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Localization of free and bound aromatic compounds among skin, juice and pulp fractions of some grape varieties

by

E. GOMEZ[1]), A. MARTINEZ[1]) and J. LAENCINA[2])

[1]) Centro Regional de Investigaciones Agrarias, Murcia, Spain
[2]) Universidad de Murcia, Tecnologia de los Alimentos, Murcia, Spain

S u m m a r y : The localization and distribution of free and glycosidically-bound aroma compounds among the skin, pulp and juice of three red grape varieties (*Vitis vinifera* L. var. Monastrell, Cabernet Sauvignon and Tempranillo) have been studied. An Amberlite XAD-2 column was utilized to separate and extract the free aroma compounds and the glycosidically-bound aromatic compounds. A commercial enzymatic preparation (Pectinol C) was used to release the bound aroma compounds. Gas chromatography and gas chromatography-mass spectrometry were used for the analysis of the compounds.

The results showed that the major compounds in these varieties were alcohols and aldehydes, the skin being the fraction with the largest quantities of aromatic compounds. After the enzymatic hydrolysis, the analysis of the liberated compounds showed that they were mainly alcohols and monoterpenes. Two monoterpenes that did not appear as free aroma compounds could be found.

K e y w o r d s : aroma compounds, red grapes, glycosidically-bound compounds, enzymes.

Introduction

The intensity and diversity of the volatile constituents in grapes are very important as the source of flavor in wines. The aroma is determined by several compounds with very different chemical natures and includes hydrocarbons, esters, ketones, aldehydes and alcohols. It is usually accepted that the varietal aroma of grapes is mainly localized in the internal cell layers of the skin and in less quantity in the pulp and juice (CASTINO 1988) but the distribution of volatile compounds is not the same in all varieties (GUNATA *et al.* 1985 b).

BAYONOVE *et al.* (1984) suggested for the first time the possible existence of glycosidically-bound aromatic compounds in *Vitis vinifera* cv. Muscat of Alexandria. After these findings, different techniques to release these bound compounds and increase the aroma of grapes have been developed (GUNATA *et al.* 1985 a; STRAUSS *et al.* 1986).

The existence of these bound aromatic compounds has been also demonstrated in other fruits and different plant species (STAHL-BISKUP 1987; SCHWAB *et al.* 1989; KRAMMER *et al.* 1991). Most of the studies on the distribution and localization of free and bound volatile compounds have been done with very aromatic varieties such as the Muscat varieties. The non-aromatic varieties, with neutral flavor, have been less studied although they are economically much more important since more than 90 % of world wine production is from non-aromatic varieties.

We report the localization of free and bound volatile compounds in three red varieties of *Vitis vinifera* L.: Cabernet Sauvignon, Tempranillo and Monastrell, and how these free and glycosidically-bound compounds are distributed within the berry.

Material and methods

Grapes at optimum maturity for winemaking were harvested. Tab. 1 shows the grape quality characteristics at the moment of analysis. Total soluble solids were measured using a hand-held refractometer and titratable acidity was calculated following titration of 10 ml of juice to pH 7.

T a b l e 1

Quality characteristics of grapes

	Monastrell	Cabernet Sauvignon	Tempranillo
Weight (100 grapes)	221.0	128.0	172.0
Solible solids (°Brix)	20.6	18.4	22.0
Titratable acidity (mg/l)	6.1	7.3	5.1

The different berry fractions were obtained following the method described by WILSON *et al.* (1986). Frozen grapes (500 g) were hand peeled and the skinned berries (400 g) homogenized for 1 min and then centrifuged at 6000 *g* for 2 h at 5 °C. The supernatant represented the juice fraction. The pulp pellet was rehomogenized with 300 ml of phosphate buffer pH 7 and continuously agitated for 24 h at room temperature. The pulp extract was then centrifuged with the same conditions mentioned above and the supernatant represented the pulp fraction. The skins were homogenized in 300 ml of phosphate buffer pH 7 and after 24 h they were centrifuged to obtain the skin fraction. 2-Octanol (100 µm/kg) was added to each fraction as an internal standard.

