Methyl trans geranate and farnesoate as markers for Gewürztraminer grape skins and related distillates

by

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Summary: Methyl ester of trans geranate and farnesoic acids, farnesol and two α-farnesene isomers are remarkable compounds in skins of mature grapes as well as in marc distillates of Traminer variety. Considerations about their level in both products of other floral varieties, like Yellow and Rose Muscats and Müller-Thurgau, as well as about their relationships with the main skin monoterpens and other compounds, including two unidentified stereoisomeric sesquiterpenes present only in distillates, were discussed. Finally, results of PCA data treatments as for the distillates are shown.

Key words: methyl trans geranate, methyl farnesoate, sesquiterpenes, marc distillates, grape varieties, Traminer.

Introduction

In a recent study dealing with compounds characterizing monovarietal marc distillates with floral aroma, a peculiar and remarkable compound - its peak was not at all or only partially resolved from that of α-terpineol by using capillary columns coated with CW-20M phase - was observed in Gewürztraminer products (Versini et al. 1993).

After improving GC separation and coming to the identification of the compound, we investigated its presence and that of other interesting compounds in berries and relevant marc distillates of some aroma-rich grape varieties, above all of those characterized by a considerable content of both free and bound geraniol and trans geranic acid.

Materials and methods

Berries from grapes collected in different vintage-years in vineyards of the Institute and stored in a freezer at -30 °C, and raw marc distillates produced in the last vintage by several pot still distilleries in the Italian region of Trentino-South Tyrol, were considered in this research.

Aroma analysis of grape: As for compounds of grape skins, 100 g of frozen berries were peeled and the skins immediately deeped in 25 ml of ethanol. After about 15 h, the ethanol solution was decanted, the skins rinsed with 5 ml of ethanol, which was on its turn decanted and added to the first. This solution, added with 25 µg of 2-octanol as internal standard and diluted with water to 400 ml, was percolated on equal part through two columns (40 x 1 cm) filled with purified XAD-2 resin (0.15-0.25 mm; Serva, Heidelberg) for 12 cm and the free compounds eluted with 80 ml of pentane/dichloromethane, 2:1, v/v (Versini et al. 1989).

The residual skins, added with 5 ml of ethanol, were distilled with water steam, thus simulating the process for obtaining grappa, until 100 ml of solution were obtained; after addition of the same internal standard, the components were extracted by using XAD-2 resin as above mentioned.

Compounds of remaining pulps, which were added with 100 ml of water, well homogenized and centrifuged, were enriched with the same adsorption method.

Aroma analysis of grappa: The main compounds were analyzed through direct injection of the distillate in packed and capillary columns as previously reported (Versini et al. 1993). Components present at less than 0.1 mg/l were enriched by extracting 10 ml of distillate, diluted 1:10 with water and added with internal standard, with pentane/dichloromethane, 2:1, v/v (3 x 20 ml). The extract was concentrated to about 0.5 ml before the GC-MS analysis.

GC-MS analysis: GC-EIMS (70 eV) analysis was performed on a HP 5890 gas chromatograph, coupled with a HP 5979 Mass Detector connected with a HP 59943B Wiley Database, and equipped with an apolar PS-264 fused silica capillary column (Mega, Milan; 25 m x 0.25 mm i.d.; df= 0.15 µm).

Experimental conditions: injector temperature: 220 °C; splitless injection; carrier gas: He; programmed temperature: 1 min at 40 °C; 10 °C/min up to 60 °C; 0.5 min at 60 °C; 2.5 °C/min up to 190 °C; 30 min at 190 °C. In many cases, methyl trans geranate and farnesoic acid, as well as farnesol of different extracts, were quantified by integrating the common ion at m/z=69 and relating the values to that of geraniol, which was usually well found also by FID-GC.

Statistical evaluation of data was carried out by using the SAS package.

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Results and discussion

1. Identification of methyl trans geranate and methyl farnesoate. The GC-MS spectrum (Fig. 1a) of the above-mentioned peak, well separated in Traminer distillate extract by using the P5-264 capillary column, corresponds to that of methyl trans geranate of the Wiley library as well as to that of the same compound synthesized by reacting trans geranic acid with diazomethane. The ester is characterized by a lime-like scent and was already found by Stevens et al. (1966) as a trace compound in aroma fraction of distilled grape juice of Muscat of Alexandria and by Schreier et al. (1976) in different grapes including the Traminer, but supposing all of the few esters found in grapes as coming from possible biochemical reactions during the extraction process. In any case, methyl trans geranate was never cited as a quantitatively interesting compound in whatever grape derivate.

