

GRAPE STEMS AS PRESERVATIVE IN TEMPRANILLO WINE



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Danielle Pires Nogueira¹; Nerea Jiménez-Moreno¹; Irene Esparza¹; Jose Antonio Moler²; Carmen Ancín-Azpilicueta¹

¹Department of Sciences, Institute for Advanced Materials (INAMAT²), Universidad Pública de Navarra, Campus Arrosadía s/n, 31006 Pamplona, Spain

²Department of Statistics and Operational Research, Universidad Pública de Navarra, Campus Arrosadía s/n, 31006 Pamplona, Spain

INTRODUCTION

SO₂ is the most widely used preservative in the wine industry. However, there are several drawbacks related with the use of SO₂ in wine, such as, its toxicity and the unpleasant odor in case of excess [1]. These reasons justify the importance of searching alternatives to reduce or eliminate this preservative from wine. Polyphenol rich extracts from agri-food industry by-products have been studied as a replacement for their high antioxidant activity, and positive results reported [2]. The grapes stems are discarded early on in the winemaking process, in spite of containing large amounts of polyphenolic compounds with antioxidant activity. The aim of this work was to determine whether the ground stem and its extract had the potential to totally or partially replace SO₂ in wine.

MATERIAL & METHODS

For this work, five Tempranillo red wines were made. The treatments were: positive control (PC) with SO₂ (60 mg/L); negative control (NC) without any preservatives; Tempranillo stem extract (TE) (200 mg/L); combination of Tempranillo stem extract (100 mg/L) and SO₂ (TM) (20 mg/L); and ground Tempranillo stem (TS) (310 mg/L). The resulting wines were analyzed to determine their antioxidant capacity by ABTS [3], Total polyphenolic content by Folin-Ciocalteu [4] and colorimetric measure of total anthocyanin content [5]. High performance liquid chromatography (HPLC) was also conducted to quantify the individual polyphenols. The results were statistically analyzed by ANOVA. The sensory analysis was a triangular test [6].

RESULTS & DISCUSSION

Table 1 presents the results for ABTS, total anthocyanins and total polyphenolic content. The evolution over time of these spectrophotometric results was as expected, since most polyphenolic compounds are extracted in the early stages of fermentation [7]. In this table it is possible to observe that negative control wine, after one year in the bottle, presented significantly lower antioxidant activity ($p < 0.05$) than the wine with SO₂. The remaining samples presented ABTS values similar to SO₂ wine. Concerning the amount of total anthocyanins, the SO₂ sample, after a year in the bottle, presented a superior concentration than the rest of the samples. Less polyphenols were extracted from the wine without preservatives than the rest of the samples, which presented similar total polyphenolic concentration among them.

Figure 1 shows the time concentration evolution of the predominant phenolic compounds. The concentration of malvidin-3-glucoside dropped after the end of malolactic fermentation, probably due to complexation with tannins, precipitation and even degradation [8]. After a year in the bottle the wine with SO₂ presented a superior concentration of this compound, when comparing with the other samples. The variation of the individual polyphenols followed the behavior described in the literature [9]. By comparing the treatments, it is possible to see that positive control wine tends to have higher concentrations of this compounds than the other samples. It is even more perceptible in earlier times, reinforcing SO₂ effect in extracting polyphenols [1].

Table 2 has the results for the triangular sensory analysis. This analysis showed that positive control wine was only perceptibly different from Tempranillo extract wine and the negative control at 99% confidence level. Tempranillo stem wine only differed from the wine that combined SO₂ and extract. Negative control wine differed from all treatments, except Tempranillo stem.

Table 1. ABTS, Total anthocyanin content and Total polyphenolic content.

