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Impact of protein fining agents and bentonite on rotundone concentration in red wine made from *Vitis vinifera* L. cv. Tardif

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ABSTRACT

Rotundone, the main aroma compound responsible for peppery notes in red wine, has a strong affinity with solids and may be affected by wine fining. This work aimed to study the impact of several fining agents on this compound using a red wine made from Tardif. *Egg albumin, gelatine, vegetable protein (pea protein)* and sodium *bentonite* were investigated at the same dosage (D1, 20 g/hL) to compare their effect under similar conditions and at a second dosage, which corresponds, depending on the agents, either to half of D1 or twice D1 to reflect more common winemaking practices. Overall, the fining agents had a minor, although significant effect on rotundone, with the greatest impact, which might not be detectable sensorily speaking, observed for *vegetable protein (pea protein)* used at 40 g/hL (–12.1 %). At D1, although not statistically different from *gelatine* and *vegetable protein (pea protein)*, *egg albumin* induced the largest decrease in rotundone, which could be attributable to its higher surface charge density. A correlation (R^2 = 0.85) was noticed between mean values of rotundone concentrations (3 replicates) and A_{620}, suggesting that this proxy can be used to predict the impact of fining on rotundone in Tardif red wines. Though further work is necessary to investigate the impact of additional fining agents and to better understand the mechanisms involved, notably using computational chemistry, our results encourage winemakers to use fining agents in their wine if required, without having to fear excessive rotundone losses.

KEYWORDS: sesquiterpene, gelatine, egg albumin, vegetable protein (pea protein), montmorillonite clay

INTRODUCTION

Rotundone is the only aroma compound found to impart black pepper notes in red wine (Wood *et al.*, 2008). This sesquiterpene is perceived positively by consumers, with the exception of younger panellists, who prefer fruit-driven red wines (Geffroy *et al.*, 2024). Consequently, this compound is generally regarded as a desirable aroma that contributes to the overall quality of wine. Rotundone is a very versatile compound whose concentration in wine is greatly affected by environmental factors, with notably cool and wet climatic conditions enhancing rotundone accumulation in grapes (Caputi *et al.*, 2011). Indeed, on the same vineyard, rotundone concentration can vary by a 25 to 40-fold factor in wine and grape, respectively, between the least and most favourable seasons (Geffroy *et al.*, 2020). To limit inter-vintage variations and produce red wines with a stable rotundone concentration, several viticultural practices have been proposed, such as the use of pre-veraison irrigation, combined or not with a cutting of the fruit-bearing cane 3 weeks before harvest, delayed harvest, defoliation in cool climate vineyards or differential harvesting (Geffroy *et al.*, 2020). More recently, Baerenzung dit Baron *et al.* (2025) highlighted that cultivating late-ripening rotundone-producing grape varieties such as Grenache, in wine regions nearing their thermal requirement limits, could be another promising approach.

A limited number of studies have investigated the impact of winemaking techniques or fermentation variables on rotundone concentration in red wine. Surprisingly, given that rotundone is mainly located in the berry skin (Caputi *et al.*, 2011) and its extraction could be enhanced by practices favouring maceration, research conducted at a laboratory scale showed that pectolytic enzymes, the increase in temperature or length of maceration did not promote rotundone extraction (Geffroy *et al.*, 2017). While sulphur dioxide addition or storage temperature had also no impact on rotundone concentration, fortification, elevated ethanol concentrations and moderate amounts of oxygen during ageing could have a small beneficial effect (Petrozziello *et al.*, 2021; Zhang *et al.*, 2017). A significant loss was observed during racking and filtration, likely due to the compound's hydrophobic nature and its tendency to bind to other particles (Caputi *et al.*, 2011).

