DETERMINATION OF FREE AND BOUND TERPENES AND NORISOPRENOIDS DURING ALCOHOLIC FERMENTATION OF PORT WINE

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INTRODUCTION

Monoterpenes and some norisoprenoids exist in grapes and wines in two forms: free and glycosidically conjugated forms (1-3). The free volatile compounds (linalool, α-terpineol, citronellol, nerol, geraniol, damascone, β-damascenone, α-ionone, β-ionone, vitispirane, 2,6,6-trimetilciclohexenona) are the most important for wine aroma. Glycosidically bound monoterpenes and norisoprenoids make no direct contribution to wine aroma, nevertheless, they can be acid or enzymatic hydrolysed, releasing their aglycons (4-6). Previous studies performed with glycosides indicate the importance of this fraction in wine bouquet, in particular after a period of wine storage (7). As Port wine suffers long periods of ageing (> 4 years), enzymatic and chemical reactions can occur during this period, in particular the aromatic evolution of vintage category wines. Thus, the aim of this work was to identify and quantify free and bound monoterpenes and norisoprenoids, in grapes, musts during alcoholic fermentation using two different vinification methods — tank versus « lagar » (foot trodde), and on the respective wines.

MATERIALS AND METHODS

• Grapes, musts and wines

All samples come from « Douro Superior sub-region », in Portugal. Wines were obtained by two different vinification methods (« Lagar »-foot trodden and Tank) from a black grapevine variety *Vitis vinifera* L. cv. Touriga Francesa. Musts and Grapes were frozen on each sampling time.

• Sample preparation – Grapes

200 g of frozen berries was used. After removal of seeds, the berries were crushed, centrifuged and filtered.

90 mL were centrifuged, after thawing in cold water, and then were filtered.

• Wines

50 mL of wine were filtered.

• Resin and column preparation

Were performed according to (4).

• Extraction of bound fraction

To 20 mL of sample, ultra pure water was added to make up to 50 mL. The extraction of the bound fraction was adapted from previous works (4, 5). The dried samples were dissolved in 20 mL of citrate-phosphate buffer (pH 5, 100 mmol·L^{-1}), containing 46,7 mg·L^{-1} of pectolytical enzyme Lallzyme Beta (Lallemand), and incubated at 40ºC over 14 hours. The sample obtained was submitted to SPME analysis and injected on GC-MS.

RESULTS AND DISCUSSION

During alcoholic fermentation there was a progressive increase of the bound fraction, being this increase more significant for the norisoprenoids and after neutral grape spirit addition, both in lagar and tank vinification processes (*tables I and II*). In fact, during the vinification process, in lagar or tank, the alcoholic fermentation occurs in the presence of grape skin, where, the most of the volatile compounds and their precursors are located. Thus, there is an increase of the bound fraction, by the continuous extraction of these compounds from the grape skin, over time, overtaking the fraction that usually hydrolyses. There was also an enhancement of the free terpenes and norisoprenoids. However, this observation was not linear within the time, as should be expected. Several factors during fermentation could account for this behaviour. A decrease in the free terpene concentration after neutral grape spirit addition was observed, which can be explained by a dilution as a consequence of this spirit addition. The reason why this occurrence was not verified immediately after neutral grape spirit addition in the concentration of norisoprenoids, might be due to the fact that a higher hydrolysis of the bound fraction in this fermentation stage occurred concomitantly with the exponential increase of the bound fraction. Higher levels, either in free and bound fractions, both in tank and lagar, occurred after the beginning of fermentation (30-35 hours). Until this moment the changes in free and bound fractions, both in vinification in tank and in « lagar », were not relevant. In fact, the increase in temperature and movement of the solid parts caused by alcoholic fermentation can improve the extraction of the bound fraction from grape skins, on the other hand, the enzymatic action of yeast can influence the variety aroma development by hydrolysis of its precursors, thus, enabling the release of aromatic terpenes and norisoprenoids.

***Table I:** Concentration (µg·L^{-1}) of free and bound terpenes and norisoprenoids, by SPME technique, in Touriga Francesa variety, during alcoholic fermentation performed on "Lagar" vinification*

