Foliar spray application of specific inactive dry yeast at veraison: effect on berry skin thickness, aroma and phenolic quality

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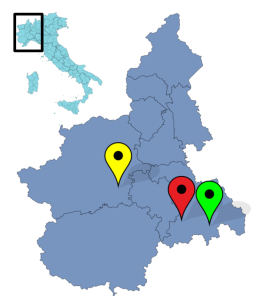
Achieving a satisfactory aroma and phenolic maturity at harvest is of key importance for the production of quality wines. To this purpose, a foliar spraying treatment with specific yeast derivatives was tested on grapes (*Vitis vinifera* L.) cv. Chardonnay, Cortese, Barbera, and Nebbiolo. The treatment was carried out at veraison with two different formulations for white and black varieties (LalVigne™ Aroma and LalVigne™ Mature, respectively) to enhance aroma and phenolic quality (Villango et al. 2015; Portu et al. 2016; Šuklje et al. 2016). In addition, the influence of the treatments on the berry skin thickness parameter was evaluated, since this parameter was seen to be correlated with higher resistance of grapes to pests (Gabler et al. 2003). The analyses were carried out on grapes at harvest, and then experimental wines were produced and analyzed.

Samples



Vitis vinifera L. cv.	Location	Vines age	Vine spacing
Chardonnay	Chieri	6	2.8 × 0.9 m
Cortese	Novi Ligure	40	2.4 × 1.2 m
Barbera	Acqui Terme	40	2.2 × 0.9 m
Nebbiolo	Acqui Terme	12	2.4 × 0.9 m

Calcareous soils. Vertical trellis and Guyot-type pruning. Fertilization in 2014/15 for Cortese with 250 kg/ha Emonatural NPK 8.5.15 (Fertben, Poggio Rusco, IT). For all the analysis, the grapes were sampled at harvest on the "treated" parcels, and compared with grapes harvested from "not treated" parcels (control) in the same vineyard, with adequate buffer space between them.

Control sample (blue)

"Not treated" parcels in the same vineyard for each variety, adequate buffer space between parcels

Specific inactive dry yeast treatment (red)

Two products based on ***Saccharomyces cerevisiae* specific inactive dry yeast derivatives** (Lallemand Inc., Montreal, Canada) were tested:

LalVigne™ Aroma	LalVigne™ Mature
white varieties	red varieties
(3 kg/ha each application)	(1 kg/ha each application)

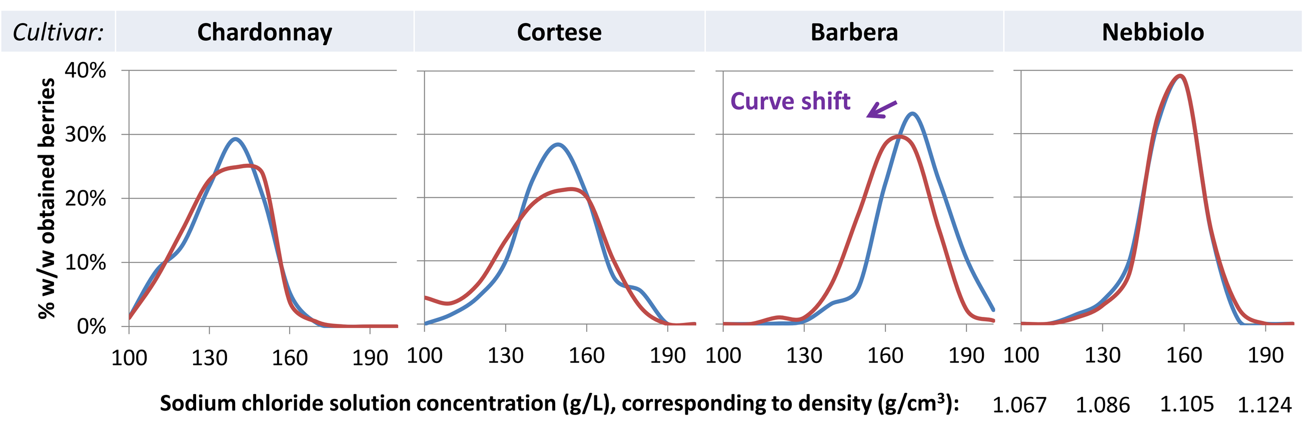
Prior to the treatment, the product was dissolved in 10 volumes of water and then further diluted for a treatment volume of 600 L/ha.

