

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

by

G. VERSINI¹), A. RAPP²), A. DALLA SERRA¹), U. PICHLER¹) and M. RAMPONI¹)

1) Laboratorio di Analisi e di Ricerca, Istituto Agrario di San Michele all'Adige, San Michele all'Adige, Italia

2) Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Deutschland

S u m m a r y : Methyl ester of *trans* geranic 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 monoterpenols 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.

K e y w o r d s : 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.

A r o m a a n a l y s i s o f g r a p e : 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 μ g 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.

A r o m a a n a l y s i s o f g r a p p a : 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.

G C - M S a n a l y s i s : 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 μ m).

E x p e r i m e n t a l c o n d i t i o n s : 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 farnesoate, 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.

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 PS-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 crustaceans, e.g. in lobsters (LAUFER *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 SHIOTA (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)- α -farnesene: 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)- α -farnesene: 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: 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

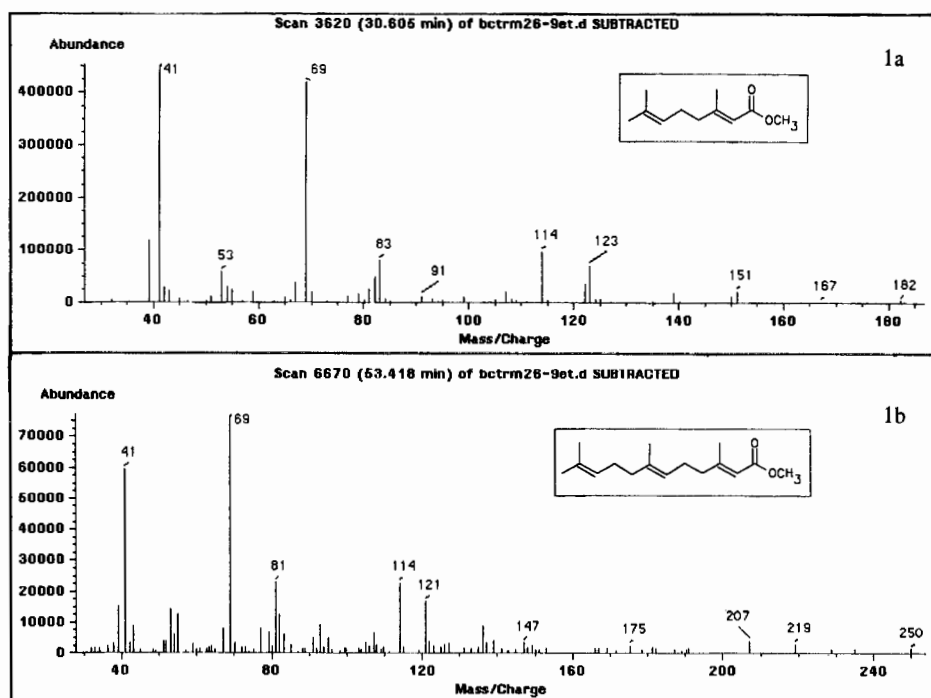


Fig. 1: MS spectra of methyl *trans* geranate (1a) and methyl farnesoate (1b) found in Traminer berry skin.

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).

Compounds (µg/Kg)	Traminer (cl. 48)						Rose Muscat						Yellow Muscat (cl.R5)			Müller Thurgau		
	1990, Aug. 20 (14.3 °Bx)			Sept. 26 (24.3 °Bx)			1989, Aug. 18 (16.2 °Bx)			Oct. 13 (27.8 °Bx)			1989, Sept. 18 (17.7 °Bx)			1993, Sept. 15 (18.7 °Bx)		
	P	Sk.1	Sk.2	P	Sk.1	Sk.2	P	Sk.1	Sk.2	P	Sk.1	Sk.2	P	Sk.1	Sk.2	P	Sk.1	Sk.2
nerol + citronellol	24.5	345	71	48	720	62	2.7	31	14	21.1	127	44	7.3	95.4	10.5	2.2	70	1.0
geraniol	88	982	148	177	2360	199	15	117	21	50.5	500	17.1	23.5	353	42.5	11.6	97	3.5
tr. geranic acid	60	1295	135	180	2750	196	6.5	51.5	28	7	135	43	39.5	735	92.5	2.4	39	4
tr. methyl geranate	-	5	2.7	1.2	255	61	-	-	-	-	-	+	-	1.6	-	-	+	-
methyl farnesoate	-	+	-	-	132	5	-	-	-	-	-	-	-	-	-	-	-	-
farnesol	-	10	-	-	215	21	-	-	-	-	-	-	-	1.4	-	-	-	-
α-farnesene (1)	-	+	-	-	92	-	-	-	-	-	-	-	-	-	-	-	-	-
α-farnesene (2)	-	+	-	-	225	-	-	-	-	-	-	-	-	-	-	-	-	-

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).

