis (mixture of Pektolase and Hemicellulase in aqueous PH 5 buffer heated at 40°C for 16 hours. 10µl of 4-nonanol (3.2 g/l) was then added, and the hydrolysate was isolated by extraction with pentane / dichloromethane azeotrope (2/1 v/v).

The free fraction and the aglycons were identified by gas chromatography coupled to mass spectrometry and quantified by GC/FID. Extraction yield in this condition was performed using reference glycoconjugate of nerol, phenol, eugenol, linalool and methyl salicylate.

Each analysis was made in triplicate.

Statistical analysis

Principal Component Analysis (PCA) and Factorial Discriminant Analysis (FDA) and Hierarchical Clustering Analysis (HCA) were performed on Statlab 2.1 software (S.L.P. Dallas-USA).

RESULTS & DISCUSSION

The procedures used in the analytical study of the free and bound fraction were similar to those described previously (REF) but using methanol / water elution instead of ethylacetate for the bound fraction. Quantitative determination was carried out using internal standard. The concentration reported in Table 1 therefore may be considered as relative data only. They relate to actual amounts in the berries only for the compounds with the same extraction properties as the internal standard. The average extraction yields were performed using reference glycoconjugates of nerol (98%), phenol (81%), eugenol (88%), linalool (66%) and methyl salicylate (74%). To evaluate the reproducibility of the method, three experiments (extraction, fractionation, concentration) were performed for each analysis. Mean values and standard deviation are shown in tables.

Odour evaluations of components (sniffing test) were performed on a polar column (DB-WAX) and an apolar column (DB5) using reference compounds.

I / IDENTIFICATION AND QUANTIFICATION OF THE AROMATIC POTENTIAL OF THE RED GRENACHE GRAPES FROM 1995 VINTAGE.

24 compounds in the free fraction and 84 in the bound fraction of the grape juice were identified and quantified. The free compounds and the liberated aglycons were classified in 5 different chemical groups: C6 compounds, alcohols, phenols, monoterpenols and C13 norisoprenoids. The mean levels of each chemical group in the grapes of the 1995 vintage from the 19 vineyards are shown in polar coordinates in figures 1a and 1b.

Free fraction from grapes:

The free fraction was quantitatively dominated by the C6 compounds (unsatured alcohols, aldehydes and hexanol from oxidation of linolenic and linoleic acids in relation with the crushing of the berries) ranging from 452 to 1745 µg/l. The volatile phenols levels (mainly gaiacyl derivatives) and alcohols levels (mainly 2-phenylethanol and benzyl alcohol) were ranging respectively from 53 to 100 µg/l and from 278 to 754 µg/l. The identified phenols could arise from oxidation of the cinnamic derivates (DUBOIS P., 1984), whereas alcohols could come from the catabolism of the amino acids. The monoterpenols contents were very low (from 2 to 15 µg/l) compared to the Muscat cultivars (500 to 1700µg/l) (GÜNATA, 1985 a).

Bound fraction from grapes:

As observed in other cultivars (GÜNATA *et al.*, 1984; VOIRIN *et al.*, 1992), the bound fraction (table 2) is more abundant qualitatively and quantitatively than the free one (table 1), except for the C6 compounds. This fraction was dominated by the phenol class, consisting mainly of gaiacyl and hydroxyphenyl derivatives, ranging from 108 to 327 µg/l, and the monoterpen class, consisting of 14% monoterpenols at the oxidation state of linalool versus 86% monoterpenols at higher oxidation state, ranging from 121 to 256 µg/l. The total content of the C13 norisoprenoids (arising from degradation of carotenoids) was ranging from 96 µg/l to 210 µg/l. The alcohol class was mainly made up of 2-phenylethanol and benzyl alcohol ranging from 533 to 2481 µg/l.

As observed in other non-Muscat varieties (Abbot N.A. *et al, 1990*), Grenache grapes contain low levels of volatiles. The aroma compounds are mainly "locked up" as non-volatile, odourless glyconconjugates, which could generate at the wine pH volatile odorants, as reported previously (ABBOTT *et al.*, 1991; FRANCIS *et al.*, 1994; ORMIÈRES *et al.*, 1997; STRAUSS *et al.*, 1987; WILLIAMS, 1993).

Impact of the terroir (vintage 1995)

A Principal Component Analysis was performed using the 19 vineyards as individuals and the total concentrations of the chemical classes of bound compounds as variables.

The first two axes explained 81% of the total variance. Figure 2 shows the projection of the individuals and of the variables on the plane of these first two axes.

The vineyards could be classified in 5 groups using and HCA analysis:

- very low level in the overall bound fraction (562, 638, 941).
- low level in C6 compounds (422, 789, 867).
- high level in C6 compounds (412, 456, 652).
- high level in phenol compounds (103, 162, 235, 383, 318, 597, 736).
- high level in the overall bound fraction (205,840,986).

