Bartlett pear unsaturated ethyl decanoates and C₉ compounds among components characterizing cv. Catalan roxo grape marc distillates

by

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S u m m a r y : Catalan roxo marc distillates contain compounds at an unusual level in a grape derivate. The most peculiar are several unsaturated ethyl decanoates typical of Bartlett pear distillates and derived from ethyl esters found in the grape skins, some of which partially modified in the stereoisomery probably by the fermentation process. Remarkable compounds are unbranched aliphatic C_9 compounds at different oxidation state as well as ethyl nonanoate. At sensorially interesting levels methyl and ethyl salicylate and ethyl cinnamate, monoterpenols typical of floral-like varieties, vitispiranes and 4-ethylguaiacol are detected. Methyl salicylate is found in the berry as free and bound compound as several monoterpenols.

K e y w o r d s : unsaturated ethyl decanoates, C_9 compounds, methyl and ethyl salicylate, metabolism, marc distillate, Catalan roxo grape variety.

Introduction

Among the marc distillates typical of Galicia in Spain, those obtained from the red grape variety Catalan roxo, are singled out and particularly appreciated for the aroma complexity including fruity, floral and spicy scents.

It is worth mentioning that the Catalan roxo cultivar, on the basis of some ampelographic characteristics, was recently reclassified by Pérez and MARTINEZ (pers. communication) as a hybrid rather than a *Vitis vinifera* variety, as it was reported in an official vine variety catalogue (ALLEWELDT and DETTWEILER 1992), probably referring to the previous classification of Hidalgo and CANDELA (1971).

In this work - which is part of an analytical study aiming at comparing marc distillates produced in Galicia (aguardiente de orujo) with those of the Italian region of Trentino (grappa), both having the European Community recognition for the origin denomination - peculiar compounds were identified and quantified in the marc distillates of the mentioned variety. At the same time, an investigation about possible precursors for some of them in grape was begun.

Material and methods

Raw distillates produced in 1990, 1992 and 1993 with traditional equipment (alambique and arraste por vapor) by a small distillery and at the Experimental Station of Leiro, stored at a rather low temperature until the analysis, and grapes picked close to ripeness (14.5 °Brix) in 1993 in the vineyards of the same Station and stored frozen, were analyzed.

A n a l y s e s of d i s t i l l a t e s : FID-GC analysis through direct injection of distillates in packed and capillary columns to quantify compounds with a concentration higher than 0.1 mg/l, as previously reported (VERSINI *et al.* 1993), was used.

GC-EIMS (70 eV) analysis was performed for the compound identification and the quantification of those not well resolved or present at lower concentration, after extraction of the distillate (10 ml with 10 μ g of 2-octanol in ethanolic solution as internal standard, diluted 1:10 with water, extracted with 3x20 ml of pentane/dichloromethane, 2:1 v/v, and concentrated to 0.5 ml) and splitless injection of 1 μ l on a HP 5890 GC instrument, coupled with a HP 5979 Mass Detector, connected with a HP 59943B Wiley Database and equipped with an apolar PS-264 fused silica column (Mega, Milan; 25 m x 0.25 mm i.d.; df= 0.15 μ m) (VERSINI *et al.* 1994).

A roma analysis of berry parts (VERSINI et al. 1994): 50 g of frozen berries were peeled, the skins depeed in ethanol and the aroma fraction of the properly diluted ethanolic as well as of the water solution from steam distilled residual skins enriched by using XAD-2 resin; the same preparation was done changing the ethanol with methanol.

Aroma of pulp, which was well homogenized, clarified with bentonite (80 mg/l) and gelatine (5 mg/l) and centrifuged, was obtained on the basis of the same adsorption method. Bound forms were eluted from XAD-2 in both cases with 80 ml of ethyl acetate/methanol, 9:1 v/v, then concentrated to dryness and reacted with Rohapect C enzyme in 4 ml of a pH 5 buffer citrate solution for 15 h to produce aglycones, which were extracted with 3x3 ml pentane/dichloromethane, 2:1 v/v (2-octanol as internal standard).