As a consequence, it can not be discarded that fining agents frequently used by winemakers to improve red wine stability and quality, by reducing excessive astringency and bitterness mainly through polyphenol protein interactions, might interact with rotundone and cause additional losses (Kumar & Suhag, 2024). Though it was demonstrated that mineral or protein fining agents could decrease varietal thiols, monoterpenes and ethyl esters in white wines with an effect depending on the agent used, its stage of incorporation and dose (Kemp *et al.*, 2022; Rihak *et al.*, 2022; Vela *et al.*, 2017), the literature concerning the impact of such oenological practice on the aroma of red wine remains scarce. The only studies conducted on Pinot noir and Zweigelt red wine were inconclusive, with, in most cases, no impact observed (Kemp *et al.*, 2022; Wimalasiri *et al.*, 2022).

This work aimed to assess the impact of several protein fining agents frequently employed in red winemaking namely *egg albumin*, *gelatine*, *vegetable protein (pea protein)* and bentonite, at two doses of use, on rotundone concentration in red wine from Tardif, a grape variety in which the presence of rotundone has been previously reported at a substantial concentration, up to 204 ng/L according to Geffroy *et al.* (2020).

MATERIALS AND METHODS

1. Chemicals

Rotundone (≥ 99 %) and d_5-rotundone (≥ 96 %) were sourced from Firmenich (Geneva, Switzerland) and Eptes, Food & Flavours Analytical (Vevey, Switzerland), respectively. The solvents (≥ 99.9 % ethanol, ≥ 99.0 % *n*-pentane, ≥99.8 % ethyl acetate and ≥ 99.9 % methanol), as well as tartaric acid (≥ 99.9 %) and potassium tartrate (≥ 99.8 %), were purchased from VWR Chemicals (Rosny-sous-Bois, France) or Sigma-Aldrich (Darmstadt, Germany). Sodium hydroxide (≥ 99.0 %) was obtained from Carlo Erba (Val de Reuil, France). Ultrapure water was obtained using PURELAB® Classic (ELGA LabWater, Veolia Water Solutions & Technologies, UK).

2. Red wine

The red wine was kindly provided by Plaimont Producteurs, a cooperative cellar located in the southwest of France (199 route de Corneillan, Saint-Mont, France). It was obtained from 100 % *Vitis vinifera* L. *cv* Tardif grapes, an almost extinct grape variety from southwest France known for producing wines with high concentrations of rotundone and that is gaining increasing popularity (Geffroy *et al.*, 2020). The grapes harvested during the 2023 vintage were fermented in a 30 hL stainless steel tank for 8 days at 25 °C. After performing a first racking, malolactic fermentation, a second racking combined with a sulphur dioxide addition of 50 mg/L and a third racking, 30 litres of wine were aliquoted in a stainless steel beer keg in February 2024, which corresponds to the usual bottling period of such wine. The sampled wine was stored at 4 °C during the duration of the study, with the empty space in the container filled with nitrogen.

3. Classical oenological analysis

Oenological classical parameters were determined on the control wine and the test samples using a Wine Scan™ SO_2 equipment (Foss, Denmark). These parameters include alcohol content, titratable acidity (TA), pH, tartaric acid, volatile acidity, sum of glucose and fructose, A_{420}, A_{520}, A_{620} (absorbances measured at 420, 520 and 620 nm, respectively), colour hue (calculated as the ratio between A_{420} and A_{520}), modified colour intensity (MCI) that provides an integrated estimate of wine colour intensity and is calculated as the sum of A_{420}, A_{520} and A_{620} in a cuvette of 1 mm optical

path length, and Total Phenolic Index (TPI) measured spectrophotometrically at 280 nm.

4. Rotundone analysis

Rotundone was extracted from wine samples with Solid Phase Extraction (SPE) cartridges, containing styrene-divinylbenzene (SDB-L) (500 mg/6 mL) (Phenomenex, Torrance, California, USA), then concentrated with a polydimethylsiloxane-divinylbenzene fibre (PDMS/DVB, 65 µm, 1 cm, Sigma-Aldrich, Darmstadt, Germany) by direct immersion. Analyses were conducted using an UltraTRACE gas chromatograph with a split/splitless injector, coupled to an ITQ900 ion trap mass spectrometer (Thermo Scientific, Courtaboeuf, France) and equipped with a TriPlus autosampler (Thermo Scientific, Courtaboeuf, France).

