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Evaluation of glutathione content in white grape varieties

J. Martínez, S. García and L. Alti

Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja), Logroño, Spain

Summary

The aim of this work was to evaluate the content of glutathione in the grape, must and wine of nine white varieties authorized in the Qualified Designation of Origin Rioja (Spain): 'Chardonnay', 'Garnacha Blanca', 'Malvasía', 'Maturana Blanca', 'Tempranillo Blanco', 'Turruntés', 'Sauvignon Blanc', 'Verdejo' and 'Viura'. The results obtained showed important varietal differences in the concentration of GSH. 'Sauvignon' and 'Tempranillo Blanco' presented high levels of this compound in grape, must and wine. 'Verdejo' and 'Turruntés' registered high values in grape, which decreased notably in must and wine. In general, the GSH content decreased in musts due to oxidative processes. In 'Chardonnay', 'Garnacha Blanca', 'Maturana Blanca' and 'Viura' an increase was observed in wines, which could be caused by the inoculated yeast. The 'Malvasía' and 'Maturana Blanca' varieties registered the lowest level of this compound. The ratio of hydroxycinnamic acids to glutathione, indicator of the susceptibility of oxidation of musts, showed significant varietal differences, was lower in 'Sauvignon Blanc' and higher in 'Garnacha Blanca'.

K e y w o r d s : glutathione; hydroxicinnamic acids; must; wine; white varieties.

Introduction

Glutathione (L-glutamyl-L-cysteinyl-glycine) is a tripeptide that is mainly found in reduced form (GSH) in grape, must and wine. It plays a relevant role in the prevention of enzymatic oxidations (Kritzinger *et al.* 2013a). In grape, it was quantified for the first time by Cheynier *et al.* (1989), and its content is influenced by many factors (variety, vintage, cultural practices, nitrogen nutrition, etc.). It generates during the maturation of the grape, increasing significantly after veraison (Adams *et al.* 1993). Its accumulation in the berry is parallel to that of soluble solids until it reaches 16 °Brix, and from this moment it remains stable. Also, the glutathione present in the grape is closely related to the level of nitrogen nutrition of the vineyard, with lower levels having been observed in the case of vineyards with nitrogen deficiency (Choné *et al.* 2006). In musts and wines, it undergoes modifications due to oxygen exposure, tyrosinase activity, yeast strain, etc. (Du Toit *et al.* 2007, Gabrielli *et al.* 2017). This compound is also involved in the stress response of *Saccharomyces cerevisiae* and it can be absorbed or excreted during alcoholic fermentation (Mikaye *et al.* 1998). The content of glutathione after fermentation may depend on the strain of yeast used (Lavigne *et al.* 2007), although the differences observed in some studies were insignificant (Fracasseti *et al.* 2010). In musts, glutathione reacts with caftaric acid, generating a colorless compound called GRP (2-S-glutathionylcaphatic acid) which prevents subsequent enzymatic browning reactions of said acid (Singleton *et al.* 1985). These reactions can lead to the deterioration of the final quality of musts and wines. Additionally, GSH exerts a protective effect on wine aroma, avoiding the oxidation of certain volatile compounds with a high sensory impact and inhibiting the formation of atypical aging characteristics (Kritzinger *et al.* 2013a). This compound also manifests beneficial effects on human health due to its antioxidant, immune and detoxifying activity, because it is considered a powerful, versatile and important self-generating defense molecule. In recent years, glutathione has been the subject of numerous studies, although so far many aspects of its behavior and evolution in musts and wines are unknown.

The aim of this work was to evaluate the content of glutathione in the grape, must and wine of nine white varieties of the Qualified Designation of Origin Rioja (Spain).

Material and Methods

P l a n t m a t e r i a l : The experiment was carried out in an experimental vineyard located in La Grajera (42°26' 18''N, 2°30'4.6''W), Logroño (Spain), planted in 2007 in a 2.90 x 1.10 m framework, trellis system, double Royat cordon training system (2.90 x 1.10 m) and a statistical design of random blocks with four repetitions of thirty vines per variety. The study was carried out during the 2017 vintage on the nine white varieties (*Vitis vinifera* L.) authorized in the Qualified Designation of Origin Rioja (Spain): 'Chardonnay', 'Garnacha Blanca', 'Malvasía', 'Maturana Blanca', 'Tempranillo Blanco', 'Turruntés',' Sauvignon Blanc', 'Verdejo' and 'Viura'.