Correspondence to: Dr. ENCARNA GOMEZ, U.S.D.A./A.R.S., 2021 South Peach Avenue, Fresno, CA 93727, USA

Each fraction was passed through an Amberlite XAD-2 column (50 cm x 0.8 cm o.d.) following the method of GUNATA *et al.* (1985 a), with the modifications proposed by SCHWAB *et al.* (1990). The Amberlite XAD-2 column was conditioned before the extraction for 24h using methanol acetonitrile and diethyl ether, each for 8 h. The free volatile compounds were eluted with diethyl ether (50 ml). The excess of solvent was removed with a low stream of nitrogen before the chromatographic analysis. The glycosidically-bound fraction was eluted with methanol (50 ml). This methanolic extract was evaporated under vacuum until dryness. The residue was then dissolved with a phosphate-citrate buffer pH 5 (10 ml) and five extractions with diethyl ether (5 ml) were made to assure that no free volatile compounds were present. To hydrolyze the glycosidically-bound compounds, Pectinol C (Rohm, Darmstadt, Germany) was used. To eliminate the polyphenolic compounds that could interfere in the enzymatic action, 1 g of polyvinyl-pirrolidone (PVP) was added to the buffer extract before the addition of the enzyme (DI STEFANO 1991). After 1 h, the PVP was separated out by centrifugation. The enzyme was added in a phosphate-citrate buffer pH 5 (0.4 mg of Pectinol C in 0.1 ml of buffer) and allowed to act for 24 h at 35 °C. The generated free volatile compounds were then extracted with diethyl ether, concentrated and analyzed.

The chromatographic analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (250 °C) and a Carbowax 20M column (50 m x 0.25 mm). Injections were made in split mode (split ratio 80:1; sample size 1 µl). The injector temperature was 250 °C and the oven was programmed from 60 to 200 °C at a rate of 2 °C/min. The carrier gas was hydrogen at a flow rate of 1.5 ml/min (60 °C). The identification of the compounds was determined using a Hewlett-Packard 5970 A mass spectrometer, and comparing the retention times with those of authentic compounds.

Results and discussion

Free volatile compounds: Tab. 2 shows the distribution of the free volatile compounds within the berry for Monastrell, Cabernet Sauvignon and Tempranillo varieties. Only those compounds appearing at a concentration higher than 1 µg/kg have been displayed. For the three varieties, the free volatile compounds were more abundant in the skins, which agrees with other studies in Muscat grapes (GUNATA *et al.* 1985 b; WILSON *et al.* 1984). These compounds occurred in the lowest concentration in the juice fraction. These results suggest that the aromatic compounds are associated with the solid parts of the berry, where they are synthesized and/or stored. Comparing the three varieties, the skins from Monastrell were the most aromatic and the juice of Tempranillo the less aromatic.

The distribution of the studied compounds was not the same in each fraction or within the different varieties. The C_6 compounds (hexanal, (E)-2-hexenal, hexanol, (Z)-3-hexenol and (E)-2-hexenol) were more abundant in the

Table 2

Localization of the free volatile compounds for Monastrell, Cabernet Sauvignon and Tempranillo varieties (µg/kg)

Compound	Monastrell			Cabernet Sauvignon			Tempranillo		
	Juice	Pulp	Skin	Juice	Pulp	Skin	Juice	Pulp	Skin
Propyl acetate	--[a]	17	15	--	--	44	1	--	--
2-Butanol	--	6	28	1	--	--	1	--	--
Pentanal	2	3	18	1	8	--	--	--	--
Hexanal	158	238	200	63	86	142	72	5	83
Isobutanol	--	104	22	--	--	--	--	--	--
Butanol	7	24	9	3	18	--	--	--	5
(E)-2-penten-1-ol	2	3	--	9	--	--	--	--	5
2-Methyl-1-butanol	--	1	--	--	--	--	--	--	--
Isoamyl alcohol	46	408	117	108	123	110	31	112	118
(E)-2-hexenal	135	--	258	61	--	--	83	--	21
Amyl alcohol	11	13	45	7	27	9	11	14	8
2-Heptanol	6	8	--	5	--	--	--	--	6
Hexanol	33	11	87	20	109	312	33	284	161
(Z)-3-Hexenol	--	16	21	4	--	--	18	--	11
(E)-2-Hexenol	36	5	236	21	20	25	28	34	71
Heptanol	--	2	15	12	--	31	--	--	--
Geraniol	56	34	104	25	49	50	13	107	33
Benzilic alcohol	26	--	204	10	23	67	--	18	12
2-Phenylethanol	26	56	155	34	42	86	--	57	306
Total	544	849	1534	384	537	571	291	631	840
%	18	31	51	26	36	38	17	36	47

The results are the average of two replications

a: concentration less than 1 µg/Kg

skins and pulp of all varieties. This is due to the presence of the lipoxygenase enzyme and the fatty acid precursors of these C_6 compounds in the solid parts of the berry (BAYONOVE *et al.* 1987). Monastrell was the variety that contained the highest concentrations of these compounds. Monastrell also showed the highest concentrations of isoamyl and amyl alcohol, and also isobutanol (104 µg/kg in the pulp fraction) which did not appear in any other fraction of the other varieties. Among the compounds with higher molecular weight, geraniol, benzilic alcohol and 2-phenylethanol appeared at concentrations greater than 1 µg/kg. Geraniol appeared in large amount in Tempranillo pulp (107 µg/kg) and Monastrell skins (104 µg/kg). Monastrell skins also had the highest levels of benzilic alcohol.