Assay	Sample	PC	NC	TE	TM	TS
ABTS (mM Trolox equivalent)	Must	3.1 ± 0.2 ^b	2.6 ± 0.3 ^a	2.7 ± 0.2 ^{ab}	2.7 ± 0.2 ^{ab}	2.5 ± 0.2 ^a
	50%AF	14.6 ± 0.6 ^b	12.8 ± 0.6 ^a	14.5 ± 0.7 ^b	14.4 ± 0.2 ^b	13.8 ± 1.1 ^{ab}
	AF	17.4 ± 1.1 ^b	15.6 ± 0.8 ^a	17.6 ± 0.9 ^b	17.8 ± 0.8 ^b	18.1 ± 1.1 ^b
	MLF	16.2 ± 1.2 ^a	14.7 ± 1.1 ^a	15.0 ± 0.2 ^a	15.3 ± 0.7 ^a	15.3 ± 0.9 ^a
	YB	16.8 ± 0.4 ^b	13.5 ± 2.1 ^a	15.6 ± 0.6 ^{ab}	15.4 ± 0.8 ^{ab}	15.2 ± 0.9 ^{ab}
Total anthocyanins (mg/L malvidin-3-glucoside)	Must	77 ± 10 ^c	39 ± 6 ^b	32 ± 1 ^{ab}	29 ± 1 ^a	30 ± 2 ^{ab}
	50%AF	368 ± 10 ^b	337 ± 18 ^a	371 ± 3 ^b	361 ± 5 ^b	370 ± 5 ^b
	AF	366 ± 35 ^c	313 ± 9 ^a	330 ± 25 ^{ac}	326 ± 14 ^{ab}	357 ± 3 ^{bc}
	MLF	272 ± 13 ^b	244 ± 15 ^a	254 ± 10 ^{ab}	242 ± 4 ^a	259 ± 6 ^{ab}
	YB	192 ± 8 ^c	107 ± 14 ^a	111 ± 4 ^a	127 ± 2 ^b	115 ± 4 ^{ab}
Total polyphenols (mM of gallic acid)	Must	2.7 ± 0.2 ^b	2.2 ± 0.2 ^a	2.2 ± 0.1 ^a	2.2 ± 0.1 ^a	2.1 ± 0.1 ^a
	50%AF	9.8 ± 0.4 ^b	8.9 ± 0.5 ^a	10.2 ± 0.3 ^b	9.7 ± 0.4 ^b	9.8 ± 0.4 ^b
	AF	10.5 ± 0.5 ^a	9.9 ± 0.3 ^a	11.4 ± 0.7 ^b	11.8 ± 0.3 ^b	11.9 ± 0.3 ^b
	MLF	10.9 ± 0.3 ^b	9.6 ± 0.9 ^a	9.6 ± 0.4 ^a	9.9 ± 0.1 ^a	9.8 ± 0.7 ^a
	YB	10.4 ± 0.6 ^b	8.0 ± 1.0 ^a	9.5 ± 0.3 ^b	9.7 ± 0.4 ^b	9.5 ± 0.6 ^b

PC: Positive control; NC: Negative control; TE: Tempranillo extract; TM: Tempranillo extract with SO₂; TS: Tempranillo stem. 50%AF: mid alcoholic fermentation; AF: end alcoholic fermentation; MLF: end malolactic fermentation; YB: one year in bottle. Results followed by the same letter, in the same line, are not statistically different at 95% significance.

