

## Varietal thiols in Pošip wines: Effect of clone selection

### Abstract

Varietal thiols are extremely volatile and desirable wine aroma compounds that contribute to tropical fruit notes. Their concentration depends on numerous factors, from agricultural practices to winemaking procedures and storage conditions. Among them, grape variety is one of the most important factors, and diversity among clones of a single variety has also been highlighted. The aim of this work was to determine the varietal thiols in wines produced from different clones of Pošip (*Vitis vinifera* L.) grape. The results showed significant differences between clones, with the highest concentrations of varietal thiols detected in wine produced from clone POŠ-124. The results suggest clone selection as an important step in diversifying the styles of produced wines.

**Keywords:** varietal thiols, Pošip, clone selection, wine aroma

### Introduction

Varietal thiols are highly odoriferous molecules present in wine at very low concentrations (Roland et al., 2011; Tominaga et al., 2000). Three thiols are important for wine aroma: 4-sulfanyl-4-methylpentan-2-one (4MSP), responsible for boxwood and blackcurrant bud notes, 3-sulfanylhexas-1-ol (3SH), responsible for grapefruit and passion fruit notes; and 3-sulfanylhetyl acetate (3SHA), responsible for passion fruit and grape fruit notes (Darriet et al., 1995; Tominaga et al., 1998). They are practically absent in grapes and musts because they are released during alcoholic fermentation by the enzyme activity of certain yeast strains from cysteinylated and glutathionylated precursors (Capone et al., 2018). Numerous factors, from viticultural practices to bottle storage conditions, influence the final concentration of these compounds in wine. From a winemaking perspective, the concentration of varietal thiols in wines is highly dependent on grape composition and applied winemaking practices (Roland et al., 2011). Different grape varieties result in wines with different concentrations of these compounds (Capone et al., 2015; Capone et al., 2018; Tominaga et al., 2000; Carlin et al., 2022), with higher concentrations typically found in Sauvignon blanc wines (Capone et al., 2015). Apart from the obvious varietal differences in thiol production, concentrations of thiol precursors in grape juice as well as free thiols in wines may also vary at the clone level. For example, Capone et al. (2011) demonstrated that concentrations of thiol precursors differed significantly among five Sauvignon blanc clones. The same authors also found differences in 3SH concentrations between different grape varieties, but these differences were not consistent with the trends estimated for precursor concentrations, implying that higher precursor concentrations do not necessarily lead to wines with higher thiol concentrations. In addition, Šuklje et al. (2016) demonstrated that clone selection significantly affected the concentrations of 3SH and 3SHA in Sauvignon blanc wines. Recently, Chen et al. (2018) found that the (S):(R) ratio of chiral 3SH and 3SHA can also be affected by clone type. Generally, the clonal selection is performed to improve vine health and

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production traits through vegetative propagation. This process takes advantage of the genetic variability within the grape variety and focuses on selecting the most promising genotypes for specific quantitative traits such as yield, aroma complexity, acidity, anthocyanins, etc., while always maintaining diversity within grapevine varieties (Lemos et al., 2020).

## Material and methods

### Chemicals

Ethanol was HPLC grade and purchased from J.T. Baker (Deventer, Netherlands), while ethyl acetate, hydrochloric acid (37%), and sodium sulfate anhydrous were purchased from Carlo Erba (Val de Reuil, Spain). Sodium acetate trihydrate was purchased from Gram-mol (Zagreb, Croatia) and cysteine hydrochloride hydrate, dichloromethane, *p*-hydroxymercurybenzoate (*p*-HMB), 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), Dowex exchange resin and butylated hydroxyanisole (BHA) were purchased from Sigma Aldrich (St. Louis, USA). In addition, 4-methyl-4-sulfanylpentan-2-one and 3-sulfanylhexyl acetate were purchased from Alfa Aesar (Ward Hill, USA), while 3-sulfanylhexanol and 4-methoxy-2-methyl-2-mercaptobutane were purchased from Endeavour Chemicals (Northamptonshire, UK).

### Plant material

Four different clones of Pošip vines were studied: POŠ-022, POŠ-068, POŠ-089, and POŠ-124, all selected from local vineyards on the Island of Korčula, Croatia, grafted onto the Kober 5BB rootstock, and grown on the experimental vineyard of the Faculty of Agriculture in Baštica, sub-region Northern Dalmatia, Croatia. The vineyard was planted in 2007, on the total area of 0.7 ha. Soil is a fertile deep sandy loam, and the viticultural climatic zone classified as C<sub>3</sub> with yearly insolation up to 2500 h, and annual precipitations of around 730–1050 mm. Plants were grown on a vertical trellis with 2.2 m between the rows and 1.1 m between plants and pruned according to the single Cordon training system, leaving cca 10 buds per vine (5 spurs with 2 buds each).