Comparison of the total concentrations of compounds occurring in these varieties showed the highest concentrations of volatile compounds being found in Monastrell grapes. The proportion in which these volatile compounds were distributed was 38-51 % in the skins, 31-36 % in the pulp and 17-26 % in the juice fraction. These results suggest that any steps in juice processing that promotes a transference of the compounds from the skin are likely to enhance the flavor of the juice or wine. Although extended skin contact may have a deleterious effect due to the extraction of undesirable components, such as the C_6 compounds, optimal conditions could be attained.

Glycosidically-bound volatile compounds: The grape berry has a low ß-glucosidase activity mainly in the pulp and juice fraction (AYRAN *et al.* 1987), but this activity is so low that it is not capable of liberating the glycosidically-bound compounds. The addition of ß-glucosidase from other sources had been tested but it has been proven that the best results are obtained with enzymatic preparations that have a residual ß-glucosidase activity, without aglycone specificity (AYRAN *et al.* 1987; GOMEZ *et al.* 1990; GUNATA *et al.* 1990).

The compounds that appeared in the methanolic extract in higher concentrations are shown in Tab. 3. Whereas in Muscat varieties the presence of terpenic alcohols are the most important compounds, in these non-aromatic varieties the presence of aliphatic alcohols is very important and this is the first time these compounds are described to appear among the glycosidically-bound compounds of grapes although they have been described in other fruits (SCHWAB and SCHREIER 1990).

The glycosidically-bound aromatic compounds were abundant in the skin fraction of the grapes. The highest concentrations were found in Cabernet Sauvignon and Monastrell and the lowest in Tempranillo. The pulp fraction was richer than the juice except for Monastrell juice where there was a greater liberation of aliphatic alcohols. Two very aromatic alcohols that also appeared in Muscat varieties, were benzilic alcohol and 2-phenylethanol.

Comparing the liberated compounds in the different fractions with the free volatile compounds, benzilic aclohol and 2-phenylethanol were present in higher concentrations from the bound aromatic compounds in almost every fraction in the three varieties. Geraniol could not be detected from the methanolic extract. The most important difference found was the apparition of two terpenic alcohols, linalool and α-terpineol, in the bound fraction. These alcohols are very characteristic of Muscat varieties. Their concentration was very important in Cabernet Sauvignon (112 and 218 µg/kg). The distribution of the glycosidically-bound compounds varied within varieties, the biggest difference was found between the Cabernet Sauvignon and Monastrell juice. The percentages of distribution were 58-64 % in the skins, 19-30 % in the pulp and 6-23 % in the juice fraction.

Although some of the liberated compounds appeared at high concentrations they are not important enough technologically (only linalool appeared at a concentration higher than its odor threshold). This is specially due to the difficulties of their liberation because the ß-glucosidase enzyme

Table 3

Localization of the bound voalitle compounds for Monastrell, Cabernet Sauvignon and Tempranillo varieties (µg/kg)

Compound	Monastrell			Cabernet Sauvignon			Tempranillo		
	Juice	Pulp	Skin	Juice	Pulp	Skin	Juice	Pulp	Skin
2-Butanol	--[a]	--	--	--	--	71	--	--	--
Butanol	15	--	29	--	9	--	--	--	--
Isoamyl alcohol	15	22	20	--	11	--	--	--	--
Amyl alcohol	11	--	--	--	11	50	14	14	--
Hexanol	40	--	72	--	6	--	--	--	--
(E)-2-Hexenol	11	--	35	--	--	--	--	--	--
Linalol	--	--	25	--	--	112	--	--	18
α-Terpineol	--	--	43	--	--	218	--	--	48
Benzilic alcohol	67	88	171	38	12	58	29	--	41
2-Phenylethanol	18	39	43	18	191	72	--	47	73
Total	177	149	438	57	260	581	43	61	180
%	23	19	58	6	30	64	15	22	63

The results are the average of two replications

a: concentration less than 1 µg/Kg

has its maximum activity at pH 5 (AYRAN *et al.* 1987) and its activity is reduced at must and wine pH. Also, glucose (present in must) and ethanol (present in wine) inhibit the enzyme activity. It has been demonstrated that the acid hydrolysis, used by other authors (WILLIAMS *et al.* 1984), is not a good solution either because it causes rearrangements of the compounds and disturbs the original glycosidically-bound compounds. Neither in the Muscat varieties, where these bound aromatic compounds are so important quantitatively, has been found a good method to release these compounds and increase the aroma. More studies in this area have to be done.

