Grape aroma precursors in cv. Nebbiolo as affected by vine microclimate during ripening.

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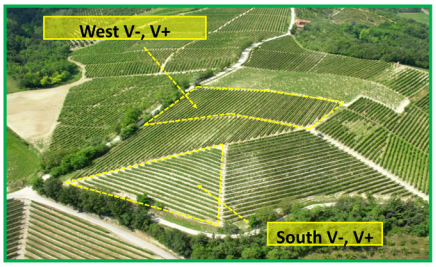
Precursors of grape aroma that generate varietal aroma compounds during wine fermentation and aging, are very important in winemaking, especially for red non floral varieties, such as cv. Nebbiolo (Di Stefano *et al.*, 1998). Vine and cluster microclimate, in particular different levels of light exposure and temperature, may modify berry aromatic composition (Lee *et al.*, 2007, Scafidi *et al.*, 2013). This work reports the results about the profile and content of norisoprenoid precursors in cv. Nebbiolo grapes during ripening, as well as about the influence that vine vigour and vineyard exposure may have on their accumulation in order to evaluate the aroma potential and when the maximum potential is achieved.

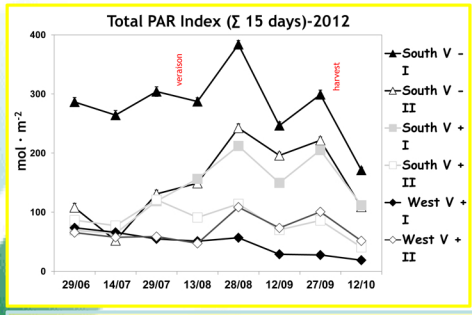


The data were collected, during two consecutive seasons, in two nearby Nebbiolo planted vineyards (North-West Italy), characterized by high vigour heterogeneity and by a different aspect (South or West). Each vineyard parcel was sorted in 2 vine vigour classes (V- and V+) while berry samplings were collected from 15 days after flowering until harvest. Photosynthetically Active Radiation (PAR) and air temperature (T, °C) inside the bunch zone were measured *in continuous* from pea size stage to harvest time and temperature index (TI) was calculated.

Solid phase extraction (SPE) and subsequent enzymatic hydrolysis have permitted to isolate the glycosylated grape norisoprenoids prior their GC-MS determination (Mateo. *et al.*, 1997).

A further step of acid hydrolysis was performed in order to simulate norisoprenoid aromas generation and/or transformation during the wine aging process (Mateo. *et al.*, 1997).

Microclimate characterization



TI depends on both the value and the duration of the temperature level. During the post veraison period, the highest value of TI above the threshold of 35 °C was registered for all treatments and the most notable differences between the parcel West V + (milder microclimate) and the South V- and V + (warmer microclimate) were observed.

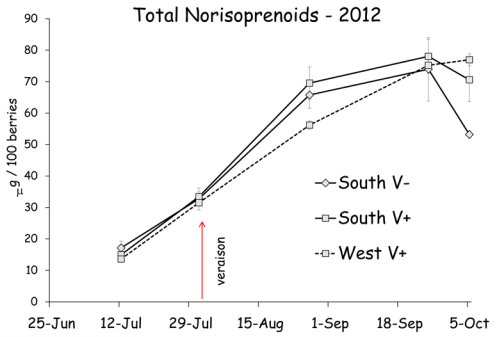
The maximum value of the index PAR was recorded in the second half of August (after-veraison) for all three parcels.

The South plot has always registered higher PAR and temperature values compared to the West exposure in both 2012 and 2013 vintage years.

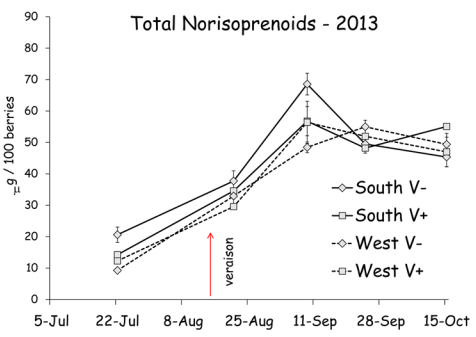
TI: Number of hours of fruit zone exposition to different thresholds of temperature (2012)