H a r v e s t a n d w i n e m a k i n g : The grapes were hand harvested at their optimal ripening stage and in good sanitary condition. The wines of each variety were vinified in triplicate (80 kg of grape per repetition) in the

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Correspondence to: Dr. J. Martínez, Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja), Carretera Burgos Km. 6, 26007 Logroño, La Rioja, Spain. E-mail: jmartinezg@larioja.org

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ICVV experimental winery. The must was extracted by crushing and gentle pressing in a pneumatic press. After adding 60 mg·L^{-1} of SO_2 and pectolytic enzymes (1 g·hL^{-1}, Lafazym CL, Laffort S.L.), the juice was clarified at 10 °C for 20 h. The clean must was transferred to 50 L tanks, and inoculated with commercial yeasts (Zymaflore X16, Laffort S.L.). Fermentations were carried out at 18 °C. At the end of the fermentation, the wines were racked, 40 mg·L^{-1} of SO_2 was added and they were then stored at 5 °C for one month to favor their stabilization.

Sample preparation and analysis of GSH: Ten clusters of grapes from each sample were collected in the vineyard before the harvest. A total of 150 intact berries were randomly selected, cut from the cluster with scissors at the pedicel level, and frozen at -20 °C for GSH analysis. The frozen berries were crushed in a ThermomixR TM5 for 10 sec and then saved frozen. Five grams of the frozen crushed grape were weighed and rinsed, placed in a 20 mL tube, with 15 mL 0.1 N HCl and 0.01 % EDTA, and then kept for two hours with horizontal agitation at room temperature. The extract was centrifuged at 5000 x g for 10 min at 5 °C. Then, 5 mL of the supernatant was taken for the determination of GSH by HPLC. The analysis was carried out by HPLC in an Agilent 1100 HPLC equipment, by automatic derivatization in precolumn with o-phthaladehyde (OPA). The separation was conducted in a Hypersil ODS column (250 x 4.0 mm 5µm). The eluents used were A: 75 mM sodium acetate buffer, 0.018 % triethylamine (pH 6.9) + 0.3 % tetrahydrofuran and B: methanol/acetonitrile/water (45/45/10). Detection was done by fluorescence (excitation = 340 nm and emission = 450 nm) (Martínez et al. 2014).

Analysis of hydroxycinnamic acids: In musts and wines, the analysis of hydroxycinnamic acids (caffeic, caftaric, coumaric, coutaric, ferulic and fertaric acids) was performed in an Agilent 1100 HPLC, equipped with a DAD photodiode detector. The samples, after filtration (0,45 µm, polyester membrane), were injected (30 µL) in a reversed-phase column Zorbax Eclipse Plus C18 (300 mm x 150 mm x 3.9 µm), thermostated at 25 °C. Solvent A was water/acetic acid (98:2, v/v), solvent B was water/acetonitrile/acetic acid (78:20:2, v/v/ v) and solvent C was methanol. A constant flow of 0.900 mL·min^{-1} was used according to the following program: 0 to 80 % of B, from 0 to 65 min; 80 % of B from 65 to 85 min; 100 % C from 86 to 90 min. The detection was carried out at 310 and 325 nm.

Statistical analysis: The statistical analysis of results was performed using the SPSS program version 22.0 for Windows. Analysis of variance (ANOVA) was applied, and Tukey test was applied for mean comparison, at $p \leq 0.05$.

Results and Discussion

The glutathione (GSH) content of the grape in the white varieties studied showed significant varietal differences (Figure). The values recorded fall within a range between 5.33 and 19.2 µg·g^{-1}. The highest concentration was obtained in the 'Verdejo', Sauvignon Blanc' and 'Tempranillo Blanco' varieties, although without significant differences in comparison to 'Chardonnay', 'Turruntés' and 'Viura', while the lowest levels corresponded to 'Malvasía'. In the case of the 'Sauvignon Blanc' variety, Cheynier et al. (1989) indicated a higher GSH content than in other varieties with values of up to 114 mg·kg^{-1} in grapes. They also observed high concentrations in 'Chardonnay' (75 mg·kg^{-1}) and lower in 'Macabeo', synonym of 'Viura', (57.9 mg·kg^{-1}) and in 'Garnacha Blanca' (58 mg·kg^{-1}). In other of the varieties studied in this work, there are no bibliographical references on the GSH content, since they are minor cultivated varieties, and the content of GSH in grapes has been quantified in this work for the first time.

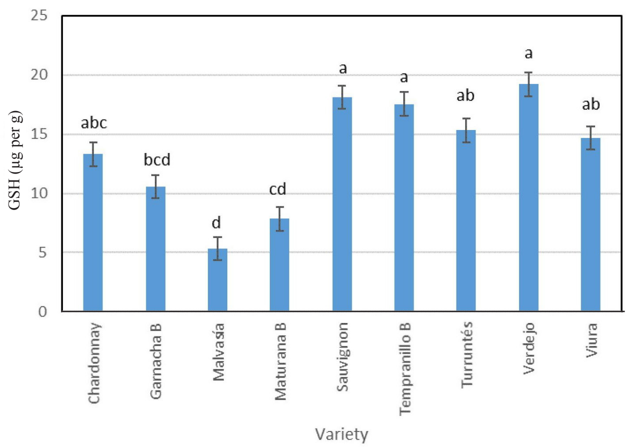

Figure: Glutathione (GSH) content (µg·g^{-1}) in white grape varieties. Standard deviation n = 3. Different letters indicate significant differences according to Tukey test $p < 0.05$.