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HPLC analysis of salicylic acid: The pentane/dichloromethane solutions from the XAD-2 resin after percolation of the above quoted berry part solutions - the skin extract after elimination of ethanol with Rotavapor - both acidified to pH 1.8 with sulfuric acid as the washing water, were concentrated to dryness and redissolved in 0.15 ml of ethyl acetate. 6 µl were injected in an HP-1090M chromatograph, coupled with a photodiode array detector and equipped with ODS-Hypersil C_{18} (5 μ m) precolumn (20 x 2.1 mm) and column (200 x 2.1 mm). A linear gradient from 0 to 20% of B (A=H,PO, 10⁻³ M, B=CH₂CN) in 10 min with a flux of 0.6 ml/min was used, being both columns heated at 40 °C. The quantification was carried out with the external standard method at 298 nm wavelength with 4 nm of band width. The detection limit was 6 μ g/kg of berries.

Results and discussion

Compounds typifying the marc distillate: In all distillate samples, several ethyl esters of differently unsaturated decanoic acid were found. Their MS spectra, reported in the Figure, fitted very well with those with the same retention times found by analyzing a Bartlett pear distillate. They are identified - for some of them being only proposed the stereoisomery of certain double bounds - as ethyl esters of (2E,4E)-, (2E,4Z)-, (2Z,4E)(?)- and (2Z,4Z)(?)-decadienoates, of (2E,4Z,7Z)and (2E,4E,7Z)-decatrienoates, and of (4Z)- and (4E)(?)decenoates on the basis of literature data (HEINZ and JENNINGS 1966; CREVELING and JENNINGS 1970; BRICOUT 1977), of spectrum similarities and relative retention times, also considering that the cis form usually elutes before the trans in the apolar capillary column used for GC-MS analysis. Among all these esters, the first two mentioned are markedly higher than the others (Tab. 1) as in pear distillates: the (2E,4Z)-isomer is in the average twice higher than the (2E,4E)-isomer and their concentrations can reach one tenth of the mean values measured in commercial Bartlett pear distillates (POSTEL and ADAM 1989 a). The relevant methyl esters, typical of Bartlett pears, were not detected in these products.

We recall that the here found ethyl esters are considered co-responsible for the particular aroma of Bartlett pear and in part are present, but to a lower extent, in other fruits as in some apple varieties (KOLLMANNSBERGER and BERGER 1992). Most of such esters were put into evidence by SCHREIER and PAROSCHY (1981) also in grapes of *Vitis labrusca* or with *Vitis labrusca* crossed varieties, so that a parentage of Catalan roxo with such *Vitis* species could be suggested.

As from the results in Tab. 1, which also reports other compounds useful to describe the peculiar typology of such

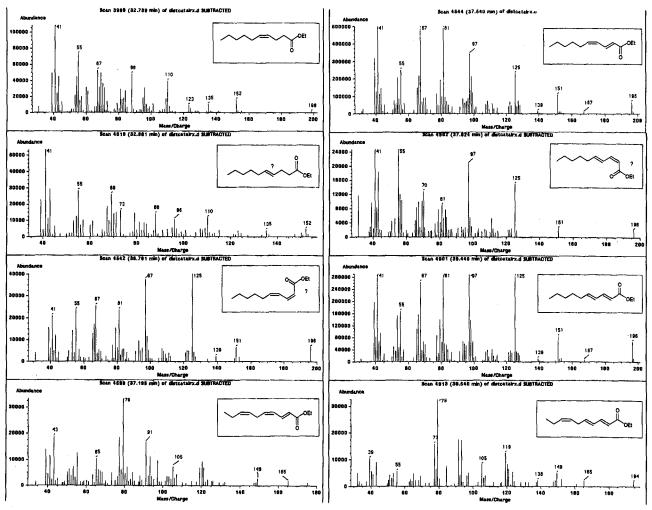


Figure: GC-MS spectra of unsaturated ethyl esters in a Catalan roxo distillate extracted with pentane/dichloromethane, 2:1 v/v.

distillates, remarkable contents unusual for a grape-marc distillate are for the C_9 compounds nonanal, 1-nonanol, nonanoic acid and its ethyl ester, as well as for methyl and ethyl salicylate, and ethyl cinnamate.