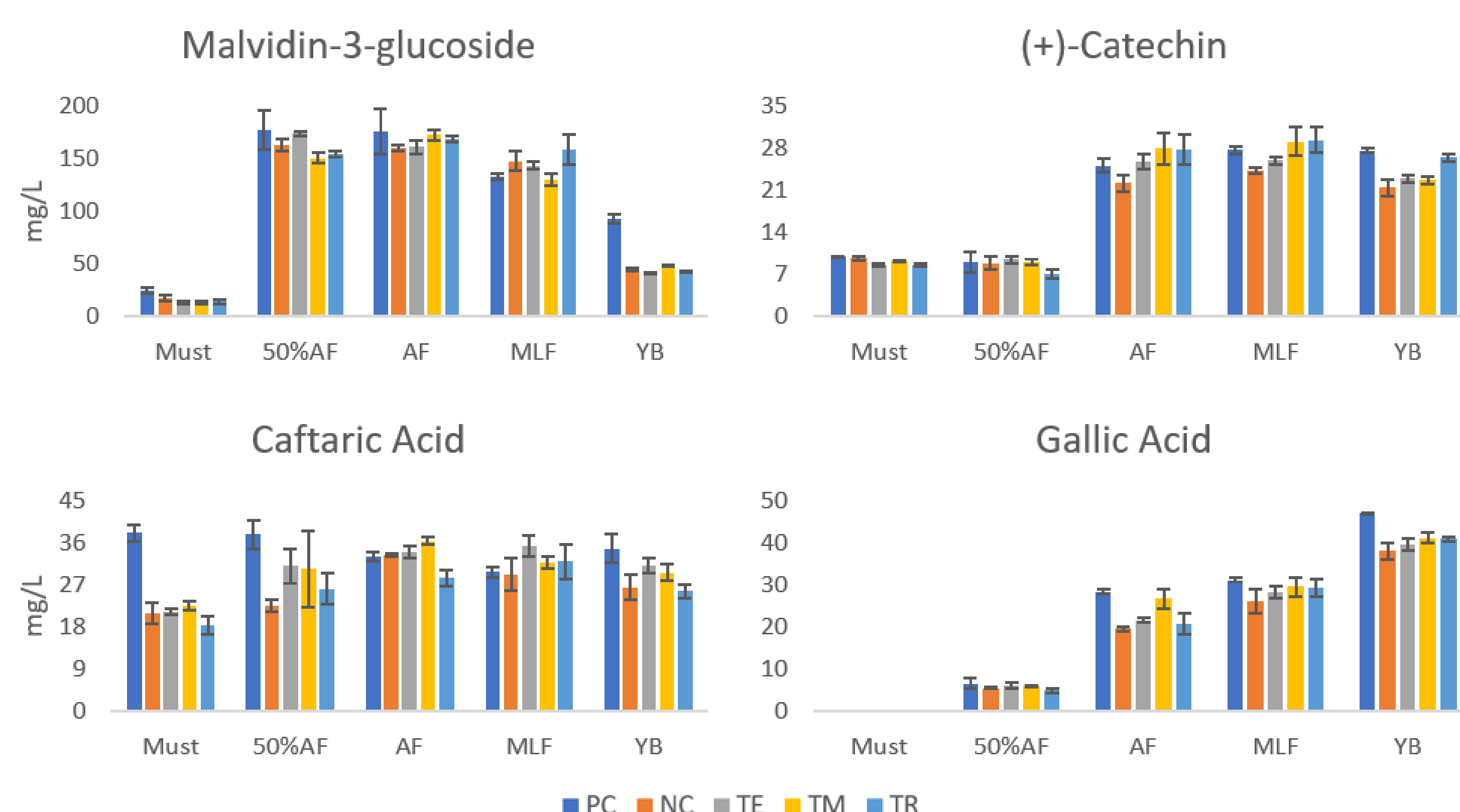


Figure 1. Concentration (mg/L) of major polyphenolic compounds.

PC: Positive control; NC: Negative control; TE: Tempranillo extract; TM: Tempranillo extract with SO₂; TS: Tempranillo stem. 50%AF: mid alcoholic fermentation; AF: end alcoholic fermentation; MLF: end malolactic fermentation; YB: one year in bottle.

Table 2. Triangular sensory analysis.

	PC x NC	PC x TM	PC x TS	TE x TE	TE x TS	TM x TS	NC x TM	NC x TE	NC x TS
n	11	11	11	11	11	11	11	11	11
Correct responses	8	7	6	8	7	4	10	9	8
Result*	≠	=	=	≠	=	≠	≠	≠	=

PC: Positive control; NC: Negative control; TE: Tempranillo extract; TM: Tempranillo extract with SO₂; TS: Tempranillo stem. * $\alpha = 0,01$. =: no difference detected. ≠: difference detected.

CONCLUSIONS

The partial or total replacement of SO₂ by grape stem or grape stem extract:

- Reduced in about 50% the anthocyanins content of bottled wines stored for one year, while the remaining compounds Content was similar or slight lower than in the wines treated with SO₂
- Allowed for wines with sensory properties more similar to the wines treated with SO₂ than the untreated ones
- could be an adequate strategy to formulate sulfite free wines

REFERENCES

- Esparza, I.; Martínez-Inda, B.; Cimminelli, M.J.; Jimeno-Mendoza, M.C.; Moler, J.A.; Jiménez-Moreno, N.; Ancín-Azpilicueta, C. Reducing SO₂ doses in red wines by using grape stem extracts as antioxidants. *Biomolecules* **2020**, *10*, 1–15.
- Gouvinhas, I.; Queiroz, M.; Rodrigues, M.; Barros, A.I.R.N.A. Evaluation of the Phytochemistry and Biological Activity of Grape (*Vitis vinifera* L.) Stems: Toward a Sustainable Winery Industry. In *Polyphenols in Plants*; Academic Press: London, UK, **2019**; pp. 381–394 ISBN 9780128137680.
- Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.
- Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Sci. Hortic.* **1999**, *213*, 152–178.
- Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278.
- European Committee for Standardization. BS EN ISO 4120:2021 Sensory Analysis - Methodology - Triangle Test **2021**, 23.
- Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Phenolic Compounds. In *Handbook of Enology*; John Wiley & Sons, Ltd: Chichester, UK, **2006**, 141–203.
- Raposo, R.; Chinnici, F.; Ruiz-Moreno, M.J.; Puertas, B.; Cuevas, F.J.; Carbú, M.; Guerrero, R.F.; Ortiz-Somovilla, V.; Moreno-Rojas, J.M.; Cantos-Villar, E. Sulfur free red wines through the use of grapevine shoots: Impact on the wine quality. *Food Chem.* **2018**, *243*, 453–460.
- Jackson, R.S. Chemical Constituents of Grapes and Wine. In *Wine Science*; Academic Press: San Diego, USA, **2000**, 232–280.