POŠ-022 was selected from the location Njivice (Smokvica, Korčula) and is characterized by lower fertility potential, medium bunch size and bunch density, lower sugar content, and significantly higher total acidity.

POŠ-068 is also selected from the location Njivice site (Smokvica, Korčula). It is characterized by very high fertility potential and stable yield, higher total acidity, average or slightly higher sugar content, higher aromatic compounds content, higher berry size, and dense bunch.

POŠ-089 comes from the location Dračevka (Smokvica, Korčula) and is characterized by very small berries (50% smaller than the average of the Pošip variety), looser and lower weight bunches; lower yield, average sugar content, lower total acidity and higher content of aromatic compounds.

POŠ-124 is selected from the location Mindel (Čara, Korčula). It is characterized by higher yields, higher and medium dense bunches; the highest sugar content among the selected clones, lower total acidity, and average content of aromatic compounds.

### Wines

The wines were produced from healthy grapes of *Vitis vinifera* L. Pošip clones grown in Dalmatia (Baštica, sub-region of Northern Dalmatia) in the 2020 vintage. The grapes were destemmed, crushed and pressed, and the must obtained was clarified for 24 hours. After racking, alcoholic fermentation began by inoculation with Lalvin Sauvvy™ yeasts and the addition of Go-Ferm Protect™ and Stimula Sauvignon Blanc™ fermentation nutrient (all purchased from Lallemmand, Germany). Fermaid E™ nutrient (Lallemmand, Germany) was added to the ferment-

ing must to ensure the completion of alcoholic fermentation. After alcoholic fermentation was finished, wines were racked and the samples were frozen at  $-20\text{ }^{\circ}\text{C}$  until laboratory analyses. Conventional wine analyses of these wines were performed according to official OIV methods using standard equipment (OIV, 2009), and the physicochemical parameters of the wines produced are listed in Table 1.

**Table 1.** Physicochemical parameters of produced Pošip wines  
**Tablica 1.** Fizikalno-kemijski parametri proizvedenih vina Pošip

Parameter	POŠ-022	POŠ-068	POŠ-089	POŠ-124
Alcohol/ Alkohol (vol %)	9.7	9.0	10.7	9.5
Total extract/ Ukupni ekstrakt ( $\text{g L}^{-1}$ )	19.8	22.2	18.8	20.6
Reducing sugars/ Reducirajući šećeri ( $\text{g L}^{-1}$ )	< 1.0	< 1.0	< 1.0	< 1.0
pH	3.03	3.03	3.22	3.10
Total acidity/ Ukupna kiselost ( $\text{g L}^{-1}$ as tartaric acid/ kao vinska kiselina)	7.7	8.6	6.4	7.3
Volatile acidity/ Hlapljiva kiselost ( $\text{g L}^{-1}$ as acetic acid/ kao octena kiselina)	0.37	0.32	0.41	0.31
Free $\text{SO}_2$ / Slobodni $\text{SO}_2$ ( $\text{mg L}^{-1}$ )	34	30	38	3
Total $\text{SO}_2$ / Ukupni $\text{SO}_2$ ( $\text{mg L}^{-1}$ )	101	89	121	104