SPE cartridges were conditioned using a series of solvents: *n*-pentane/ethyl acetate (4:1, 6 mL), followed by methanol (6 mL), and then a hydroalcoholic solution (12 % aqueous ethanol, buffered to pH 3.4 with tartaric acid/sodium tartrate; 2 × 5 mL). The wine samples to analyse (100 mL) were then gradually added and allowed to percolate through the SPE column, which was washed with MilliQ water (4 × 5 mL) before being eluted with *n*-pentane/ethyl acetate (9:1, 10 mL). The solvent was evaporated under a nitrogen stream. The residue was dissolved in ethanol (0.5 mL) and then diluted with MilliQ water (9 mL). SPME conditions, as well as GC/MS analytical parameters and identification of the target molecule, were presented by Baerenzung dit Baron *et al.* (2025) as part of the analysis of rotundone in grapes.

Calibration curves were prepared from the base wine. It was spiked with triplicate addition of rotundone at 6 concentrations (between 0 and 200 ng/L) and with 100 µL of internal standard (d_5-rotundone at 700 µg/L) and then analysed by SPE-SPME-GC/MS.

The calibration curve was linear between the limit of quantification (LOQ) (30 ng/L, estimated value for the lowest concentration associated with a signal-to-noise ratio of 10) and the upper limit of the calibration range (R^2 = 0.9992). Repeatability was assessed by triplicate addition of rotundone at 3 concentration levels (2 × LOQ, 5 × LOQ and 8 × LOQ). Relative standard deviations (RSD) were less than 10 %. Recovery rates ranged from 98 % to 109 %.

The rotundone determination of the base wine revealed a low concentration of 33 ng/L, which could be the consequence of the particularly warm climatic conditions experienced in 2023 during maturation and harvest that took place in mid-October.

5. Fining trials

The four fining agents selected for the study, specifically *egg albumin* (Ovaline®), non-hydrolysed pork *gelatine* (Gelfine®), *vegetable protein (pea protein)* extracted from *Pisium sativum* (Greenfine® Must) and sodium *bentonite* (Bentosol Poudre) were provided by Lamothe-Abiet (Canéjan, France). The molecular weight (MW) distributions provided by the manufacturer were mainly 30–75 kDa for *vegetable protein (pea protein)* and *ovalbumine*, and predominantly 75–150 kDa with fractions up to 300 kDa for *gelatine*. The zeta potential at pH 3.8, which reflects the surface electrical charge of the fining agents in suspension at the red wine pH, exhibited a slightly negative value for *vegetable protein (pea protein)* (–3.21 ± 0.40 mV), while *gelatine* and *albumin* showed positive values (9.70 ± 1.80 mV and 10.90 ± 0.40 mV, respectively).

The fining agents were all tested at the same dose of 20 g/hL (D1) as proposed by Lagarde (2022) to compare their effect under similar conditions of use, and at a second dose (D2) reflecting more common winemaking practices, which was either half of D1 or twice D1, depending on the fining agent. D2, determined in close collaboration with experts from Lamothe-Abiet who had decades of practical experience with fining agents, were 10 g/hL for *egg albumin* and *gelatine*, and 40 g/hL for *vegetable protein (pea protein)* and *bentonite*. These treatments were compared with a *control* that did not receive any addition of fining agents. Each treatment, including the *control*, was assessed in triplicate.

Wine samples of 210 mL were placed in 250 mL polypropylene containers (Nalgene, Rochester, USA), then spiked with rotundone to target a concentration of 120 ng/L. This concentration was chosen to be well above the limit of quantification and representative of usual Tardif wine concentrations (Geffroy *et al.*, 2020).

Then, each sample was shaken at 160 rpm for 15 min with a Polytest 30 device (Fisher Scientific, Reinach, Switzerland) before performing the fining agent addition using a 10 % w/v solution prepared from ultrapure water obtained using PURELAB® Classic (ELGA LabWater, Veolia Water Solutions & Technologies, UK) 24 h before the trial and continuously stirred until use. The gelatine solution that gelled after 24 h was heated at 40 °C for 1 h to be restored to a liquid state.

