CHARACTERIZATION AND DIFFERENTIATION OF SEVEN *VINHOS VERDES* GRAPE VARIETIES ON THE BASIS OF MONOTERPENIC AND C_{13}-NORISOPRENOID COMPOUNDS

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ABSTRACT

The aromatic profiles of the 7 recommended cultivars for *Vinho Verde* wine production have been established with respect to monoterpenic and C_{13}-norisoprenoids compounds, present either in free or in glycosidically bound fractions. Eighteen monoterpenic compounds have been identified and quantified in the free form and 22 in the glycosidically bound form. Fifteen C_{13}-norisoprenoids compounds have been identified and quantified in the glycosidically bound form. *Loureiro* variety can be easily differentiating from the 6 other varieties by important levels of linalool in the free fraction, above the odour perception threshold; in contrast, *Alvarinho, Avesso*, *Arinto, Azal* and *Trajadura*, geraniol prevails. *Batoca* variety showed a very poor monoterpenic profile in the free form. In the glycosidically bound form *Alvarinho* is the richest in total concentration of monoterpenic compounds, followed by *Loureiro*.

RÉSUMÉ

Les profils aromatiques des 7 cépages recommandés pour la production de *Vinho Verde* ont été établis en ce qui concerne les C_{13}-norisoprenoïdes et les composés monoterpéniques, trouvés dans les deux fractions de l´arôme, livre et glycosillée. Dix-huit monoterpènes ont été identifiés et quantifiées, dans la fraction libre, et d'autres 22 attachés à des glycosides. Quinze C_{13}-norisoprenoïdes ont été identifiés et quantifiés dans la fraction glycosillée. Le cépage *Loureiro* peut facilement être différencié des autres 6 variétés par les niveaux importants de linalol dans la fraction libre, au-dessus du seuil de perception olfactive; par contre, le geraniol prédomine pour *Alvarinho*, *Avesso*, *Arinto*, *Azal* et *Trajadura*. Le cépage *Batoca* a montré un très pauvre profil monoterpénique dans la fraction libre. Pour la fraction glycosillée, le cépage *Alvarinho* est le plus riche, en ce qui concerne la concentration totale en composés monoterpéniques ; le *Loureiro* est le deuxième cépage plus riche.

INTRODUCTION

The Demarcated Region of *Vinhos Verdes* is located in the northwest of Portugal. There are seven recommended white grape varieties for of *Vinho Verde* production, named *Alvarinho, Arinto, Avesso, Azal, Batoca, Loureiro* and *Trajadura*.

It is well known that monoterpenic compounds are important to discriminate between grape varieties. Oliveira *et al.* [1] discriminate five grape varieties from *Vinhos Verdes* region. Other authors [2,3] also could differentiate *Riesling* wines from different regions, or select between *Riesling* and *Gewürztraminer* clones, based on the monoterpenic composition of grapes. Strauss *et al.* [4] have also classified *Vitis vinifera* cultivars in Muscat varieties, in non-Muscat aromatic varieties and in neutral varieties, based on global concentration of monoterpenic compounds (free plus glycosidically bound).

MATERIALS AND METHODS

Grape Samples: Grapes from *Alvarinho, Arinto, Avesso, Azal, Batoca, Loureiro* and *Trajadura* varieties were manually harvested in 2005 vintage in two different vineyards. Because *Vinhos Verdes* Demarcated Region is a rather wide region, two sub-regions inside it were selected: Lima and Sousa.

Extraction of Volatiles and Glycoconjugates from Wines: To 150 mL of must, previously centrifuged (25 min, *RCF* = 9660, 4 ºC) were added 5 µl of solution of 4-nonanol (internal standard). The must was passed through an Amberlite XAD-2 resin (20−60 mesh, Supelco) column. The free and bound fractions of the aroma were then eluted successively with 50 mL of pentane-dichloromethane (2:1) and 50 mL of ethyl acetate. The extracts of both fractions were then concentrated to a final volume of 200 µL. Analyses were made in triplicate.