In the same analysis, methyl farnesoate was also identified, its MS spectrum (Fig. 1b) well fitting on that of the (2E,6E) isomer in the Wiley library. To our knowledge, this compound was never found in plant or fruit, but only as reproductive hormone in crustaceous, e.g. in lobsters (Lauffer et al. 1992), or as juvenile hormone in different insects (see Borovsky et al. 1992).

2. Methyl esters and other peculiar compounds in grapes of different varieties and in relevant distillates.

Presence in berries: What we found in Traminer marc distillates, led us to investigate in the presence of the mentioned methyl esters in grapes of different varieties, especially in berry skins.

In order to avoid the problems of a preferential adsorption of both esters on the skin waxes during homogenization of the whole berries as found by Versini et al. (1988) to happen partially for monoterpenols, and to avoid also enzymatic reactions, another extraction process was performed as above described.

In the ethanolic extract of skins of well ripened grape of Gewürztraminer, we found at remarkable content (Tab. 1) besides methyl trans geranate and methyl farnesoate - other interesting compounds as farnesol, probably the (2E,6E) form, and two α-farnesene isomers, probably the (3Z,6E) and the (3E,6E) - all components already found in La France pears by Šhota (1990) and the second α-farnesene isomer corresponding to the α-farnesene reported by Schreier et al. (1976). Their MS spectral data and retention times (RT) referring to geraniol, are summarized as follows:

\[(3Z,6E?)-\alpha\text{-farnesene}: \text{RT: 1.48; m/z: 41(100); 55(33); 69(43); 77(31); 79(34); 91(35); 93(78); 105(23); 107(29); 119(51); 123(2); 133(5); 147(3); 161(5); 189(2); 204(1).}\]

\[(3E,6E)-\alpha\text{-farnesene}: \text{RT: 1.50; m/z: 41(100); 55(38); 69(39); 77(25); 79(38); 91(30); 93(71); 105(21); 107(32); 119(23); 123(22); 133(3); 147(2); 161(4); 189(2); 204(1).}\]

farnesol: \text{RT: 1.88; m/z: 31(5); 41(93); 55(14); 69(100); 81(29); 93(19); 95(12); 107(9); 109(7); 121(7); 123(7); 136(8); 161(3); 179(2); 191(2); 207(1); 222(<1).}\]

We emphasize that farnesene compounds are present only in mature grapes of Gewürztraminer and, at that time, methyl trans geranate and farnesoate are markedly increased in comparison with the other mentioned monoterpenes.

All such sesquiterpene compounds are absent in residual skin distillates, differently from the so called skin-monoterpenes geraniol, nerol and trans geranic acid and from methyl trans geranate.

This different behaviour could be explained by a different distribution of the compounds in the skin, being the sesquiterpenes probably included in wax layers and well...
Compounds and some relevant ratios in different monovarietal distillates (number of samples in brackets; Rose Muscat distillates

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Traminer (cf. 48)</th>
<th>Rose Muscat</th>
<th>Yellow Muscat (cf.RS)</th>
<th>Müller Thurgau</th>
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</thead>
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<tr>
<td>Sk. 1</td>
<td>Sk. 2</td>
<td>Sk. 1</td>
<td>Sk. 2</td>
<td>Sk. 1</td>
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<tr>
<td>nerol + citronellol</td>
<td>24.5(23)</td>
<td>34.1(32)</td>
<td>2.7(2)</td>
<td>3.1(2)</td>
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<td>geraniol</td>
<td>88(13)</td>
<td>88(13)</td>
<td>15(1)</td>
<td>15(1)</td>
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<tr>
<td>tr. geranic acid</td>
<td>60(13)</td>
<td>126(13)</td>
<td>6.5(1)</td>
<td>7.1(1)</td>
</tr>
<tr>
<td>tr. methyl geranate</td>
<td>-</td>
<td>5(2)</td>
<td>-</td>
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<tr>
<td>farnesol</td>
<td>-</td>
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<td>-</td>
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<td>-farnesene (2)</td>
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<td>9 a-farnesene (1)</td>
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<tr>
<td>8 a-farnesene (2)</td>
<td>8 a-farnesene (2)</td>
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<td>7 nerol (II)</td>
<td>563(45)</td>
<td>1549(42)</td>
<td>95(0)</td>
<td>140(0)</td>
</tr>
<tr>
<td>6 geraniol</td>
<td>1246(45)</td>
<td>4366(50)</td>
<td>140(0)</td>
<td>230(0)</td>
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<td>5 tr. hexanol (g.p.a.)</td>
<td>3502(56)</td>
<td>1765(55)</td>
<td>2917(120)</td>
<td>1470(120)</td>
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<td>3860(80)</td>
<td>1324(120)</td>
<td>323(12)</td>
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<td>481(40)</td>
<td>760(45)</td>
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<td>520(10)</td>
<td>216(5)</td>
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<td>1549(42)</td>
<td>95(0)</td>
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**Table 2**

Compounds and some relevant ratios in different monovarietal distillates (number of samples in brackets; Rose Muscat distillates are single reported-samples; sample 1 is a final product).