In the musts (Table) the highest GSH value was found in 'Tempranillo Blanco', with 'Sauvignon Blanc' in second place, while the lowest levels were recorded in 'Garnacha Blanca' and 'Malvasía'. The range of concentrations obtained was between 0.84 and 19.15 mg·L^{-1}. These results confirm those obtained in other studies conducted with some of the varieties studied, in which a high level of GSH was also observed in 'Tempranillo Blanco' (Martínez et al. 2014). The content of GSH in the musts of 'Tempranillo Blanco' was 19.15 (mg·L^{-1}), while in other studies the values reached were between 5.33 and 10.40 mg·L^{-1} (Martínez et al. 2014). In 'Garnacha Blanca' and 'Viura', the GSH content in musts (Table) was lower than that observed by Martínez et al. (2014), and in 'Maturana Blanca' and 'Turruntés' the values reached were within the same range. In 'Sauvignon Blanc' musts, the GSH content was quite high (17.40 µg·g^{-1}), being within the range between 10.6-42.3 mg·L^{-1} indicated by Fracassetti et al. (2011). The 'Chardonnay' musts showed 9.61 mg·L^{-1} of GSH, coinciding with that observed by Fracassetti et al. (2015). However, Cheynier et al. (1989) obtained much higher values in musts of this variety and others, such as 'Garnacha Blanca' and 'Macabeo'.

The GSH concentration in musts can also be strongly affected by the conditions during the cycle. Pons et al. (2015) observed great variability in the GSH content of 'Sauvignon Blanc' musts from different vintages, noting a significant reduction in years with high temperatures and a deficit of water during ripening. Moreover, Martínez

Table 1

Glutathione and hydroxycinnamic acids concentration ($mg \cdot L^{-1}$) in musts and wines of white grape varieties

Variety	Musts			Wines		
	GSH	HAc	HAc/Glutathione	GSH	HAc	HAc/Glutathione
Chardonnay	9,61 ± 0,74 c	11,24 ± 2,10 de	1,17 ± 0,19 de	21,29 ± 2,41 a	10,93 ± 1,15 cd	0,51 ± 0,09 c
Garnacha B	0,84 ±0,25 f	60,83 ± 5,91 a	72,42 ± 6,09 a	13,47 ± 1,81 b	60,94 ± 6,23 a	4,52 ± 0,27 b
Malvasía	1,43 ± 0,12 f	39,28 ± 2,10 b	27,47 ± 5,09 b	1,32 ± 0,47 d	37,42 ± 0,99 b	28,35 ± 8,19 a
Maturana B	3,14 ± 0,88 de	10,82 ± 0,84 e	3,45 ± 0,39 cd	6,09 ± 0,36 c	11,62 ± 0,75 cd	1,91 ± 0,19 bc
Sauvignon	17,4 ± 1,02 b	11,98 ± 0,92 de	0,69 ± 0,20 e	17,97 ± 3,65 ab	8,90 ± 0,98 d	0,50 ± 0,06 c
Tempranillo B	19,15 ± 0,42 a	57,88 ± 3,24 a	3,02 ± 0,31 d	21,23 ± 0,60 a	50,97 ± 4,21 ab	2,40 ± 0,19 bc
Turruntés	4,44 ± 0,40 d	20,26 ± 2,63 c	4,56 ± 0,22 cd	3,21 ± 0,36 cd	14,08 ± 0,75 c	4,39 ± 0,35 b
Verdejo	3,18 ± 0,21 de	10,73 ± 1,51 e	3,37 ± 0,19 cd	3,30 ± 0,37 cd	6,35 ± 0,87 de	1,92 ± 0,46 bc
Viura	2,28 ± 0,11 ef	14,92 ± 1,84 de	6,54 ± 0,31 c	6,55 ± 0,22 c	13,23 ± 1,39 c	2,02 ± 0,25 bc

Standard deviation n = 3. Different letters indicate significant differences according to Tukey test $p < 0.05$.

et al. (2014) found that GSH decreased in musts when the vines suffered water stress. The climate conditions of the 2017 growth cycle, characterized by very low rainfall and a much earlier ripening period of the grapes, could have had a significant influence on the GSH content of the different varieties studied.