Some or all of C_9 compounds has been found to characterize only the cherry (TUTTAS 1977) and the plum juice (ISMAIL *et al.* 1981) as well as the plum distillates (POSTEL *et al.* 1975; VERSINI 1983).

Table 1

Compounds (mg/100 ml pure alcohol) present in Catalan roxo distillates from different
vintages and distilleries (A=1990, industrial; B=1991, experimental; C=1993, industrial;
D=1993, experimental). Unsaturated ethyl esters are evaluated as ethyl caprate

Compounds	Α	В	С	D 50.5
alcohol (% Vol.)	56.3	55.5	60.2	
methanol	515	1010	356	380
1-propanol	39	77	55	69
2-butanol	0.9	5.5	3.0	0.1
higher alcohols	318	353	398	284
1-hexanol	9.3	20.5	17.1	6.1
(E) 3-hexen-1-ol	0.10	0.15	0.12	0.06
(Z) 3-hexen-1-ol	0.35	2.0	1.7	0.60
(E) 2-hexen-1-ol	0.35	2.3	1.0	0.15
1-heptanol	0.10	0.46	0.60	0.12
1-octanol	0.13	0.30	0.39	0.09
1-nonanol	1.45	2.8	3.9	0.36
1-decanol	0.07	0.06	0.12	0.09
benzyl alcohol	0.04	0.28	0.15	0.13
phenethyl alcohol	6.2	14.6	9.5	3.45
ethyl lactate	78.5	103	69	1.8
diethyl succinate	1.9	3.0	9.6	0.09
ethyl C6+C8+C10	36.3	11.9	58.2	17.1
ethyl heptanoate	0.03	0.12	0.19	0.02
ethyl nonanoate	0.38	1.1	1.55	0.09
nonanoic acid	0.13	0.50	0.45	0.02
methyl salicylate	0.08	0.19	0.28	0.25
ethyl salicylate	0.075	0.18	0.29	0.09
ethyl cinnamate	0.13	0.10	0.09	0.05
styrene	0.01	0.15	0.01	0.04
nonanal	0.29	1.4	2.1	0.40
benzaldehyde	0.60	1.2	0.60	0.10
furfural	1.4	1.9	1.5	0.90
vitispiranes	0.06	0.07	0.18	0.055
eugenol	0.032	0.023	0.030	0.010
4-ethylguaiacol	0.20	0.14	0.21	0.005
(E) furan linalool oxide	0.35	0.82	0.63	0.30
(Z) furan linalool oxide	0.17	0.60	0.24	0.19
(E) pyran linalool oxide	0.024	0.15	0.015	0.014
(Z) pyran linalool oxide	0.015	0.04	0.009	0.007
linalool	0.63	1.6	0.84	0.18
ho-trienol	0.008	0.010	0.010	0.005
a - terpineol	1.25	2.0	0.72	0.18
citronellol	0.81	1.25	1.05	0.51
nerol	0.37	0.92	0.33	0.09
geraniol	0.43	1.3	0.55	0.05
ethyl (4Z)-decenoate	0.23	0.04	0.075	0.04
ethyl (4E) (?) -decenoate	0.080	0.023	0.045	0.013
ethyl (2Z,4E) (?) -decadienoate	0.080	0.023	0.045	0.013
ethyl (2E,4E) (2) -decadenoate ethyl (2E,4Z)-decadienoate	2.5	0.032	0.045	0.012
ethyl (2Z,4Z) (?) -decadienoate	0.090	0.29	0.48	0.24
ethyl (2E,4Z,7Z)-decatrienoate	0.090	0.012	0.010	0.000
ethyl (2E,4E)-decadienoate	1.0	0.018	0.018	0.010
ethyl (2E,4E,7Z)-decatrienoate	0.20	0.10	0.30	0.006