For each variety, two applications of the product were done in the vineyard ("treated" parcels): at 5% véraison and 10 days later.

Grape analysis at harvest

Samples were sorted by densimetric flotation in saline solutions to obtain **berry density distribution curves** by weight percentage (Fournand et al. 2006; Kontoudakis et al. 2011; Rolle et al. 2011).

For white varieties, the treatment seemed to have induced a less-narrower Gaussian-shape curve. In Barbera, a shift towards lower density values was found, while Nebbiolo distribution was not affected.

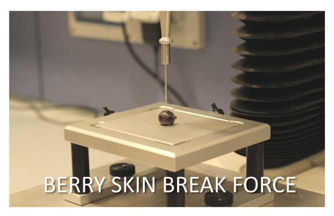


Cultivar	Sample	Average berry weight	Must composition (whole sample, not densimetrically sorted)					
			°Brix	pH	Titr. acidity	Citric acid	Tartaric acid	Malic acid
		g			g tartaric acid/L	g/L (HPLC)	g/L (HPLC)	g/L (HPLC)
Chardonnay	Control	1.70	21.7	3.35	5.18	nd	6.91	1.17
	Treated	1.60	21.7	3.32	5.48	nd	7.11	1.25
	Sign.	ns	ns	ns	ns	-	ns	ns
Cortese	Control	2.38	22.5	**3.21**	**5.08**	0.11	7.19	0.95
	Treated	2.24	22.5	**3.13**	**6.04**	0.11	7.23	0.97
	Sign.	ns	ns	*	*	ns	ns	ns
Barbera	Control	2.61	**26.5**	3.14	8.18	0.25	9.01	2.51
	Treated	2.70	**25.8**	3.18	8.36	0.25	8.95	2.74
	Sign.	ns	*	ns	-	ns	ns	ns
Nebbiolo	Control	2.20	**23.8**	**3.19**	**5.33**	0.11	6.70	0.90
	Treated	2.17	**24.6**	**3.28**	**4.61**	0.11	6.99	0.91
	Sign.	ns	*	*	**	ns	ns	ns

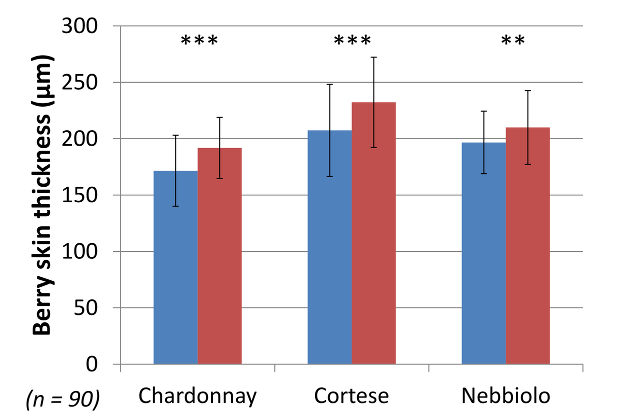
No significant effect on average berry weight was found. The **must composition** reported **no effect on sugars accumulation on white varieties**, and a different trend for Barbera and Nebbiolo.

Due to the abnormal ripeness shift previously seen, which might have caused by external factors, the **Barbera experiment is not included in the following results**; the behavior will be investigated with new trials.

