

Ripening effect on the concentration of polyfunctional thiol precursors in 'Gewürztraminer'

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Summary

The effect of ripeness on the concentration of polyfunctional thiol precursors was investigated in 'Gewürztraminer' juices in two vintages characterised by very different climate conditions. An incremental trend of glutathionyl-3-mercaptohexan-1-ol and cysteinyl-3-mercaptohexan-1-ol during ripening was observed. The increase in the last phase of maturation was noticeable in both vintages. The concentration of 4-S-glutathionyl-4-methylpentan-2-one and 4-S-cysteinyl-4-methylpentan-2-one was confirmed to be low in 'Gewürztraminer', although the latter was found at quantifiable levels in a couple of samples. The management of the harvest date appears to be highly important in order to exploit the potential grapefruit-like note related to polyfunctional thiols of little aromatic 'Gewürztraminer' wines, particularly in case of challenging vintage years.

Key words: 'Gewürztraminer'; 3-mercaptohexan-1-ol; 4-mercapto-4-methylpentan-2-one; ripening.

Introduction

'Gewürztraminer' (GWT) is an international wine grape variety native to Tramin (South-Tyrol, Italy) well known for its richness in terpenes (MANDERY 1983, VERSINI 1985, MARAIS 1987, VERSINI *et al.* 1988, 1990 a, b, RAPP 1990, MARAIS and RAPP 1991). In the last decade, DUBOURDIEU and TOMINAGA (2009), ROLAND *et al.* (2010 a, b) and CONCEJERO *et al.* (2014) reported the presence of glutathionyl-3-mercaptohexan-1-ol (GSH-3MH) and cysteinyl-3-mercaptohexan-1-ol (Cys-3MH), precursors of the so-called varietal thiols also in this variety. A high concentration of these precursors in GWT pomace and leaves has also been observed (ROMÁN VILLEGAS *et al.* 2016), as well as a significant difference between GWT monoclonal grape musts (NICOLINI *et al.* 2019). GSH-3MH and Cys-3MH are precursors of 3MH and its acetate formed during fermentation, resulting in an increase of tropical, passion fruit and grapefruit-like notes that play a positive role in the aroma intensity and typicality of GWT wines (ROMÁN *et al.* 2018).

Previous works have already investigated the course of thiol precursors' concentration during ripening but showing different trends according to the varietal and geographic origin of the grapes (CAPONE *et al.* 2011, CERRETI *et al.* 2015 and 2017, KOBAYASHI *et al.* 2010, ROLAND *et al.* 2010c). This work investigates the effect of ripening on polyfunctional thiol precursors concentration and monoterpenoids in 'Gewürztraminer' juices obtained from non-clonal grapes grown in Trentino (North-East Italy).

Material and Methods

The survey was carried out in the 2014 and 2018 vintages. In 2014, grapes were hand-harvested from 8:00 a.m. to 10:00 a.m. from six vineyards located at an altitude of between 205 and 525 m a.s.l. in different locations across the whole Trentino growing area (supplemental material S1). Five different sampling times were carried out, one every 7 d, during the last month before harvest, which occurred between September the 10th and the 24th according to altitude. In 2018 instead, the work just focused on the most typical cultivation areas for DOC Gewürztraminer wine in Trentino and in South-Tyrol, sampling one week before and at technological harvest in twelve vineyards sited between 200 and 280 m a.s.l. in the municipalities of Roveré, San Michele, Salorno and Magré. The vineyards were trained with the usual systems in the region: pergola trentina and Guyot. Grape samples (about 5 kg per sample) were hand collected picking 35 basal bunches from central fruit canes of just as many vines all over the plot, avoiding plants with uneven vigour and production (FREGONI 2005). Each sample was destemmed (Ares 15, OMAC s.r.l., Corridonia, MC, Italy) and pressed twice (150 atm, 60 seconds) with a R70 hydraulic press (Meccanica Arturo Rossi, Verla di Giovo, TN, Italy). Juice (25 mL) was then supplemented with 25 mL methanol and kept at -20 °C until precursor analysis according to LARCHER *et al.* (2013).