A representative vineyard of each group (underlined in the groups shown above) was selected for the rest of this study. The same analysis of the bound fraction was performed in 1996 and 1997 on these representative vineyards.

To find which compounds could differentiate the individuals according to the vineyard or the vintage effect, two FDA were performed.

In the first one the vineyards were group according to the three vintages, including all experiments and the thirty-six significantly different variables. The two first axes explain the full variance. Figure 4 show the projection of the vineyards in the plane of these two first axes.

The first axis, was correlated to 13 variables with correlation coefficients higher than 0.89. It clearly separates the 1995 vintage from the two other ones, with higher level in 3-hydroxy-7,8-dihydro-? -ionol, unknown 35W, 3-oxo-? -ionol and zingerone. These compounds, detected in GC/Olfactometry using two capillary columns of different polarity and reference solutions, displayed olfactive notes described as floral for the unknown 35W, animal-dusty for 3-oxo-? -ionol or spicy for the zingerone. The second axis was correlated to 6 compounds with a coefficient higher than 0.89 and separated the 3 vintages.

Whereas the 1997 vintage was characterized by higher content in zingerol, phenol, octan-2-ol and vanilloyl methyl ketone, the 1996 vintage was characterized by higher content in methyl 2,6-dihydroxybenzoate (described as pepper, spicy) and *trans*-furan linalool oxide (smoky). Grapes of the 1995 vintage had average levels in these compounds.

In the secondary, the individuals were group according to the five vineyards groups, including all experiments and the thirty-six significantly different variables. The two first axes explained 92% of the variance. Figure 5 show the projection of the vineyards in the plane of these two first axes.

The first axis, explaining 48% of the variance, clearly separated the 986 vineyard characterized by higher levels of *trans*-furan linalool oxide, 3-oxo-? -ionol, oct-3-en-1-ol, methyl 2,6-dihydroxybenzoate, 3-hydroxy-? -damascone and zingerone.

The second axis, explaining 45% of the variance, was correlated to linalool, 4-methylbenzyl alcohol, 2(4'-gaiacyl)-ethan-1-ol and nerol, and opposed the 162 and 638 vineyards (low levels in these compounds) to the 652 and 789 vineyards (high levels).

CONCLUSION

Grenache variety is known to be a neutral cultivar. This study on the analytical characterisation of the berries have shown that its aromatic potential, mainly made up of glycoconjugates of phenols, C13 norisoprenoids and monoterpenols was important and dependent on vintage but also on vineyard. Complementary studies have shown the odour impact of the bound fraction, characterising this cultivar as an aromatic potential for the future.

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Figure.1 (a) Total concentrations (µg/l) of the three main chemical classes of the volatiles identified in the red Grenache grapes of the 1995 vintage from the nineteen selected vineyards.

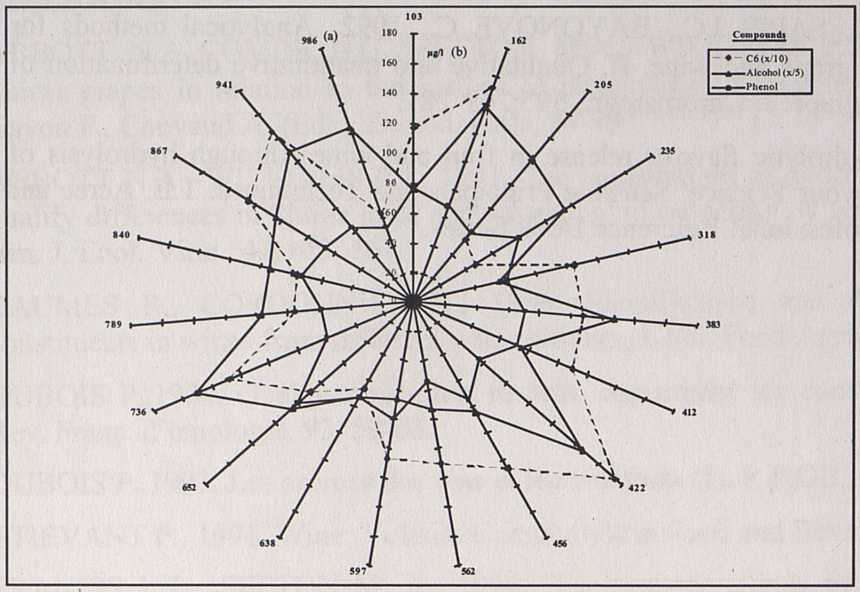

Figure.(1b) Total concentrations (µg/l) of the five main chemical classes of the aglycones identified in the red Grenache grapes of the 1995 vintage from the nineteen selected vineyards.

(a): identification code of the vineyard. (b): mean totals of the levels of the chemical groups (µg/l).