Gas-chromatography-Mass Spectrometry (GC-MS): Gas chromatographic analysis of volatile compounds was performed using a GC-MS composed by a Varian 3400 Chromatograph and an ion-trap mass spectrometer Varian Saturn II. Each 1 µL injection was made in a capillary column, coated with CP-Wax 52 CB or CP-Wax 57 CB (50 m x 0.25 mm i.d., 0.2 µm film thickness, Chrompack), respectively. The temperature of the injector (SPI – septum-equipped programmable temperature) was programmed from 20 ºC to 250 ºC, at 180 ºC/min. The temperature of the oven was held at 60 ºC, for 5 min, then programmed from 60 ºC to 250 ºC, at 3 ºC /min, then held 20 min at 250 ºC and finally programmed from 250 ºC to 255 ºC at 1 ºC/min.

Identification and Quantification of Volatile Compounds: Identification was performed using the software Saturn, version 5.2 (Varian), by comparing mass spectra and retention indexes with those of pure standard compounds. All the compounds were quantified as 4-nonanol equivalents.

RESULTS AND DISCUSSION

The monoterpenic profile of the free fraction and the monoterpenic and C_{13}-norisoprenoid compounds of the glycosidically bound form were established.

Free fraction: Linalool is the leading compound for *Loureiro* variety, with levels higher than its odour threshold. *Batoca* is very poor variety respecting monoterpenic compounds of the free fraction. Respecting the other 6 cultivars, *Alvarinho, Arinto*, *Avesso*, *Azal* and *Trajadura,* the major compound is geraniol. Geraniol is represented at levels beyond its odour threshold for *Alvarinho*, *Arinto* e *Trajadura* (Sousa). The Principal Component Analysis, made separately for the two sub-regions Lima and Sousa, showed the differentiation of the 7 varieties, with respect to specific compounds (figure 1). For the two sub-regions, the first Principal Component -Prin 1- (the more discriminated one) discriminates *Loureiro* from the other 6 varieties. Meanwhile Prin 2, in sub-region of Lima, divides the rest 6 grape varieties in 2 groups (Figure 1A) and in sub-region of

Sousa in 3 groups (Figure 1B). Nevertheless, it is obvious that *Avesso, Azal* and *Batoca* are very identical between each other and differ from *Alvarinho*, *Arinto* and *Trajadura,* although *Trajadura* is in an intermediate position.

Bound fraction: As much as monoterpenic compound of the bound fraction again linalool is the major component for *Loureiro* cultivar, with levels higher than its odour threshold (Sousa sub-region). Linalool is also the major monoterpenol for *Alvarinho* cultivar (on the contrary of the free fraction where the major terpene was geraniol) and is in concentrations higher than its odour threshold (Sousa sub-region). *Alvarinho* differ also from *Loureiro* by showing higher concentrations of (*Z*)-8-hydroxy-linalool. Tespecting the remaining cultivars, *Alvarinho* differentiates by high levels of 3,7-dimethylocta-1,5-dien-3,7-diol. Concerning the bound fraction, Principal Component Analysis could differentiate the grapes varieties in three groups. For the varieties from the Lima sub-region, the formed groups (Figure 2A) were as following: 1- *Arinto*; 2- *Alvarinho*; 3- *Avesso, Azal, Batoca, Loureiro* and *Trajadura*. Meanwhile these groups for the samples from Sousa sub-region (Figure 2B) were: 1- *Alvarinho*; 2- *Loureiro*; 3- *Arinto*, *Avesso, Azal, Batoca* and *Trajadura*.

Regarding C_{13}-norisoprenoids, all the cultivars showed a rich profile, difficulting the possibility of discrimination between the samples. Anyway, the Principal Component Analysis showed the same group formation like for the monoterpenol profile of the bound fraction, *i. e.* Lima sub-region (Figure 3A): 1- *Alvarinho*; 2- *Arinto*; 3- *Avesso, Azal, Batoca, Loureiro* and *Trajadura*; Sousa sub-region (Figure 3B): 1- *Alvarinho*; 2- *Loureiro*; 3- *Arinto*, *Avesso, Azal, Batoca* and *Trajadura*.