The varietal differences found in musts (Table) were greater than those observed in grapes (Figure), and it could be explained by the consumption of glutathione in the oxidative processes which occur during the crushing and pressing of the grape. Depending on the quality of the grape, the length of maceration time of the must with the skins can raise the glutathione concentration considerably, since this compound is mainly located in the skins (Pons *et al.* 2015).

The content of hydroxycinmic acids (HAc) in musts (Table) showed important varietal differences, with values between 10.73 and 60.83 $mg \cdot L^{-1}$. The highest concentration was reached in the musts of the varieties 'Garnacha Blanca', 'Tempranillo Blanco' and 'Malvasía', and the lowest in 'Verdejo' and 'Maturana Blanca'. The ratio of hydroxycinnamic acids to glutathione of the must was very high in 'Garnacha Blanca' and 'Malvasía', and lower in 'Sauvignon Blanc'. The varietal differences in hydroxycinnammic acids and polyphenoloxidase (PPO) content determine their oxidative susceptibility and it can influence the glutathione content of the musts. For this reason, the GSH concentration in 'Garnacha Blanca' was the lowest. Cheynier *et al.* (1989) observed that the ratio between hydroxycinnamic acids and glutathione is higher in 'Garnacha Blanca' than in other varieties. The polyphenoloxidase (PPO), mainly laccase, also can influence in the glutathione content of the musts by oxidation. For this reason, the concentration of GSH in 'Malvasia' must was probably the lowest, since this variety is very sensitive to *Botrytis*, and the presence of laccase is high. No bibliography was found regarding 'Malvasia', but our results (unpublished data) indicate that this variety has a high PPO content.

In wines, the highest GSH concentration was found in 'Tempranillo Blanco' and 'Chardonnay', with little difference compared to that of 'Sauvignon Blanc' (Table). The lowest level was obtained in 'Malvasía' and was similar to that observed in wines made with 'Turruntés' and 'Verdejo'. The levels of concentration ranged widely, between 1.32 and 21.29 $mg \cdot L^{-1}$, similar to those observed in other studies with some of the white grape varieties of the Qualified Designation of Origin Rioja (Martínez *et al.* 2014). In addition, the results for the 'Sauvignon Blanc' variety were within the range recorded by various authors (Janeš *et al.* 2010, Fracassetti *et al.* 2011). In wines from 'Chardonnay', the GSH content obtained was 21.29 $mg \cdot L^{-1}$, coinciding with that observed by Fracassetti *et al.* (2015).During alcoholic fermentation, a slight increase in this compound was detected in most of the varieties, most notably in 'Chardonnay', 'Garnacha Blanca', 'Maturana Blanca' and 'Viura'. Fracassetti *et al.* (2015) monitored the concentration of GSH during winemaking and also observed an increase in wines of different varieties with respect to musts. In any case, the existing results about its evolution during alcoholic fermentation are contradictory. Some authors (Mikaye *et al.* 1998, Lalvigne *et al.* 2007) suggested that this compound is also involved in the stress response of *Saccharomyces cerevisiae* and it can be absorbed or excreted during alcoholic fermentation depending on the yeast strain, although the differences observed in some studies were insignificant (Fracasseti *et al.* 2015). Kritzinger *et al.* (2013b) observed fluctuations in the concentration of GSH during fermentation depending on the yeast and also on the initial concentration of GSH in the must. These authors suggest the *de novo* synthesis and secretion of GSH by the yeast, as well as the need for future studies with marked GSH to elucidate its evolution in alcoholic fermentation.

The concentration of hydroxycinnamic acids (HAc) in the wines (Table) was slightly lower than that observed in the must, and the varietal differences being similar. The ratio of hydroxycinnamic acids to glutathione was lower than in musts, except in 'Malvasía' and 'Turruntés', which was similar, and 'Garnacha Blanca', which decreased significantly. This decrease in the ratio was due to the notable increase in GSH in wines of said variety, and it could be related to the numerous reactions in which said compound participates in musts and wines.

Conclusions

In this work, the glutathione content of nine white grape varieties authorized in the Qualified Designation of Origin Rioja (Spain) has been evaluated for the first time. The results obtained have shown the existence of notable varietal differences in grapes, musts and wines. The 'Tempranillo Blanco' and 'Sauvignon Blanc' varieties have stood out for their high concentration in all cases. Taking into account the antioxidant properties of glutathione, these varieties manifest a high potential for the production of quality white wines with more stable sensory characteristics over time. The ratio of hydroxycinnamic acids to glutathione, indicator of the susceptibility of oxidation of musts, showed significant varietal differences, was lower in 'Sauvignon Blanc' and higher in 'Garnacha Blanca'.

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