Basic composition was assessed with a WineScan™ FT 120 Type 77310 (Foss Electric A/S Hillerød, Denmark) calibrated with the official methods (Organisation Internationale de la Vigne et du Vin 2013). Thiol precursors were quantified using an UHPLC Acquity equipped with a UPLC HSS T3 C18 column (100 mm × 2.1 mm, 18 µm particle size, Waters, Milford, MA, USA) and coupled to

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a Xevo TQ MS mass spectrometer (Waters Corporation, Milford, MA, USA) according to LARCHER *et al.* (2013), while terpenes were determined according to the GC-MSMS method proposed by PAOLINI *et al.* (2018). Statistical analysis was carried out with Statistica v. 9.0 (StatSoft Inc., Tusla, OK, USA).

Results and Discussion

In 2014 the mean pressing yield (w/w) per date of sampling, showed lower extraction yields (61 %) at the first point (-28 d) while the maximum values (73 %) were reached later. These values are consistent with the ripeness degree and the softening of grape berries during ripening as a consequence of significant modifications of specific polysaccharide components (NUNAN *et al.* 1998), and the activity of cell wall degradation-related enzymes (ISHIMA-

RU and KOBAYASHI 2002). In the 2018 samples, the mean pressing yield ranged between 70 % and 73 %, confirming the highest values obtained in 2014 for the latter samples. The average composition of juice (Tab. 1) indicates changes during ripening consistent with those expected. Climatically, the 2014 vintage in Trentino was characterized by low temperatures and substantial rainfall after veraison (Tab. 2), that resulted in a slow ripening, even stalled in some plots due to either dilution phenomena or the inability of the plant to mature further (suppl. material S1). In this situation, the risk of *Botrytis cinerea* attack led to an early harvest, with grapes averaging about 4 °Brix less than those typical for the region, while the favourable 2018 climatic conditions (Tab. 2) allowed a higher and more typical level of total soluble solids (TSS) and the highest grape production since 2000 (BOTTURA 2019). The distribution of the ripeness parameters of juice regarding the period 2007 and 2019 are supplied as suppl. material S2. The Figure

Table 1

Mean values, standard deviations (S.D.) and statistical significance regarding the basic composition of the juices (Legenda: YAN = yeast assimilable nitrogen; different letters associated with each time to harvest indicate significant differences at Tukey HSD test, $p < 0.05$)

	2014														
	28 days			21 days			14 days			7 days			Harvest		
	Mean (N = 6)	SD	sign	Mean (N = 6)	SD	sign	Mean (N = 6)	SD	sign	Mean (N = 6)	SD	sign	Mean (N = 6)	SD	sign
TSS (°Brix)	14.4	2.2	d	15.6	2.0	cd	16.6	1.5	bc	17.6	1.8	ab	18.7	1.4	a
pH	2.85	0.16	d	2.95	0.13	c	3.05	0.15	b	3.13	0.18	b	3.25	0.14	a
Titrateable acidity (g·L ⁻¹)	15.9	4.5	a	13.2	3.3	b	11.0	2.5	bc	10.4	2.1	c	8.5	1.7	c
Tartaric acid (g·L ⁻¹)	7.9	0.6	a	7.4	0.5	b	7.0	0.5	c	6.9	0.4	c	6.7	0.5	c
Malic acid (g·L ⁻¹)	10.7	3.5	a	8.3	3.0	b	6.6	2.0	bc	6.0	1.7	c	4.5	1.2	c
Potassium (mg·L ⁻¹)	1580	309	bc	1407	226	c	1631	191	bc	1664	283	b	1937	214	a
YAN (mg·L ⁻¹)	220	57	a	222	75	a	197	50	a	173	68	a	178	38	a

	2018					
	7 days			Harvest		
	Mean (N = 12)	SD	sign	Mean (N = 12)	SD	sign
TSS (°Brix)	21.9	0.7	n.s.	22.7	1.4	n.s.
pH	3.32	0.18	a	3.50	0.11	a
Titrateable acidity (g·L ⁻¹)	3.6	0.4	n.s.	4.1	0.8	n.s.
Tartaric acid (g·L ⁻¹)	5.0	0.7	b	6.0	0.5	a
Malic acid (g·L ⁻¹)	1.8	0.5	n.s.	1.6	0.3	n.s.
Potassium (mg·L ⁻¹)	1521	326	b	1941	211	a
YAN (mg·L ⁻¹)	112	59	n.s.	103	34	n.s.