CONCLUSIONS

The studied varieties were differentiated by monoterpenic profiles of their free and bound compounds, even when they were grown in different sub-regions. It is more difficult the differentiation regarding C_{13}-norisoprenoid compounds.

Loureiro, *Alvarinho* and *Arinto* cultivars are different from the remaining samples. *Trajadura* showed an intermediate position in the free fraction of aroma.

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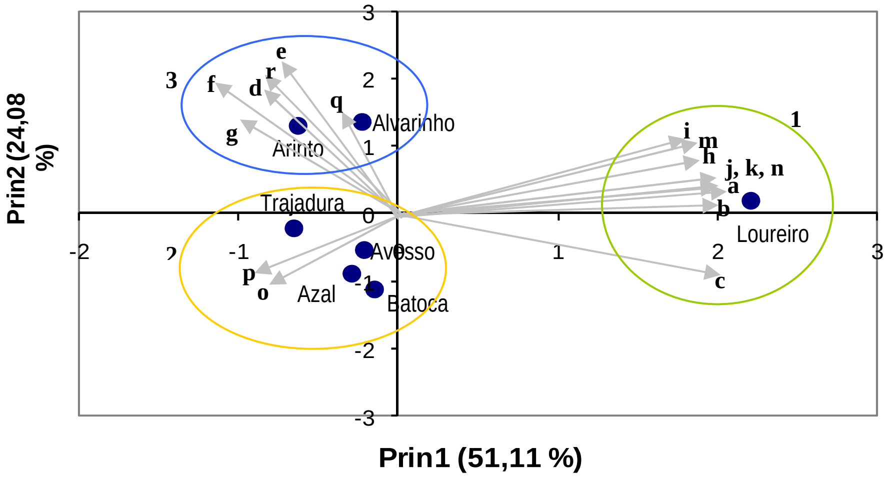

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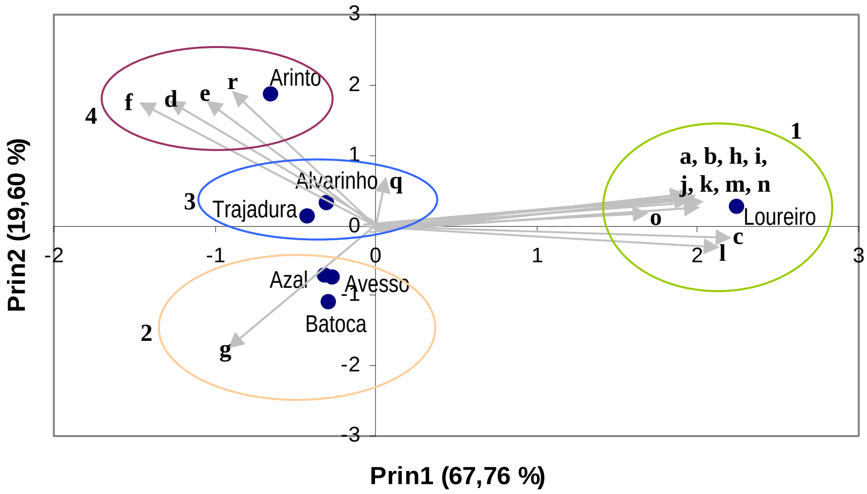
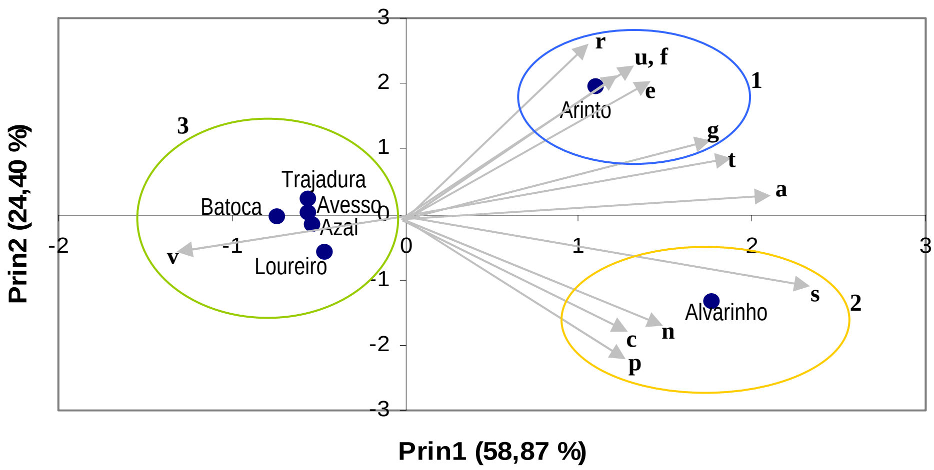