### Analysis of varietal thiols

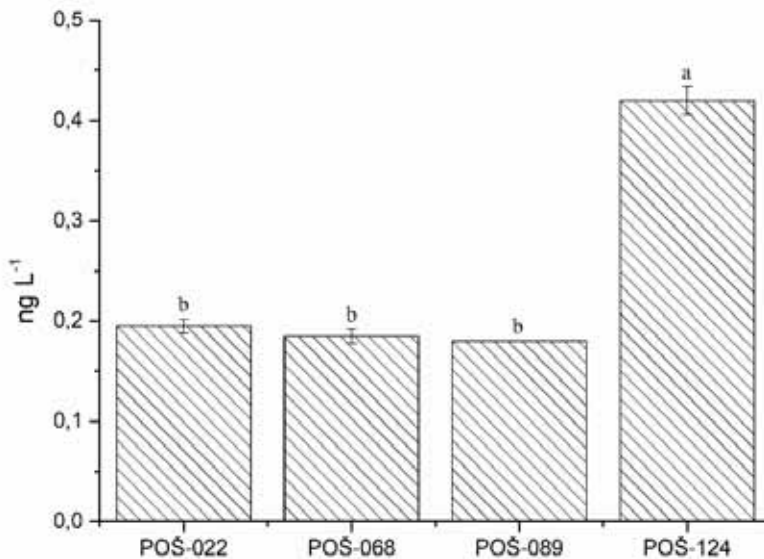
The three thiols (4MSP, 3SH, and 3SHA) were extracted following a procedure developed by Tominaga et al. (1998), with slight modifications to the original protocol described in detail by Tomašević et al. (2017). Briefly, *p*-HMB was added to the wine with internal standard and the mixture was percolated onto a strongly basic anion exchange column (Dowex 1x2, chloride form). After loading the sample, the column was washed with sodium acetate buffer. Thiols were released by percolating a cysteine hydrochloride monohydrate solution through the column. Then, 0.5 mL of ethyl acetate was added to the eluted sample and extracted with 15 and 10 mL of dichloromethane in two consecutive steps. The organic phases were collected and concentrated under vacuum and nitrogen to about 30  $\mu\text{L}$ , then GC/MS analysis was performed. The analyzes were performed in duplicate. Thiols were identified and quantified using an Agilent 6890 series gas chromatograph (Santa Clara, USA) coupled to an Agilent 5973 inert mass selective detector and an automatic injector (7683B series injector). Separation was performed with a BP20 capillary column (50 m  $\times$  220 mm, 0.25 mm) (SGE Analytical Science, Victoria, Australia). Helium 5.0 (Messer Croatia, Croatia) was used as the vector gas with a constant flow rate of 1.2 mL/min. 2 mL of sample was injected using an automatic sampler in splitless mode. The injector was heated to  $250\text{ }^{\circ}\text{C}$ , while the oven temperature was programmed at  $35\text{ }^{\circ}\text{C}$  for 10 min and increased to  $230\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$ . The acquisition was performed in Selective Ion Monitoring mode (SIM) with selected ions (*m/z*): 134 and 75 for the internal standard; 132, 75, 99 for 4MSP; 134, 100, 101 for 3SH; and 116, 101 for 3SHA. Volatile compounds were identified by GC/MS using Enhanced Chemstation software (Agilent Technologies, Palo Alto, USA). The calibration parameters, including retention time, retention index, target and qualifying ions, concentration range, and linear equation, for all analyzed compounds were presented in the previously mentioned article (Tomašević et al., 2017).

### Statistical analysis

The statistical data analysis was carried out using Statistica V.10 software (Statsoft Inc., Tulsa, USA). The analysis of Variance (ANOVA) was performed on all independent variables of varietal thiols. In order to compare variable means and to examine which wines were different, Tukey's HSD test was used as a comparison test when samples were significantly different after ANOVA ( $p < 0.05$ ) of analyzed compounds.

### Results and discussion

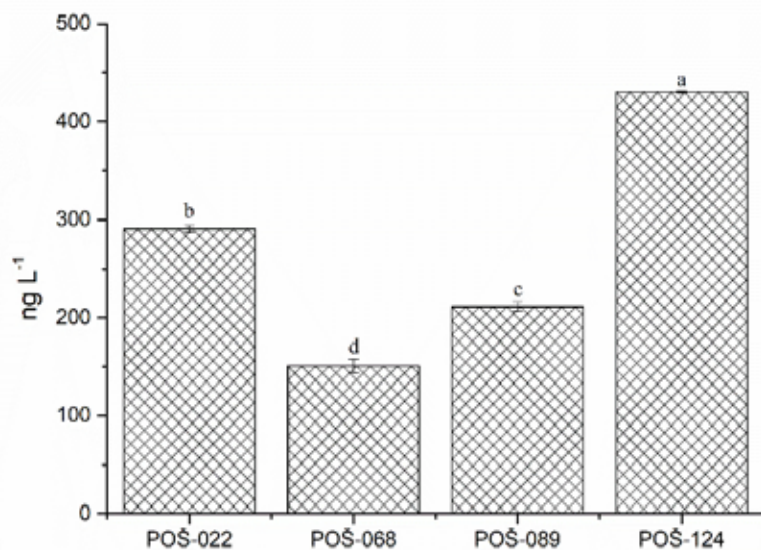
Three thiols, namely 4-sulfanyl-4-methylpentan-2-one (4MSP), 3-sulfanylhhexanol (3SH), and 3-sulfanylhhexyl acetate (3SHA) were identified and quantified in the analyzed Pošip wines and the results are shown in Figures 1-3. Among them, 4MSP is determined in the lowest concentrations, in the range of 0.18-0.42 ng L<sup>-1</sup> (Figure 1). In general, 4MSP is reminiscent of boxwood, blackcurrant bud, and cat urine (Darriet et al., 1995; Howell et al., 2004). Clone POŠ-124 is characterized by significantly higher concentrations of 4MSP, more than double those in the other samples analyzed. Nevertheless, these concentrations are below the sensory threshold of 0.8 ng L<sup>-1</sup> (Tominaga et al., 1998), which means that this compound has no influence on the varietal aroma of the Pošip wines studied. However, at lower concentrations, it has been shown to contribute to the desirable fresh character of the wines (Escudero et al., 2004). 4MSP was first identified in Sauvignon blanc wines with concentrations ranging from 0 to 120 ng L<sup>-1</sup> (Ribéreau-Gayon et al., 2006). However, other grape varieties such as Scheurebe, Macabeo, Gewurztraminer, Riesling, Muscat, Colombard, Petit Manseng, and Tokaj have also been reported to contain detectable concentrations of 4MSP (Roland et al., 2011). In addition, a wide range of concentrations (0 - 48 ng L<sup>-1</sup>) was detected in Pošip wines (Tomašević et al., 2017; Tomašević et al., 2019), suggesting its importance for the varietal character of wine from this grape variety.