N	Traminer (5)				Müller Thurgau (6)				Yellow Muscat (6)				Rose Muscat	
	Mean	Std.dev.	Min	Max	Mean	Std.dev.	Min	Max	Mean	Std.dev.	Min	Max	Sample 1	Sample 2
- ethanol (% vol)	72.21	5.45	66.85	80.50	69.34	3.98	61.60	72.10	69.41	7.66	59.40	81.10	40.10	77.70
1 methanol (mg % ml p.A.)	797.2	195.64	569	1059	932.3	290.19	569	1343	515.7	190.58	166	716	325	261
2 hexanol (µg % ml p.A.)	9654.6	3122.80	5854	13846	20144.0	7587.87	12850	29194	10473	3215.87	5362	13603	3848	6023
3 linalool	836.0	180.22	640	1120	873.3	315.83	560	1400	6543.3	1422.04	4730	8960	4500	2180
4 ho-trienol	112.0	58.91	60	210	351.7	125.29	220	570	1586.7	411.91	1100	2180	1900	230
5 α-terpineol	806.0	123.41	610	930	463.3	142.08	240	650	2941.7	1001.49	1680	4680	1900	1750
6 citronellol (C)	620.0	218.52	340	910	56.7	36.15	20	110	505.0	82.16	430	650	1000	2450
7 nerol (N)	636.0	159.47	420	800	95.0	36.74	40	140	835.0	361.48	450	1360	500	860
8 geraniol (G)	1246.0	463.82	590	1790	140.0	62.61	60	230	1581.7	704.60	860	2760	1000	1250
9 C+N+G	2502.0	756.85	1680	3500	291.7	125.13	120	470	2921.7	1107.38	1840	4770	2500	4360
10 tr. methyl geranate (tMG) (*)	560.6	212.29	360	898	23.3	24.92	1.2	68	71.8	42.50	26.5	135	30	15
11 tr. geranic acid (tG ac.) (*)	286.0	132.78	190	500	53.3	27.33	20	100	726.7	352.00	70	1140	1220	400
12 farnesol (F) (*)	114.6	34.06	67	156	23.3	15.94	10	54	32.4	16.18	15.5	61	34	53
13 methyl farnesoate (MF) (*)	26.3	9.12	14.5	36	1.5	1.10	0.6	3.4	0.57	0.370	0.3	1.3	< 0.2	< 0.2
14 α-farnesene (1) (F1) (*)	80.6	21.38	44	97	32.6	20.74	4	63	18.4	7.33	5	25.5	8	44
15 α-farnesene (2) (F2) (*)	163.0	64.64	85	231	63.7	43.19	12.5	130	26.3	15.14	6.5	52	8.5	68
16 sesquiterpene (1) (*)	17.0	2.72	14	20.5	12.6	9.05	3	29	23.8	18.32	6.5	58	2.5	36.5
17 sesquiterpene (2) (*)	32.6	31.22	13	88	19.6	6.35	11.5	27.5	34.3	18.12	19	66.5	6	46.5
- (C+N+G)/tMG	4.6	0.73	3.7	5.3	16.7	8.91	6.9	25.7	54.0	33.10	18.4	105.1	83.3	290.7
- tMG/MF	21.9	4.69	13.7	24.9	27.7	26.01	1.5	68.0	128.5	48.04	87	210	> 150	> 75
- tMG/tG ac.	2.09	0.723	1.41	3.32	0.34	0.244	0.06	0.68	0.25	0.431	0.03	1.13	0.02	0.04
- F/(F1 + F2)	0.51	0.201	0.24	0.74	0.70	1.262	0.07	3.27	0.95	0.697	0.33	2.09	2.06	0.47

* = evaluated as 2-octanol

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

Other considered skin-monoterpene rich varieties like Yellow Muscat (Moscato 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 only in skins and close to the analytical detection level.

Presence in marc distillates: Tab. 2 reports the concentrations of compounds under discussion, as well as of other monoterpenols and compounds which could characterize the marc distillates of Gewürztraminer, Yellow and Rose Muscat and Müller-Thurgau. The above cited farnesol and α-farnesenes 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. sesquiterpene 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. sesquiterpene 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 monoterpenols, 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 farnesoate. 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

($r=0.965$) with each other. No significant intervarietal difference was observed as for the content of unknown sesquiterpene isomers.

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 marcs were 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).

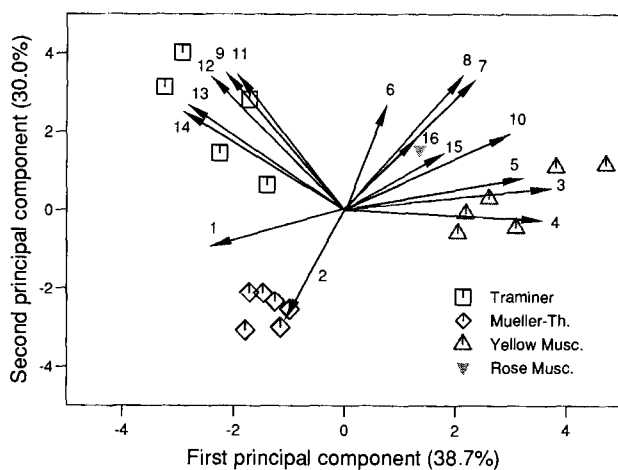


Fig. 2: Biplot for the data of distillates: score of the objects and loadings of the variables (significance of the labels are included in Tab. 2) in the plane defined by the first two eigenvectors.

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 farnesoate, 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 horthogonal 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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