A marked presence of ethyl nonanoate and/or ethyl heptanoate in distillates from grape products has been stated as an aromatization to simulate a Cognac-like scent or indicative of deviating production (Postel and ADAM 1984). The concentrations of both these odd-carbon fatty acid esters - originated and increasing during the fermentation, as the even-carbon fatty acid esters, as shown by SCHREIER et al. (1978) for apple wines - seem not to be connected, as it cannot for the ethyl caproate, to the yeast quantity at the distillation (Postel and ADAM 1989 b), probably because almost already diffused in the fermentation medium (NYKÄNEN et al. 1977). In addition to these aspects, we would like to underline that the lowest content of ethyl nonanoate and nonanoic acid - in this case, similar to those found in other grape marc distillates - and of 1-nonanol, but in presence of a marked content of nonanal, was found in the 1993 experimental distillate, where the malolactic fermentation did not happen, as demonstrated by the low content of ethyl lactate, because of the marcs acidification at the ensilage.

Therefore, a different metabolic course bearing to the C_9 compounds, perhaps connected with a possible inhibition of peculiar yeasts and bacterial spoilage, could take place.

As for both salicylates and ethyl cinnamate, they have been already identified by DI STEFANO (1986) in marc distillates of White Muscat of Piedmont and generally found in all marc distillates (VERSINI *et al.*, unpublished results). These esters are known to be present, together with styrene, in wines obtained from grape undergoing carbonic maceration, their level depending also from the variety (DELL'ORO and DI STEFANO 1990), being hypothesized a common origin from prephenic acid anaerobic metabolism in intact cells (DUCRUET 1984). Probably, such a metabolism takes place also in skin cells of the marcs during the silage period. Methyl salicylate was often also found as aglycon after enzymic (VERSINI 1991) or acid (WILLIAMS *et al.*1989) hydrolysis of grape heterosides. Ethyl salicylate appears to be markedly reduced in comparison to the methyl ester in the distillate from acidified marcs, being at the same level in the others.

It is worth mentioning the important sensorial contribution of both salicylate esters and ethyl cinnamate to a spicy-balsamic and plum-fruity note, respectively: for the latter, a threshold value at about 10-20 μ g/l was found in wine (VERSINI and TOMASI 1983). Finally, we recall that ethyl cinnamate results to characterize the above cited plum distillates in respect to those of other fruits (VERSINI 1983; BINDLER and LAUGEL 1985).

Other compounds typifying the Catalan roxo distillate aroma because of their concentrations similar to those of distillates of grape varieties with a floral aroma (VERSINI *et al.*1993), are some monoterpenols, mainly linalool, *trans* and *cis* furan linalool oxides, α -terpineol, nerol, geraniol and citronellol.

At sensorially interesting concentrations the varietal norisoprenoid vitispiranes (green-eucalyptus odor), the 4-ethylguaiacol (phenolic, horsesweat-like scent), in traces in the distillate from acidified marcs, and the eugenol (clove-like odor) may also occur.

Compounds (μ g/kg berry), as free (f) and bound (b) form, quantified in berry pulp and skin of Catalan roxo grape and in steam distillate of skins after the alcoholic extraction. All compounds are evaluated as 2-octanol (+ = about 0.2 μ g/kg of berries)

Table 2

Compounds	pulp		skin		skin distillate
	f	b	f	b	
(E) furan linalool oxide	1.4	15	1.5	30	+
(Z) furan linalool oxide	+	2	+	2.5	0.5
(E) pyran linalool oxide	6.4	4	1.3	6	0.6
linalool	0.5	1	1.5	1.7	+
α - terpineol	6.4	35	14.6	42	7.5
nerol	0.7	12	13.7	23	2.4
geraniol	9.7	24	81	60	6.2
3.7-dimethyl-1.5-octadiene-3.7-diol	59	18	68	15	2
benzyl alcohol	38.3	435	39.5	215	6
phenethyl alcohol	1705	4350	625	3160	70
ethyl (4Z)-decenoate	-	-	64	-	2.5
ethyl (4E) (?) -decenoate	-	-	-	-	-
ethyl (2Z,4E) (?) -decadienoate	-	-	6	-	2
ethyl (2E,4Z)-decadienoate	· +	-	233	-	14
ethyl (2Z,4Z) (?) -decadienoate	-	-	14	-	1.5
ethyl (2E,4Z,7Z)-decatrienoate	-	-	46	-	0.4
ethyl (2E,4E)-decadienoate	+	-	15	-	0.4
ethyl (2E,4E,7Z)-decatrienoate	-	-	6	-	-
methyl salicylate	5.5	1	10	9	30

Which linkage with grape aroma? Trying to give a first answer to this question, grape-berries, considered as skin and pulp parts, were analyzed (Tab. 2).