Table 2

Climatic indexes recorded at the weather station of San Michele all'Adige in 2014 and 2018

Year	Month	Rainfall (mm)	Mean Relative humidity (%)	Solar radiation (MJ/m ²)	Insolation (sec)	Mean Temperature (°C)
2014	June	145.4	64.2	699.35	1079040	20.47
	July	192.2	74.4	608.21	972240	20.62
	August	93.2	75.5	501.41	817080	19.66
	Sptember	26.6	73.7	442.04	757800	17.83
	October	82.2	79.4	297.24	608520	13.98
2018	June	76.4	63.6	701.93	1052460	21.26
	July	145.0	67.0	682.11	1068360	22.84
	August	93.8	64.7	609.87	998040	23.06
	Sptember	26.0	70.1	467.04	848880	18.87
	October	273.2	75.1	284.29	587340	13.35

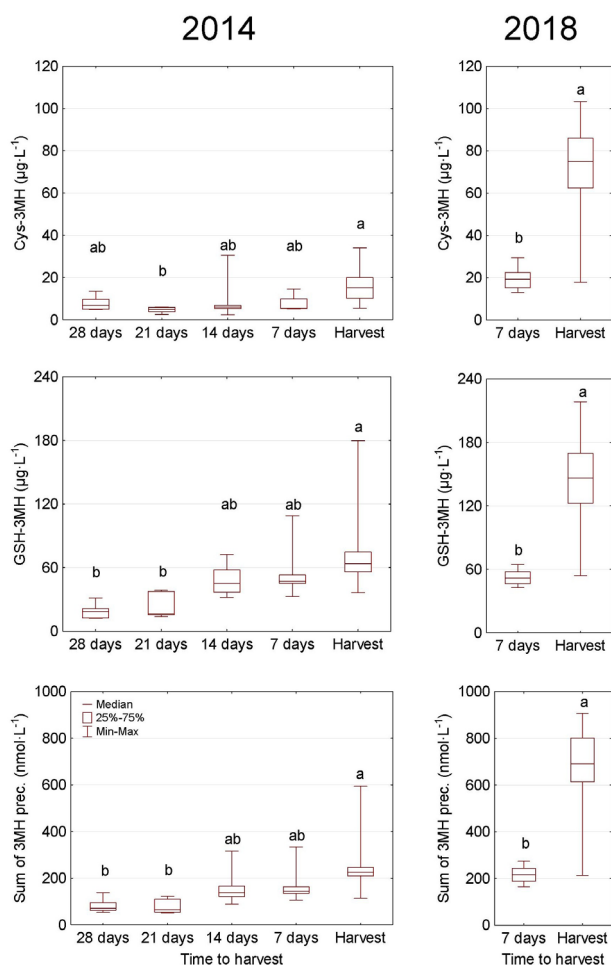


Figure: Concentration changes versus time to harvest of glutathionyl-3-mercaptohexan-1-ol (GSH-3MH), cysteinyl-3-mercaptohexan-1-ol (Cys-3MH) and relative sum in 'Gewürztraminer' grape juices of 2 vintage years. (Legenda: different letters associated with each time to harvest indicate significant differences at Tukey HSD test, $p < 0.05$).

