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A new enzymatic solution for prevention of quercetin precipitation in red wines

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Ensuring wine quality is crucial. However, the rise in quercetin deposits in red wines, particularly those made from Sangiovese grapes, is escalating due to factors such as machine harvesting and climate change[1 2 3 4]. This issue extends beyond mere visual inconveniences linked to bottled wine to concerns about potential organoleptic damage. Currently used methods, such as PVPP fining, are prohibited in organic wine production under EU regulation n. 203/2012. To address this issue, the present study explored an organic-friendly solution that involves the use of a pectolytic enzyme with secondary glycosidase activity. The enzyme was validated in Chianti wine production and was observed to significantly accelerate the hydrolysis of glycosylated quercetins, giving their aglycone form. This accelerated process enhanced deposition before bottling without causing significant wine colour depletion, thus can be considered a promising approach even for organic winemakers.

First approach: removal of quercetin by means of fining agents

Various fining agents target phenolic compounds, but there is no specific method for quercetin removal. PVPP, commonly used in winemaking, has minimal impact on aroma and polyphenols[5]. In wine, PVPP did not significantly affect quercetin glycosides (Figure 1A), but it showed a dose-dependent interaction with quercetin aglycone (Figure 1B). Other agents had limited effects, except for the PVI/PVP polymer (DW) at a higher dose. Despite endorsing PVPP as a viable option, 60 g/hL may be insufficient for aglycone removal. In another experiment in which each of the two main glycosides of quercetin were separately monitored (Figure 2), quercetin-3-glucoside naturally degraded to aglycone during fermentation; meanwhile, quercetin-3-glucuronide remained unmodified, thus is a potential source of quercetin aglycone post-PVPP treatment. PVPP can adversely affect wine quality, impacting colour and specific compound[6]. EU regulations restricting PVPP in organic wine highlight the need for alternative treatments in the organic wine market.

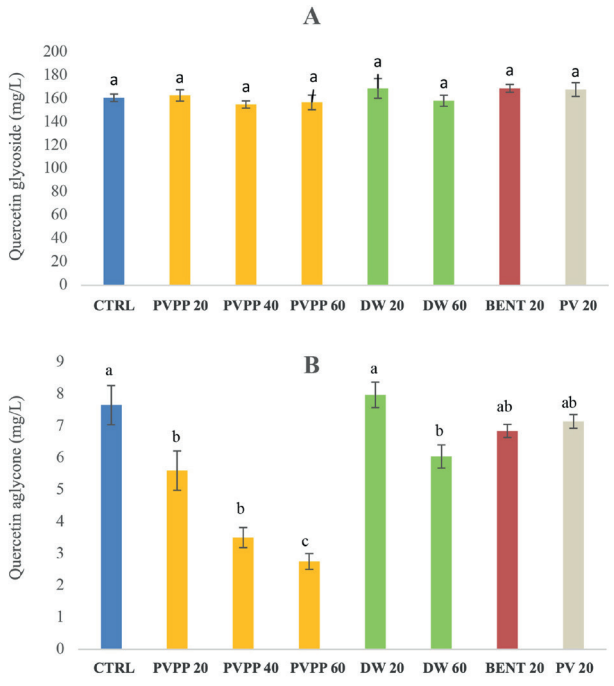

FIGURE 1. Content of quercetin glycosides (A) and quercetin aglycone (B) in wine after fining.

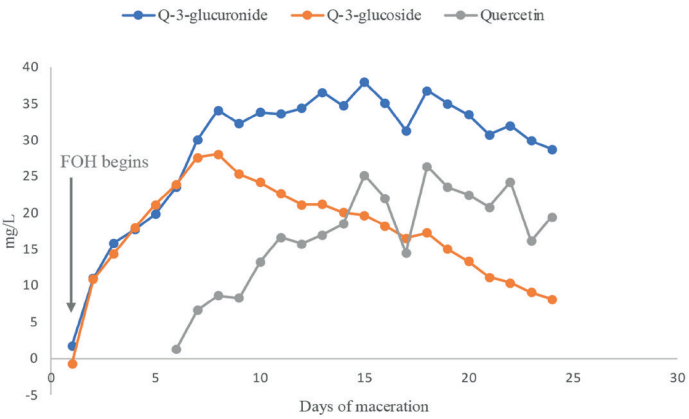

FIGURE 2. Quercetin Compounds in Sangiovese Grapes: 24-Day Alcoholic Fermentation with Skin Maceration. FOH begins: start of alcoholic fermentation.