As for the unsaturated ethyl decanoates, mainly the (2E,4Z)-, the (4Z)- and the (2E,4Z,7Z)-isomers, the first being much higher than the others, were found in skins. The same compounds were obtained by using methanol, instead of ethanol, for the skin extraction, this confirming undoubtedly the original presence of the ethyl esters. Only traces, shown through GC/SIM-MS, of (2E,4Z)- and (2E,2Z)-isomers were in the pulp as well as in two wines. The measured quantities in skins can explain those found in the distillates.

The increased presence of ethyl (2E,4E)-decadienoate in comparison to the (2E,4Z)-isomer in the distillates in respect to the skins as well as the finding out of supposed ethyl (4E)-decenoate and of ethyl (2E,4E,7Z)-decatrienoate only in distillates, could be justified, as proposed by BRICOUT (1977), by the partial switch of the *cis* double bound in 4 position into the *trans* form of the corresponding isomers due to the fermentation process.

No ethyl nonanoate was found in grape; the other C_9 compounds, including the nonanoic acid, were at a level of traces. Their marked presence in the distillates cannot be justified by prefermentative products, differently from what observed in plum and cherry; a peculiar catabolism of some fatty acid could happen during the fermentation, probably in relation to the above quoted factors.

As for the salicylate esters, we emphasize that methyl ester, but not the relevant acid at the detection limit of HPLC analysis, is present in small quantity among the free forms as well as among the aglycons from enzymatic hydrolysis of heterosides, of both skin and pulp; a residue remains in the already extracted skins as shown through their steam distillation. Anyway, the origin of the above mentioned salicylic and cinnamic esters in the distillate should be connected mainly with the anaerobic metabolism of the skin cells.

Monoterpenols are present, even if at a rather low concentration, in both considered berry parts, with a dominance in skins of 3,7-dimethyl-1,5-octadiene-3,7-diol, α -terpineol, nerol and geraniol. Their content in distillates was however higher than that foreseeable from the berry also considering what coming from the bound forms: this unexpected difference could be linked to the earlier ripening stage of the analyzed grape and therefore to a lower content of monoterpenes (WILSON *et al.* 1984).

Conclusion

Peculiar aroma compounds of Catalan roxo cv. marc distillates, which include groups of components typical of other fruits like plum and Bartlett pear, have been proved to derive from compounds mostly present in grapes or differently produced in the fermentation, also in relation to the anaerobic environment. The berry skin appears in this variety for the Bartlett esters as it was in the Traminer variety for terpenic methyl esters (VERSINI et al. 1994 a), an unexpected reserve of particular compounds which can support the aroma complexity and typicity of the distillate. It is however, at the moment, inexplicable why unsaturated ethyl esters, considered till now, above all, climacteric volatiles for the Bartlett pear and likely deriving from unsaturated C₁₈ fatty acids (JENNINGS and TRESSL 1974), can originate in a non-climacteric fruit like the grape (CHAMPAGNOL 1984), only at skin level and in some hybrid, and already on the plant and at a not full ripening stage. Another possible strange metabolism of some fatty acid in the marc fermentation medium of this variety, probably linked to the pH value and/or to the prevailing micro-organisms, seem to be involved in the formation of here found group of C_o compounds.

Interesting compounds in marc distillates are confirmed to be salicylate and cinnamate esters, probably in connection with anaerobic metabolism of skin cells during the silage period.

Acknowledgement

We wish to thank F. MATTIVI for the HPLC analyses.

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Received June 26, 1994