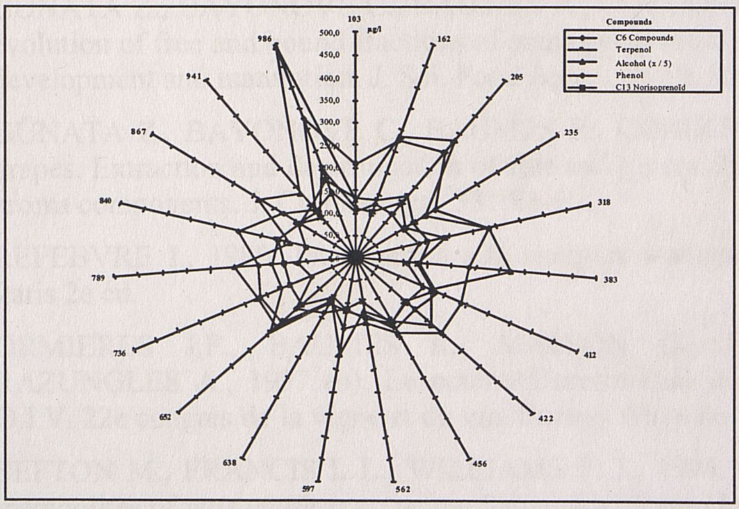

Figure.2 Projection of the nineteen vineyards and of the corresponding liberated bounds compounds on the two first principal components of the PCA.

(Axis1 : 56.28% of the variance ; Axis2 : 24.81% of the variance).

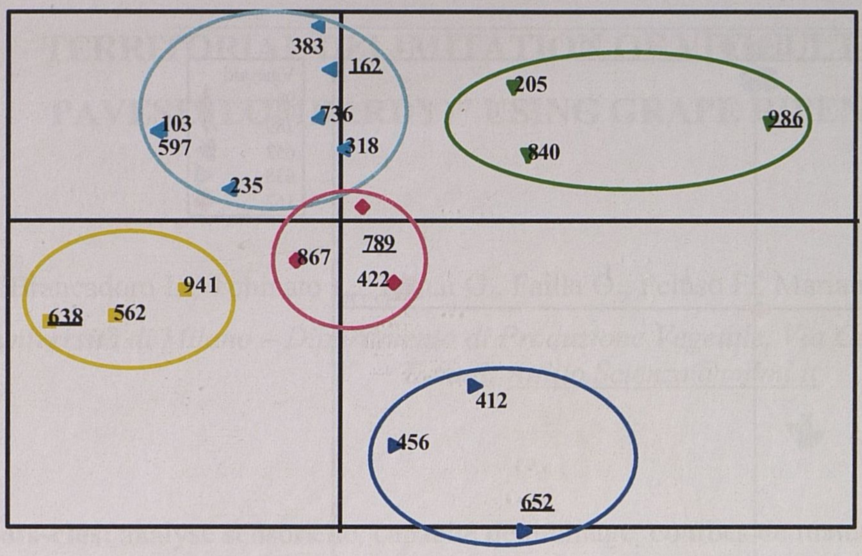

Figure.4 Projection of all the repetitions of the five selected vineyards, 1995-1997 vintages, and of the 36 selected liberated bounds compounds on the two first principal components of the FDA, according to the vintage.

(Axis1 : 56% of the variance ; Axis2 : 44% of the variance).

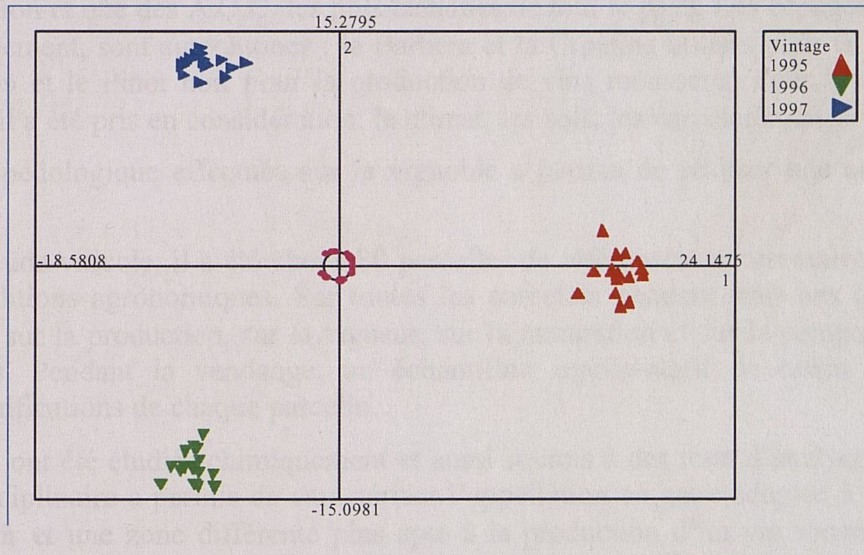

Figure.5 Projection of all the repetitions of the five selected vineyards, 1995-1997 vintages, and of the 36 selected liberated bounds compounds on the two first principal components of the FDA, according to the vineyard.

(Axis1 : 56% of the variance ; Axis2 : 44% of the variance).