B

Figure 1. Principal Component Analysis applied on the monoterpenic compounds of the free fraction of the 7 cultivars: A– sub-region Lima; B– sub-region Sousa

(a-linalool, b- HO-trienol, c- α-terpineol, d-citronellol, e- nerol, f- geraniol, g- α-tujone, h- trans-furan linalool oxide, i- cis- furan linalool oxide, j- trans-pyran linalool oxide, k- cis-pyran linalool oxide, l- *exo*-2-hydroxy-1,8-cineole, m-3,7-dimethylocta-1,5-dien-3,7-diol, n-3,7-dimethylocta-1,7-dien-3,6-diol, o-8-hydroxy-6,7-dihydro-linalool, p- (*E*)-8-hydroxy-linalool, q-(*Z*)-8-hydroxy-linalool, r-geranic acid).



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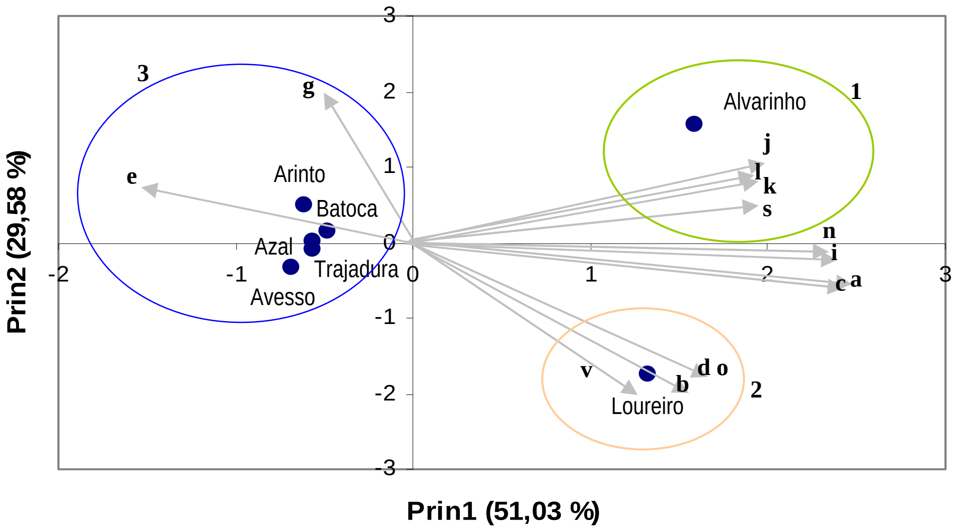

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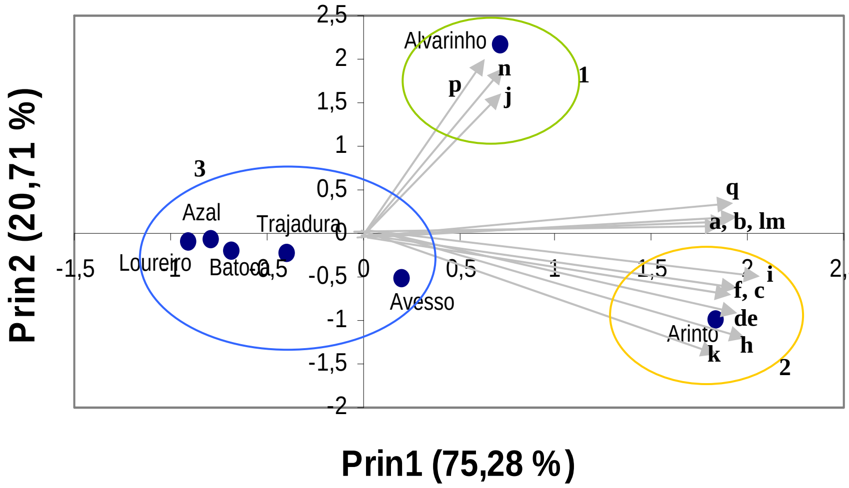
Figure 2. Principal Component Analysis applied on the monoterpenic compounds of the bound fraction of the 7 cultivars: A– sub-region Lima; B– sub-region Sousa