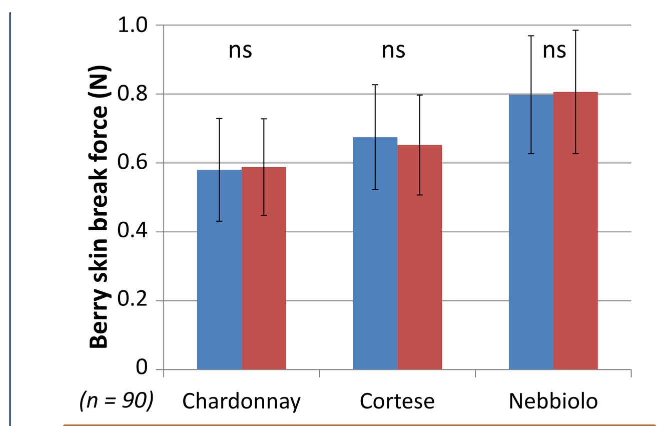
Berry skin thickness and break force



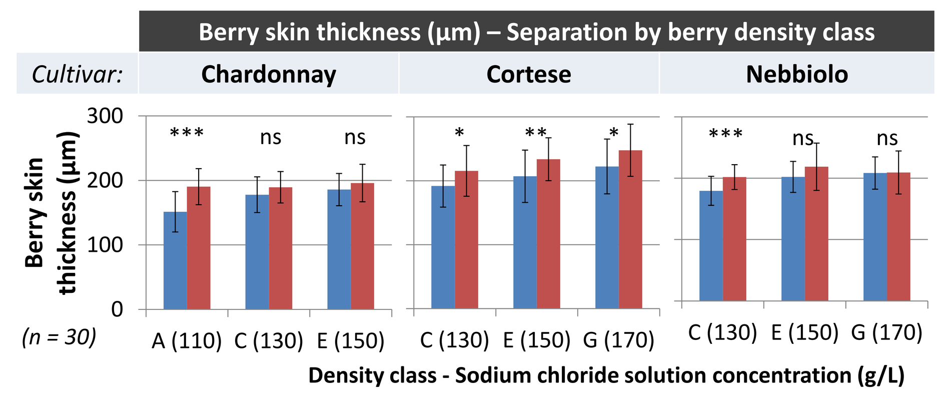
Berry skin mechanical properties were evaluated using a TA.XTplus instrument (Stable Micro Systems, Godalming, UK) according to the methods proposed by Letaief et al. (2008) and Rolle et al. (2013). The analyses were carried out on the three most represented density classes for each variety, on both control and treated samples. For each test combination 30 randomly-taken berries were tested.



Treatment effect on berry skin thickness
Positive influence



No effect on berry skin break force
No significant differences

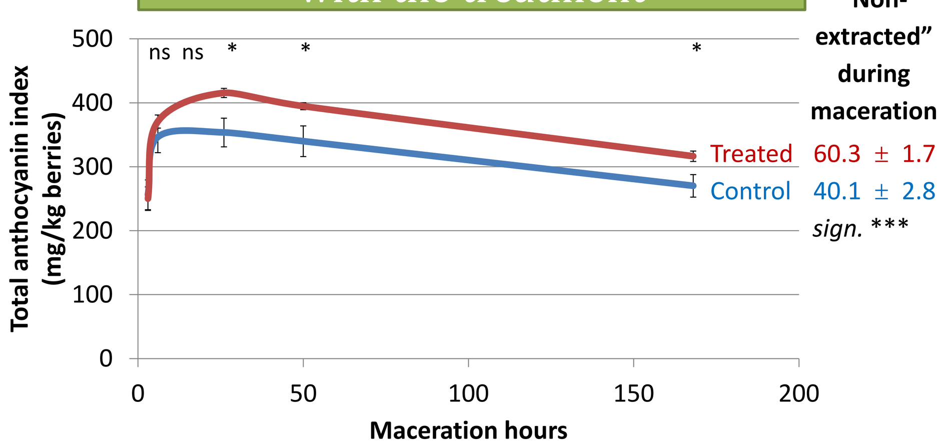


Higher positive variations between control and treated samples were found in lower density classes (less ripe berries).