**Figure 1.** Concentration of 4MSP (ng L<sup>-1</sup>) in analyzed Pošip wines  
**Slika 1.** Koncentracije 4MSP (ng L<sup>-1</sup>) u analiziranim vinima Pošip

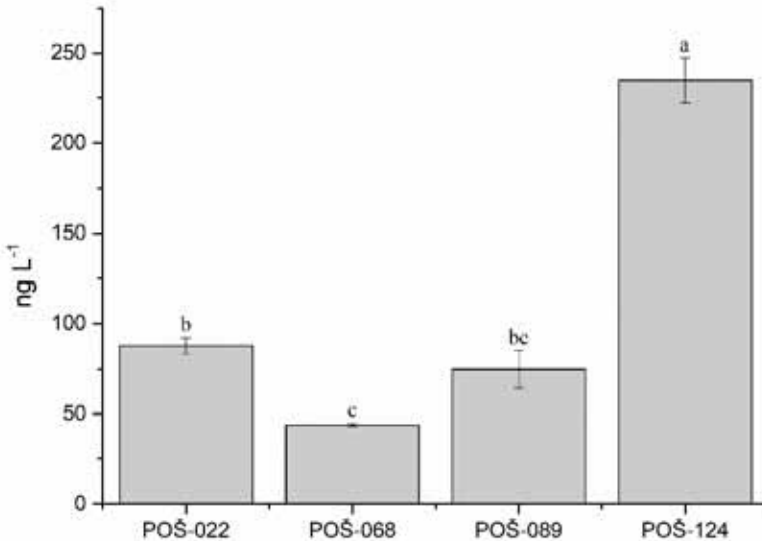
The concentrations of 3SH in the studied Pošip wines are shown in Figure 2. A significantly higher concentration of 234.97 ng L<sup>-1</sup> was detected in the wine of clone POŠ-124, followed by clones 022, 089, and finally clone 068, where the lowest concentration was detected (43.64 ng L<sup>-1</sup>). 3SH is the most abundant thiol in wines, its concentration ranging from below the sensory threshold (60 ng L<sup>-1</sup>) to 12822 ng L<sup>-1</sup> (Tominaga et al., 1998). This thiol has been detected in a wide range of varietal wines such as Sauvignon Blanc, Petit and Gros Manseng, Semillon, Petite Arvine, Melon B. and Bacchus, Verdejo, and Kosu (Roland et al., 2011), as well as in Pošip wines (Tomašević et al., 2017; Tomašević et al., 2019). As for the sensory effects of this thiol on the studied Pošip wines, it can be concluded that 3SH contributes to the varietal character of all studied wines with grapefruit and passion fruit nuances (Tominaga et al., 1998), as the detected concentrations were above the sensory threshold.

The concentrations of 3SHA are shown in Figure 3, and similar trends to 3SH were observed: the highest concentration was detected in wine POŠ-124, and the lowest was in sample POŠ-068. The aroma of 3SH is described as boxwood, passion fruit, and grapefruit, depending on the concentration with a sensory threshold of 4.2 ng L<sup>-1</sup> (Tominaga et al., 1998), and Sauvignon blanc wines are characterized by higher concentrations (up to 2500 ng L<sup>-1</sup>) (Tominaga et al., 2000).