References

AYRAN, A.; WILSON, B.; STRAUSS, C.; WILLIAMS, P.; 1987: The properties of glycosides of *Vitis vinifera* and a comparison of their ß-glucosidase activity with that of exogenous enzymes. An assessment of possible applications in enology. Amer. J. Enol. Viticult. **38**, 182-188.

BAYONOVE, C.; GUNATA, Y.; CORDONNIER, R.; 1984: Mise en évidence de l'intervention des enzymes dans le développement de l'arôme du jus de Muscat avant fermentation: la production des terpénols. Bull. O.I.V. **57**, 741-758.

- -; ROUFET, M.; CORDONNIER, R.; 1987: Données sur les pécurseurs d'arôme préfermentaire: cas des acides gras insaturés du raisin. In: SCIENZA, A.; VERSINI, G. (Eds.): Le sostanze aromatiche dell'uve e del vino. Prim. Simp. Intern. S. Michele all'Adige, Italy, 25-27 Giugno 1987, 93-112.

CASTINO, M.; 1988: Connaissance de la composition du raisin et du vin: Passage au vin des substances non-transformées par la fermentation. Apparition dans les vins des substances nées lors de la fermentation. Bull. O.I.V. **61**, 539-553.

DI STEFANO, R.; 1991: Proposition d'une méthode de préparation de l'échantillon pour la détermination des terpenes libres et glycosides des raisins et des vins. Bull. O.I.V. **64**, 219-223.

GOMEZ, E.; NUNEZ, J.; LAENCINA, J.; FLORES, J.; LOPEZ, J.; ALMELA, L.; 1990: Volatile compounds of grapes cv. Monastrell. In: XX. Intern. Symp. Fruit Juices Hot Climates, 371-379. Paris, France.

GUNATA, Y.; BAYONOVE, C.; BAUMES, R.; CORDONNIER, C.; 1985 a: The aroma of grapes. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. J. Chromatograph. **331**, 85-90.

- -; - -; - -; - -; 1985 b: The aroma of grapes. Localisation and evolution of free components of cv. Muscat during first development and maturation. J. Sci. Food Agricult. **36**, 857-862.

- -; BITTEUR, S.; BAUMES, R.; SAPIS, J.; BAYONOVE, C.; 1990: Activités glycosidases en vinification. Perspectives d'exploitation des précurseurs d'arôme du raisin, de nature glycosidique. Rev. Franc. Oenol. **30** (122), 37-41.

KRAMMER, G.; WINTERHALTER, P.; SCHWAB, M.; SCHREIER, P.; 1991: Glycosidically-bound aroma components in the fruits of Prunus species: Apricot (*Prunus armeniaca* L.), peach (*Prunus persica* L.), yellow plum (*Prunus domestica* L. ssp. Syriaca). J. Agricult. Food Chem. **39**, 778-781.

SCHWAB, M.; MAHR, C.; SCHREIER, P.; 1989: Studies on the enzymatic hydrolysis of bound aroma components from *Carica papaya* fruit. J. Agricult. Food Chem. **37**, 1009-1012.

- -; SCHREIER, P.; 1990: Glycosidic conjugates of aliphatic alcohols from apple fruit (*Malus sylvestris* MILL, cult. Jonathan). J. Agricult. Food Chem. **38**, 757-763.

STAHL-BISKUP, E.; 1987: Monoterpene glycosides, state of the art. Flavor Frag. J. **2**, 75-82.

STRAUSS, C.; WILSON, B.; GOOLEY, P.; WILLIAMS, P.; 1986: Role of monoterpenes in grape and wine flavour. In: PARLIMENT, T. (Ed.): Biogeneration of Aromas, 222-242. ACS Symposium Series 317, Washington.

WILLIAMS, P.; STRAUSS, C.; WILSON, B.; MASSY-WESTROPP, R.; 1984: Studies of the hydrolysis of *Vitis vinifera* monoterpene precursors compounds and model monoterpene ß-D-glucosides rationalizing the monoterpene composition of grapes. J. Agricult. Food Chem. **32**, 1215-1223.

WILSON, B.; STRAUSS, C.; WILLIAMS, P.; 1984: Changes in free and glycosidically-bound monoterpenes in developing Muscat grapes. J. Agricult. Food Chem. **32**, 919-924.

- -; - -; - -; 1986: The distribution of free and glycosidically-bound monoterpenes among skin, juice, and pulp fractions of some white grape varieties. Amer. J. Enol. Viticult. **37**, 107-111.

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