Anthocyanin extractability

The extraction was evaluated on berries belonging to the most represented density class according to the method proposed by Rio Segade et al. (2015). Extracts were taken during a 7-days skin maceration in model wine solution (12% ethanol, 50 mg/L SO_2, pH 3.20). At the end of the maceration, the skins were further extracted in a similar solution with the SO_2 content increased to 2 g/L, homogenized and centrifuged, to evaluate the "non-extracted" fraction. Spectrophotometric (total anthocyanin index, proanthocyanidins, vanillin assays; Di Stefano and Cravero, 1991) and HPLC (anthocyanin profile; Rolle and Guidoni, 2007) analyses were done (*n* = 3).

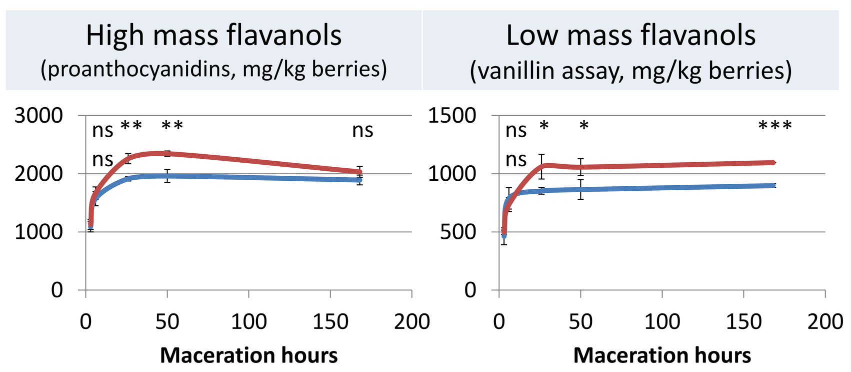
Nebbiolo: significant increase with the treatment



- \+ higher anthocyanin value extracted after 48h and at the end of the 7-day maceration
- \+ higher anthocyanin content "non-extracted" during maceration
- **= higher anthocyanin content in grapes from treated samples (mainly Peonidin-3-glucoside)**

Tannin extraction

A **significant increase in both high- and low-mass flavanols** extracted was found during the simulated maceration in the treated samples, except after 7 days for proanthocyanidins assay.



Wine production

Cortese and Nebbiolo grapes from control and treated samples were separately used for the production of experimental wines (micro scale vinifications, 100 L each).

Wines were analysed using OIV (2015) methods, Di Stefano et al. (1989), Rolle and Guidoni (2007) methods for phenolics and Di Stefano (1991), Rolle et al. (2012) methods for volatile compounds.

CORTESE
- Destemming and crushing
- Cold maceration (24h, 5°C)
- Pressing
- Low-temperature must static decanting
- Fermentation with QA23 inoculum
- Racking
- Cold stabilization
- Bottling

NEBBIOLO
- Destemming and crushing
- Maceration and fermentation at 28°C with D254 inoculum
- Devatting and pressing
- Malolactic fermentation at 20°C with VP41 inoculum
- Racking
- Cold stabilization
- Bottling

Wine composition

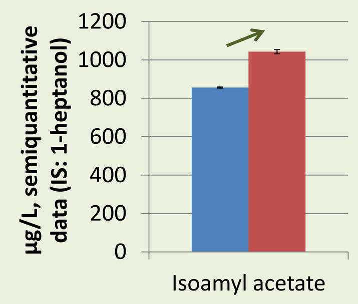
*Average of two analytical replicates. * OIV-MA-F1-05. nd = not detected*

The trend seen in berry density distribution curves seem to be related to the **ethanol content** in wines: a **smoother curve in Cortese** treated sample marked a slight reduction in ethanol, while the identical curves on **Nebbiolo evidenced almost the same ethanol content in wines**. The trend on the acidity parameters was not related to that found in the musts. No relevant changes were found for other parameters.