**Figure 2.** Concentration of 3SH (ng L<sup>-1</sup>) in analyzed Pošip wines  
**Slika 2.** Koncentracije 3SH (ng L<sup>-1</sup>) u analiziranim vinima Pošip

In general, 3SHA is formed by the esterification of 3SH with acetic acid during fermentation, and its final content depends on many factors, including the balance between the activities of the enzymes promoting esterification and those involved in hydrolysis, but also on the yeast strain used (Swiegers et al., 2006). This ester is formed above equilibrium during fermentation and is hydrolyzed to 3SH relatively quickly during storage. Based on the results obtained and the sensory threshold, it can be concluded that 3SHA also contributes to the varietal aroma of all Pošip wines studied.



**Figure 3.** Concentration of 3SHA (ng L<sup>-1</sup>) in analyzed Pošip wines  
**Slika 3.** Koncentracije 3SHA (ng L<sup>-1</sup>) u analiziranim vinima Pošip

This work represents the first evidence of the influence of the clone on some of the aroma compounds in Pošip wine. Previous research regarding the other grape varieties has also reported differences between wines produced from different clones. For example, Šuklje et al. (2016) found significant differences in the concentration of 3SH and 3SHA in wines produced from two different Sauvignon blanc clones. These authors also highlighted defoliation as one of the processes leading to higher thiol content in wines. In addition, Capone et al. (2011) studied the effects of clones on thiol precursors as well as volatile thiol concentrations during grape ripening and fermentation of Sauvignon blanc wines from Adelaide Hills. They found that precursor concentrations differed significantly among the five clones studied. In addition, the concentration of 3SH in wines from different clones also varied, but the trends were not the same as those found for the precursors. Chen et al. (2018) investigated the influence of yeast strain, commercial enzymes, and nutrient additions on the concentrations of enantiomers of 3SH and 3SHA in wines from five different clones of Sauvignon blanc. The results showed the significant influences of grapes from different clones during fermentation, beyond the effect of yeast or additive that were tested.

Moreover, the increase in the concentration of thiol precursors is usually directly correlated with the stage of grape ripeness (Peyrot des Gachons et al., 2000; Roland et al., 2010; Cerreti et al., 2015). As for the Pošip grapes studied, it can be noted that the ripeness of the clones at harvest was similar (Table 1), revealing the clonal influence on thiols in the final wine. Nevertheless, the concentrations of precursors and thus of varietal thiols in Pošip wine could possibly be increased if the grapes were harvested at their full maturity. The grapes used in this study yielded a relatively low alcohol content (Table 1) for wines from this grape variety, considering previous studies reporting values higher than 13 vol% (Tomašević et al., 2017; Jagatić Korenika et al., 2019; Tomašević et al., 2019; Radeka et al., 2022).

Finally, a possible reason for the differences between the studied samples could be the

origin of the clones: as indicated in the description of the vines in the Material and Methods section, clone POŠ-124 originated from a different microlocation (Čara) compared to the other clones studied (Smokvica). The differences in varietal thiols and their precursors in relation to different vineyards or different clones in the same vineyard have been reported previously (Capone et al., 2011; Šuklje et al., 2016; Chen et al., 2018). However, to the best of our knowledge, clone origin as a factor of diversity has not yet been investigated. However, before a general conclusion can be drawn regarding clone origin, this hypothesis needs to be further investigated and more experiments need to be conducted in the future.

## Conclusion

Based on obtained data, it can be concluded that the clone of the grape variety Pošip significantly influences the final concentration of varietal thiols in the produced wine. Herein, the clone POŠ-124 is especially emphasized, with the highest concentrations of investigated compounds found in wine produced from its grape. This study provides the first insight into the differences in aroma composition between clones of the Pošip grape and gives a future perspective in terms of modulation of wine aroma by selecting specific clones. However, further research needs to be conducted on more grape samples and in different vintages, as well as in terms of overall aroma composition and wine quality.

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Izvorni znanstveni rad

## Sortni tioli u vinu Pošip: Utjecaj klonske selekcije

### Sažetak

Sortni tioli su izrazito mirisni i poželjni spojevi arome koji doprinose mirisu vina aromama tropskog voća. Njihova koncentracija ovisi o brojnim čimbenicima, od ampelotehničkih i enoloških postupaka do uvjeta skladištenja. Sorta grožđa je jedan od najvažnijih čimbenika, pri čemu su utvrđene razlike i između samih klonova pojedine sorte. Cilj ovog rada bio je istražiti utjecaj klonova sorte Pošip na koncentraciju sortnih tiola u vinima. Rezultati su pokazali značajne razlike između klonova pri čemu su najviše koncentracije utvrđene u vinu proizvedenom od klona POŠ-124. Dobiveni rezultati ističu klonsku selekciju kao važan korak u proizvodnji vina različitih stilova.

**Ključne riječi:** sortni tioli, Pošip, klonska selekcija, aroma vina