(a-linalool, b- 4-terpineol, c-HO-trienol, d- α-terpineol, e-citronellol, f- nerol, g- geraniol, h- geranial, i- trans-furan linalool oxide, j- cis- furan linalool oxide, k- trans-pyran linalool oxide l- cis-pirân linalool oxide, m- *exo*-2-hydroxy-1,8-cineoe, n-3,7-dimethylocta-1,5-dien-3,7-diol, o- linalool hydrate, p-3,7-dimethylocta-1,7-dien-3,6-diol, q- citronellol hidrate, r-8-hydroxy-6,7-dihydrolinalool, s- (*E*)-8-hydroxy-linalool, t-(*Z*)-8-hydroxy-linalool, u-geranic acid, v- *p*-1-menthen-7,8-diol).



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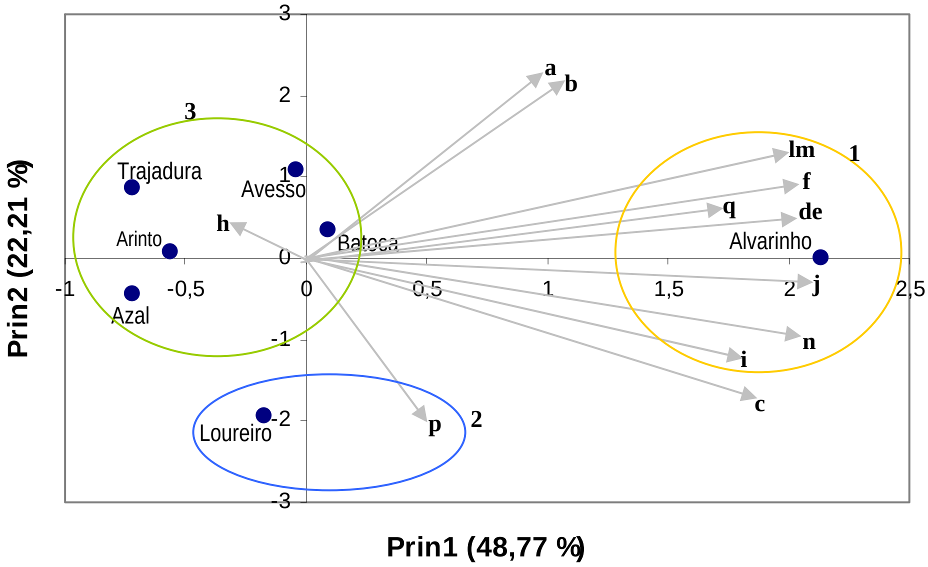

B

Figure 3. Principal Component Analysis applied on the C_{13}-norisoprenoids compounds of the bound fraction of the 7 cultivars: A– sub-region Lima; B– sub-region Sousa

(a- α-damascone, b- β-damascone, c- 3,4-dihydro-3-oxo-actinidol I, de- 3,4-dihydro-3-oxo-actinidol II + 3,4-dihydro-3-oxo-actinidol III, f- 3-hydroxy-β-damascone, g- 3,4-dihydro-3-oxo-actinidol IV, h- 3-hydroxy-7,8-dihydro-β-ionone, i- megastigma-7-eno-3,9-diol, j- 3-oxo-α-ionol, k- 3-hydroxy-7,8-dihidro-β-ionol, lm- 4-oxo-7,8-dihydro-β-ionol+ 3-hydroxy-β-ionona, n- 3-oxo-7,8-dihydro-α-ionol, o- 3-oxo-α-retroinol, p- 3-hydroxy-7,8-dehydro-β-ionol, q- vomifoliol).