**Table 1**

Some terpenes in grape berries of different varieties (P = pulp; Sk.1 = ethanolic skin extract; Sk.2 = distillate of skins after extraction. + = <1 μg/kg; - = not detectable).

Completely dissolved by ethanol, rather than in the cells, as for the monoterpene compounds.

Other considered skin-monoterpene rich varieties like Yellow Muscat (Moscatello giallo) and Rose Muscat (Moscato rosa) and, for a comparison in their respect, the skin-monoterpene poor Müller-Thurgau, may contain some of the cited methyl esters and farnesene products in skin and close to the analytical detection level.

**Present in marc distillates:** Tab. 2 reports the concentrations of compounds under discussion, as well as of other monoterpens and compounds which could characterize the marc distillates of Gewürztraminer, Yellow and Rose Muscat and Müller-Thurgau. The above cited farnesol and α-farnesene and two probable stereoisomers of a sesquiterpene, not previously reported in grape, are also included. Following RT (geraniol as reference) and MS spectral data regard such unknown isomers:

*unkn.sesquiperene 1*: RT: 1.48; 41(55); 43(41); 55(38); 69(22); 77(29); 79(16); 81(78); 91(40); 93(21); 105(46); 119(48); 133(26); 147(21); 161(100); 189(23); 204(41).

* unkn.sesquiperene 2*: RT: 1.51; 41(69); 43(27); 55(24); 69(13); 77(41); 79(54); 81(31); 91(54); 93(43); 105(61); 119(42); 133(29); 147(7); 161(100); 189(3); 204(21).

We point out that several other identified and unknown sesquiterpenes were already found by Di Stefano (1986) in marc distillates of White Muscat of Piedmont.

At first, we observed that some results performed on skins can be confirmed as tendency on the grappas, where, however, the presence of methyl esters and farnesene compounds, referred to that of skin monoterpenes, proves more remarkable than in skins. If considering the ratio of the sum of geraniol, nerol and citronellol - citronellol being the principal fermentation metabolite of geraniol (Versini et al. 1990) - in respect to methyl trans geranate, the Gewürztraminer grappas, with such mean sum similar to that of Muscat products, show values about ten-fold lower than the Yellow Muscats and even further in comparison to the Rose Muscats distillates. As for the Müller-Thurgau ones, this ratio is, even though about four-fold higher, closer to that of the Traminer than to the corresponding of the Muscats. Similar tendencies can be observed as for the ratio between methyl trans geranate and methyl farnesate. Furthermore, the degree of methylation for trans geranic acid, as can be inferred from the ratio between methyl ester and relevant acid contents, is higher in the Traminer than in the other products.

Traminer distillates are also the richest in farnesol and α-farnesene isomers, being those isomers well correlated...
The levels of all the cited compounds within every group of distillate seem not depending on the levels of methanol and of hexanol, which are raw indicators of the skin pressurage degree in different winemaking processes: in this respect, it may be useful to remember that the marc was ensiled not fermented for all considered varieties, except for the Rose Muscat.

It is worth to mention that the usual technology of distillate finishing includes water dilution to about 40-45 alcoholic proof, refrigeration and filtration processes, thus markedly reducing the content of the less polar sesquiterpene as shown by comparing their levels in the two Rose Muscat distillates. This fact has already been observed for the Bartlett pear distillates, which are characterized by a high content of both α-farnesene isomers, but with the prevalence of the (3E,6E) form (Versini et al. 1989).

Finally, we emphasize some results of Principal Component Analysis (PCA) on the data regarding the raw distillates, as shown in the biplot of Fig. 2. The scores of the objects in the plane defined by the first two eigenvectors (65.3 % of variance) and, at the same time, the loadings of the variables and their possible relationships, are reported.

A good separation among the varieties is obtained. Methyl trans geranate and farnesolate, as well as farnesol and α-farnesene isomers, all near to each other, characterize positively the Traminer distillates, while monoterpenols and trans geranic acid characterize mostly the Muscats, and hexanol the Müller-Thurgau distillates. Hexanol, Traminer characterizing compounds and monoterpenols result almost hortogonal and therefore approximately independent variables.

Conclusions

The monoterpene and sesquiterpene compounds investigated give us other chances to typify the Gewürztraminer grape and, in particular, its marc distillate. It is worth mentioning, above all, the peculiar esterase activity found in the skins in this variety and at a progressed ripening stage. Contribution of methyl trans geranate to the distillate aroma has to be proved.

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References


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