Second approach: pectolytic enzyme with secondary glycosidase activity

To address the quercetin precipitation problem, it is necessary to remove both the aglycone and its precursor. First, we tested a pectolytic enzyme using recommended (4 g/hL) and higher (40 g/hL) doses. Both treatments rapidly reduced quercetin glycosides and increased aglycone within days. Quercetin quantification showed concentration-dependent glycoside degradation (Table 1). Glycosidic enzymes impact wine compounds, colour and aroma, and can cause sluggishness under winery conditions and rapid tannin deactivation[7]. Adding glucosidase early on in the aging process could be a way of dealing with these issues. Further experiments assessed the real applicability of the enzymatic approach in winery.

To assess the effectiveness of glycosidase in reducing quercetin in young Chianti wine, we monitored quercetin glycoside and aglycone evolution during barrel aging. We confirmed the occurrence of natural glycoside degradation in the untreated control wines (TQ), with a one-third decrease in total quercetin glycosides over four months. Bentonite treatment had no significant effect on the quercetin glycosides, indicating the absence of endogenous glycosidases, as well as the occurence of slow chemical degradation. Enzyme treatment (ENZ) resulted in a consistent aglycone increase for 100 days, with quercetin glycosides dropping below control levels. While the glycosides continued to decrease, aglycone levels reached a plateau before the completion of barrel aging, a likely saturation point for precipitation.

TABLE 1. Quercetin content in Sangiovese wine with pectolytic enzyme treatment at 4 g/hL and 40 g/hL, compared to untreated wine (test).

Days after treatment	Quercetin glycoside			Quercetin aglycone		
	Test	4 g/hL	40 g/hL	Test	4 g/hL	40 g/hL
0	91.0 ± 4.0			2.8 ± 0.4		
2		82.0 ± 6.0 (- 10 %)	65.0 ± 4.0 (- 29 %)		4.2 ± 1.0 (+ 49 %)	10.4 ± 3.1 (+ 270 %)
7		75.0 ± 6.0 (- 18 %)	46.0 ± 6.0 (- 49 %)		10.1 ± 2.0 (+ 261 %)	19.5 ± 3.9 (+ 597 %)

Similar patterns were observed in wines from two independent wineries (Figure 3). A concern regarding enzymatic treatment is its impact on other glycosylated components, like anthocyanins, potentially affecting colour stability. One year after enzyme addition, a colour analysis showed a slight decrease in red colour intensity (8 % reduction); overall, the impact on colour was minor.

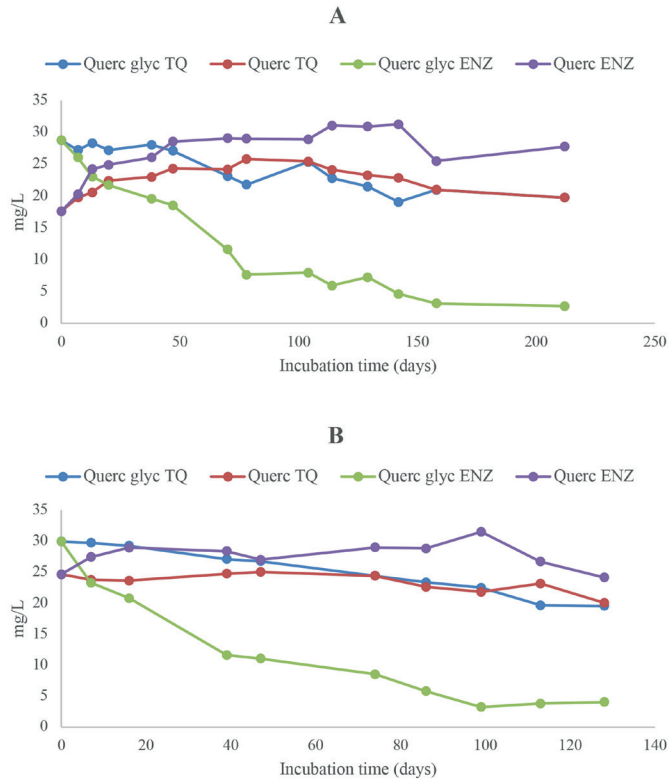

FIGURE 3. Quercetin Evolution in Wines 1 (Winery 1) and 2 (Winery 2) with and without Enzyme Treatment.

Conclusion

To summarise, while PVPP has proved to be superior to other fining agents for quercetin aglycone removal, it falls short of resolving the precipitation challenge. Our proposed enzymatic treatment results in a swift reduction in quercetin glycosides, enhancing wine stability. Applied early on in the aging process, it enables natural aglycone precipitation before bottling, as validated by experiments in two wineries. With minimal impact on anthocyanins, the enzymatic approach emerges as a promising mean of preventing quercetin precipitation without compromising wine colour. To further explore the effects of enzyme addition on wine composition, additional analyses on non-volatile phenolics and aroma compounds will be necessary. ■

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