Class of temperature	Class of vigour	pre veraison period		post veraison period				harvest period	total
		1*	2	3	4	5	6	7	
T < 15°C	South V–	0	8	0	3	58	**121**	**171**	361
	South V+	0	8	0	3	66	**125**	**176**	378
	West V+	0	9	0	4	62	**130**	**180**	385
15° C ≤ T > 25 C	SouthV–	201	208	196	174	228	165	164	1336
	SouthV+	207	213	201	178	220	165	167	1351
	West V+	210	218	214	185	236	187	171	**1421**
25° C ≤ T > 35 C	South V–	155	114	107	108	54	68	25	**631**
	SouthV+	140	103	104	106	66	69	17	605
	West V+	149	118	118	120	58	43	9	615
T ≥ 35 C	South V–	4	30	**57**	**75**	20	6	0	192
	SouthV+	13	36	**55**	**73**	8	1	0	186
	West V+	1	15	**28**	**51**	4	0	0	**99**

Glycosylated norisoprenoids



Most of C_{13}-norisoprenoid glycosides accumulation in Nebbiolo grapes occurs early, from the pre veraison period until 20-25 days post veraison (dpV), then a plateau or a decrease may be noticed until preharvest (49-53 dpV) and harvest date.



Compound	Calculated Lineary Retention Indices (LRI)[1]
3,4-dihydro-3-oxoactinidol (isomer II)	2434
3,4-dihydro-3-oxoactinidol (isomer III)	2437
3-hydroxy-β-damascone	2510
Unknown norisoprenoid	2559
3-oxo-α-ionol	2610
4-oxo-β-ionol	2613
3,9-dihydroxy mégastigma-5-ene	2628
4-oxo-7,8- dihydro-β-ionol	2648
Blumenol C	2681
Trans-5,6-epoxy-β-ionone	2699
3-hydroxy-7,8-dehydro-β-ionol	2749

Norisoprenoid compounds identified by GC-MS after enzymatic hydrolysis of precursors from Nebbiolo grape extracts. [1]calculated on a Innowax 30 m, 0.25 mm, 0.25 µm (J&W Scientific, Folsom, CA, USA).

Total norisoprenoids accumulated during 2012 season without great differences between South V- and V + plots, but in the West V+ plot norisoprenoid accumulation was delayed, maybe due to both vigour and exposure effect. At harvest time, total norisoprenoids concentration was significantly lower in the warmest plot (V-) compared to the two vigorous plots (South V+, West V+).

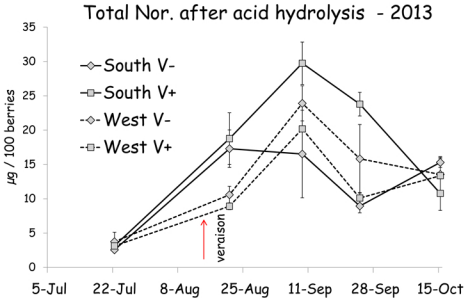
During 2013 the excessive vigour of the South V+, West V- and West V+ (unbalanced vigour/yield) probably caused a delay of the norisoprenoid accumulation in these plots if compared to the South V-. In the less vigorous and warmest plot South V- an important decrease is noticed, anticipated in this second year. At harvest no significant differences between treatments were noticed.

Norisoprenoids after acid hydrolysis

Compound	Calculated Lineary Retention Indices (LRI)[1]
Riesling acetale	1619
TDN	1724
Actinidol (isomer I)	1906
Actinidol (isomer II)	1919

Norisoprenoid compounds identified by GC-MS after acid hydrolysis of precursors from Nebbiolo grape extracts. [1]calculated on a Innowax 30 m, 0.25 mm, 0.25 µm (J&W Scientific, Folsom, CA, USA).

West exposed treatments presented significantly lower concentration of norisoprenoids if compared to the South exposed ones until after veraison. The amount of the considered norisoprenoids, decreased dramatically from 20-25 days after veraison (11Sep.) until harvest especially for the South V+ plot. The differences were not significant between treatments at harvest



In the preharvest period, the South V+ grapes had the higher potential to generate the considered norisoprenoids , while it is worth noting the fact that the excessive light and temperature in the less vigorous and warmest plot South V- probably penalized the generation of these compounds . The mild microclimate of the the most vigorous West V+ plot led at similar results.

Conclusions The degree of protection of the precursors varied according to the exposure of the vineyards and the degree of shading caused by the different conditions of foliage vigour, thus the final glycosylated norisoprenoid concentration may be also influenced by the microclimatic conditions of the bunch zone in the near harvest period. In addition to the vigour, the vineyard exposure played a probable role on norisoprenoids seasonal trend in the 2012 season. From the results obtained in the 2013 vintage, vine vigour appeared to be more determinant on norisoprenoids seasonal accumulation when an unbalance ratio between vigour and yield occurs. The grape potential in some norisoprenoids, frequently reported during wine aging, seems to be less favorite by extremely warm or mild conditions.

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