Lagar		Free fraction							Bound fraction						
TF (µ·L^{-1}) Time	G	M 0 h	M 12 h	M 35 h*	M 44 h*	W NGS** 48 h	FW 63 h	G	M 0 h	M 12 h	M 35 h*	M 44 h*	W NGS** 48 h	FW 6
Terpènes														
Citronellol	1.5	1.3	7.6	9.8	54.8	17.6	12.8	nd	0.3	0.5	0.8	3.5	6.4	6.6
Nerol	11.4	11.5	29.8	15.7	105.1	43.5	52.9	nd	7.9	11.5	18.2	6.5	7.7	21.7
Geraniol	51.5	34.9	66.4	35.2	151.2	56.5	20.7	15.9	9.5	16.7	23.4	59.6	28.9	25.9
α-Terpineol	4.9	3.2	4.0	1.4	6.0	10.7	5.9	7.6	1.4	1.6	2.0	nd	3.4	3.3
Linalool	6.4	17.3	35.7	8.6	42.7	158.5	155.8	nd	36.1	34.9	80.3	74.4	69.1	60.4
Total	76	68	143	71	360	287	248	23	55	65	125	144	116	118
Norisoprenoids														
Damascone	0.5	0.5	0.7	0.3	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Vitispirane***	nd	nd	nd	nd	nd	2.8	1.6	nd	nd	nd	nd	nd	0.5	0.4
β-Damascenone	0.3	1.5	2.6	1.5	1.8	2.5	3.0	nd	nd	0.1	0.1	0.6	3.6	4.4
α-Ionone	nd	nd	nd	nd	nd	nd	nd	nd	0.5	0.8	0.9	2.5	4.4	4.8
β-Ionone	0.5	0.2	0.7	0.2	0.7	0.3	0.1	nd	0.1	0.2	0.2	0.2	0.4	0.3
2,6,6-trimethylcyclohexanone	0.1	0.3	0.3	0.1	0.5	2.4	1.1	nd	nd	nd	nd	nd	nd	nd
Total	1.4	2.5	4.4	2.1	3.1	8.0	5.9	nd	0.6	1.0	1.2	3.4	8.8	9.9
Total (terpenes + norisoprenoids)	77	71	148	73	363	295	254	23	56	66	126	147	124	128

*: must on fermentation ; **: wine after neutral grape spirit addition; ***: express in µ·L^{-1} of β-damascenone; TF: Touriga Francesa; M: must; nd: not detected; grapes; h: hours; FW: Final wine.

Table II: *Concentration ($\mu g \cdot L^{-1}$) of free and bound terpenes and norisoprenoids, by SPME technique, in Touriga Francesa variety, during alcoholic fermentation performed on Tank vinification*

Tank	Free fraction							Bound fraction						
TF ($\mu \cdot L^{-1}$) Time	G	M 0 h	M 18 h	M 29 h*	M 47 h*	M 53 h*	FW NGS**	G	M 0 h	M 18 h	M 29 h*	M 47 h*	M 53 h*	FW NGS**
Terpenes														
Citronellol	1.5	0.5	1.0	3.1	50.9	49.4	13.1	nd	nd	0.6	0.6	nd	1.0	1.0
Nerol	11.4	3.5	6.2	13.4	68.6	71.1	49.4	nd	5.8	8.9	1.2	5.2	6.6	3.3
Geraniol	51.5	10.9	20.9	10.7	54.0	120.4	44.8	1.2	7.7	32.0	28.2	8.2	44.0	8.5
α-Terpineol	4.9	1.0	1.3	1.1	3.4	2.1	5.1	nd	nd	1.0	0.6	nd	nd	1.0
Linalool	6.4	2.0	2.9	5.0	10.0	15.8	104.9	7.7	4.9	9.4	7.9	6.0	16.1	16.6
Total	76	18	32	33	187	259	217	9	18	52	38	19	68	30
Norisoprenoids														
Damascone	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Vitispirane***	nd	nd	nd	nd	nd	nd	1.6	nd	nd	nd	nd	nd	nd	0.4
β-Damascenone	0.3	nd	0.1	0.2	1.0	1.4	4.0	nd	nd	0.1	0.1	nd	1.0	3.1
α-Ionone	nd	nd	nd	nd	nd	nd	nd	0.1	0.5	0.8	nd	0.1	nd	2.2
β-Ionone	0.5	0.1	0.1	0.2	0.6	0.9	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.2
2,6,6-trimethylcyclohexanone	0.1	0.1	0.1	0.1	0.4	0.7	nd	nd	nd	nd	nd	nd	nd	nd
Total	1.4	0.2	0.3	0.5	2.0	3.0	5.9	0.3	0.7	1.0	0.3	0.2	1.5	5.9
Total (terpenes + norisoprenoids)	77	18	33	34	189	262	223	9	19	53	39	20	69	36

*: must on fermentation; **: wine after neutral grape spirit addition; ***: express in $\mu \cdot L^{-1}$ of β-damascenone; TF: Touriga Francesa; M: must; nd: not detected; G: grapes; h: hours; FW: Final wine.

In "Lagar" vinification it was observed that, at the moment that fermentation was started, the concentration of free terpenes and norisoprenoids underwent an important decrease, which was not seen in tank vinification. The total concentration of the free fraction (terpenes and norisoprenoids), in the final wines, was similar, in both vinifications while the bound fraction was higher in the wine obtained from the "lagar" vinification, being two and half time as high as that from the tank. In final wines, vinified in tank and in "lagar", the percentage of the bound fraction of terpenes and norisoprenoids represented 14% and 33.5% of the total fraction, respectively.

CONCLUSION

A progressive increase of the bound fraction occurs during alcoholic fermentation. This increase is more significant for norisoprenoids and after neutral grape spirit addition, both in lagar and tank vinification processes. There was an enhancement of the free terpenes and norisoprenoids, which is not linear within the time. The total concentration of the free fraction, in final wines, was similar, in both vinifications while, the bound fraction was in lagar vinification two and half times higher than in tank vinification. This fact, may be very important in the Port wine ageing process, in which this bound fraction can represent an important reservoir of terpenes and norisoprenoids, that can be released by acid hydrolysis. The aromatic potential of wines obtained by "lagar" vinification process is higher than those for wines obtained by tank vinification.

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