Cultivar	Sample	Ethanol	pH	Titr. acidity	Citric acid	Tartaric acid	Malic acid	Lactic acid	Acetic acid	Glucose	Fructose	Glycerol
		% v/v		g tartaric acid/L*				g/L (HPLC)				
Cortese wines	Control	**14.21**	3.24	**5.85**	0.23	2.09	1.30	0.13	0.35	nd	1.67	7.59
	Treated	13.71	3.24	5.74	0.23	2.09	1.27	0.12	0.35	nd	1.55	7.45
	Diff.	-	=	-	=	=	~	(incl. D-isomer)	=		~	-
Nebbiolo wines	Control	13.69	3.44	5.59	nd	1.60	nd	0.90	0.43	nd	nd	7.72
	Treated	13.61	3.42	**5.76**	nd	1.69	nd	0.80	0.50	nd	nd	7.67
	Diff.	~	~	+		+		-	+			~

Cortese wine aroma

Cortese grape variety is considered somewhat a **neutral aroma variety**, with few varietal aroma compounds. With the treatment and vinification, the **aroma complexity was improved** (+8% of total free forms detected content) especially for **isoamyl acetate** (**+22%**), contributing to fruity aroma. Also isoamyl alcohol content improved, but values are lower than the odour threshold (Guth, 1997), although it could be involved in **additive or synergistic effects with other compunds**.

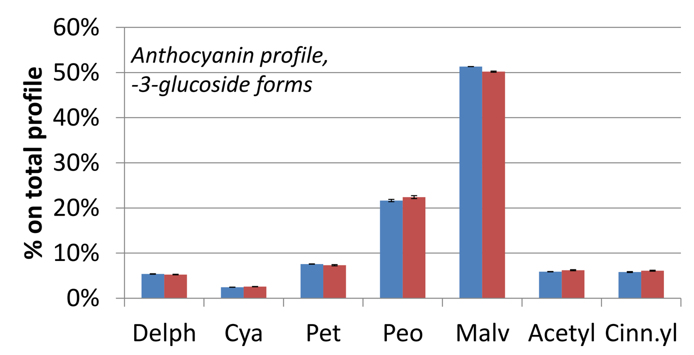


Nebbiolo wine color phenolics

Sample	Total anthocyanins index	Monomeric anthocyanins index	Proanthocya-nidins assay	Vanillin assay	Color intensity	Color hue	L*	a* (red)	b* (yellow)
	mg/L	mg/L	mg/L	mg/L	a.u, OP 10mm				
Control	101	49	2467	1128	3.07	0.76	44.2	56.9	27.5
Treated	101	48	**2617**	**1211**	**3.19**	0.75	**43.5**	**57.9**	29.1
Diff.	=	~	+	+	+	~	-	+	+

An **increase in polymeric and monomeric flavanols** (from proanthocyanidins and vanillin assays) was evidenced **in treated Nebbiolo samples**.

While no difference in wine total anthocyanins was found, a **slightly darker, red color** was detected in treated sample wines with respect to the control. Treated wine anthocyanin profile reflected the grape relative contribution increase in peonidin-3-glucoside (and acylated anthocyanin forms due to their nature), and the decrease of malvidin-3-glucoside.



Sensory analysis

Duo trio test (ISO 10399)

Control and treated Cortese wines were judged significantly different by the tasters. In Nebbiolo, the analytical differences found were not determinant by a sensory point of view after 30 days from the bottling.

Conclusions

The tested **specific inactive dry yeast treatment enhanced the berry quality**:

- Average berry skin thickness increased on Chardonnay, Cortese, and Nebbiolo. *The trend found on Shiraz by Villango et al. (2015) was confirmed also on the tested varieties.*
- Berry anthocyanin content and extraction on Nebbiolo was found higher after a 7-days maceration, mainly for di-substituted forms. Also tannin extraction improved.
- Cortese wines evidenced enriched aroma traits, leading to different products.
- On Nebbiolo wines the tannin contents and red wine color were positively affected.

Barbera grapes in this trial presented an abnormal shift of the berry density distribution, the trial will be repeated. For other varieties, further experiments could be aimed to confirm these results.

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