shows the evolution of the concentration for both 3MH precursors in juice during 2014 and 2018. The accumulation trend versus time is clear in both vintages, and particularly remarkable in the imminence of the technological harvest in 2018 vintage, with a mean concentration level significantly higher than 7 d before (ANOVA main effects: plot, sampling time; Tukey HSD test; $p < 0.05$). The correlation between TSS and the sum of total precursors is shown in suppl. material S3. These results - observed for the first time in GWT, to the best of our knowledge - confirm the increasing trend found for polyfunctional thiol precursors in other varieties, *i.e.* 'Sauvignon Blanc' (ROLAND *et al.* 2010c, CAPONE *et al.* 2011), and 'Grechetto' (CERRETTI *et al.* 2017), but do not completely agree with those found for 'Koshu' (KOBAYASHI *et al.* 2010) where 3MH precursors peaked before the complete ripeness of grapes, while CERRETTI *et al.* (2015) found different behaviours depending on the variety and the precursors under study. In terms of the aroma compounds, PINU *et al.* (2012) and CHEN *et al.* (2019) did not observe a clear relationship between the free thiols in wine and the corresponding precursors in juice, while PINE-

AU *et al.* (2011) reported that the higher maturity of grapes, the higher the concentration of thiols in the corresponding wines. The lack of the relationship between precursors and free thiols is reported taking into account different grape lots and could be related to the matrix effect (PINU *et al.* 2014; 2019) and the number of factors affecting the formation and liberation of the aroma molecules during winemaking (SCHNEIDER *et al.* 2006; MASNEUF-POMARÉDE *et al.* 2006). The concentration of total precursors measured at harvest is consistent with the mean value ($n = 5$) found by ROLAND *et al.* (2010b) for GWT (285 nM) but not in terms of distribution between precursors, since these authors reported a higher concentration of Cys-3MH. Several papers regarding GWT (ROMÁN *et al.* 2018; NICOLINI *et al.* 2019) and many works for other varieties (e.g. CAPONE *et al.* 2010; PINU *et al.* 2012) have reported instead a higher concentration of the glutathionylated precursor in juice.

Despite the very different climate conditions of 2014 and 2018, the final concentration level of the 3MH precursors in 2014 was quite similar to that of the first sampling time in 2018, showing these lasts samples 3 °Brix more on average (Tab. 1).

Neither 4-*S*-glutathionyl-4-methylpentan-2-one (GSH-4MMP) nor 4-*S*-cysteinyl-4-methylpentan-2-one (Cys-4MMP) were found over the detection limit ($0.5 \mu\text{g}\cdot\text{L}^{-1}$) in any sample of the 2014 vintage, however, Cys-4MMP was found in two samples from 2018 ($2 \mu\text{g}\cdot\text{L}^{-1}$). These compounds had already been reported in GWT by ROLAND *et al.* (2010b), at concentrations that did not exceed $0.8 \mu\text{g}\cdot\text{L}^{-1}$ and $0.2 \mu\text{g}\cdot\text{L}^{-1}$, respectively, very close to their limit of detection.

In 2018, the main GWT terpenes (geraniol, citronellol, nerol and linalool in free and bound form) were also measured in juice. As expected, the sum of these compounds increased at harvest, although differences were not statistically significant between the first and the second sampling time, averaging $337 \pm 130 \mu\text{g}\cdot\text{L}^{-1}$ and $414 \pm 191 \mu\text{g}\cdot\text{L}^{-1}$, respectively. These concentrations are quite low, both compared to previous literature (VERSINI *et al.* 1990 a, b) and our unpublished data regarding Trentino GWT juices (2012: $3348 \pm 1071 \mu\text{g}\cdot\text{L}^{-1}$, $n = 25$; 2013: $3905 \pm 1505 \mu\text{g}\cdot\text{L}^{-1}$, $n = 19$), but non unusual in the region (NICOLINI *et al.* 2013; MALOSSINI *et al.* 2006) and reasonably affected by the abundant grape production of the 2018 vintage.

Conclusions

This work is basically a survey of the polyfunctional thiol precursors' concentration in 'Gewürztraminer' grapes cultivated in Trentino, similar to those already performed concerning other wine grape varieties. However, it allows the authors to make some final observations. The material increase in concentration during the last stages of ripening highlights the importance of choosing the harvest date, particularly in the case of suboptimal climatic conditions or elevated grape productions. In such situations, usually characterised by low terpene concentrations in grapes, these precursors can be exploited to increase the grape-

fruit-like notes and typicality of GWT wines by applying adequate winemaking, e.g. using yeast strains having β -lyase, and finishing blending.

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