After the addition, the samples were shaken at 160 rpm for 15 min, then left to settle for 24 h in a temperature-controlled room at 20 °C in darkness. They were then centrifuged at 4900 *g* for 15 min at 20 °C with a Sigma 6-16K bench centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany) and filtered using 1.6 µm glass fibre filters (Whatman, Maidstone, UK).

In total, 100 mL of supernatant was used for rotundone determination (Section 4), and the remaining amount was employed for classical oenological analysis.

It must be pointed out that the wine was left in contact with the fining agent for only 24 h, a period of time sufficient to assess the impact of the treatment on chemical and sensory characteristics (Marangon *et al*., 2019). This was confirmed during preliminary experiments that showed no difference in rotundone concentration and classical parameters between samples kept in contact for 24 h and 6 days, another length of time implemented during red wine fining trials (Cosme *et al*., 2008; Lagarde, 2022). Such time reduction was particularly useful in the frame of the present research i) for

practical and logistical reasons, given the three-day period required for rotundone sampling and analysis, and ii) to limit biases that may arise from differences in dissolved oxygen levels between samples which could, for longer contact times, lead to varying degrees of oxygen consumption, a factor known to influence rotundone concentrations (Petrozziello *et al*., 2021).

6. Statistical treatment

Statistical analyses were performed using XLStat software (Addinsoft, Paris, France). All analytical data, including the wine final rotundone content, were subjected to a one-way analysis of variance (ANOVA). Fisher's least significant difference test was used as a post-hoc comparison of means at $P < 0.05$. A principal component analysis (PCA) was conducted on classical oenological analysis data, using only significant variables ($P < 0.05$) and rotundone concentration as additional data. Regression analyses were also performed to explore the linear correlations between rotundone concentration and other variables.

RESULTS AND DISCUSSION

The influence of the studied treatments on classical oenological parameters is shown in Table 1. As expected, in accordance with a recent review article (Kumar & Suhag, 2024), the use of a fining agent had no significant impact on ethanol content, residual sugar and volatile acidity. While previous works highlighted a significant increase in pH along with a decrease in TA and tartaric acid following a bentonite fining, as a likely consequence of this agent's ability to exchange cations notably Na^+ and $Ca2^+$ (Cheng & Watrelot, 2022; Maslov Bandić et al., 2022), this effect was not observed in our experimental conditions. This discrepancy may be attributed to the moderate bentonite dosage used in our work, which did not exceed 40 g/hL, unlike in earlier investigations where levels above 100 g/hL were explored. An alternative explanation could be the notable enhancement in bentonite quality in recent years, largely attributed to a selection process that excludes bentonites prone to releasing calcium into solution (G. Nogues, unpublished).

The treatments had a significant impact at $P < 0.001$ on all the spectrophotometric variables related to colour (i.e., A_{420}, A_{520}, A_{620}, colour hue, MCI) and total phenol content (TPI). As observed on the PCA plot (Figure 1), the largest effect was noticed for *vegetable protein (pea protein)*, *egg albumin* and *gelatine* used at the highest dosage. Overall, the same fining proteins used at a lower dosage had an intermediate impact, while the smallest effect was observed for the bentonite treatments. These findings are in contradiction with a previous study emphasising a remarkable effect of *bentonite*, and a lesser impact of *vegetable protein (pea protein)* and *gelatine* on colour and phenolic contents (Ghanem *et al.*, 2017). However, the comparison remains difficult as the concentrations, the commercial fining agents and their related molecular weight, as well as the varietal wine selected for the study differed, some key factors known to influence the fining efficiency (Cosme *et al.*, 2009; Karamanidou *et al.*, 2011).

Counterintuitively, though the control treatment showed the highest TPI (+ 5.5 % in comparison with the lowest value observed for *vegetable protein (pea protein)* used at 40 g/hL), A_{420}, A_{520} and MCI were greater for the wines treated with gelatine, egg albumin and vegetable proteins at the lowest dosage. This phenomenon previously described for fining may be related to a destabilisation of colour complexes, notably anthocyanins bound to proteins (Ferrentino *et al.*, 2017), thus increasing the concentration of free pigments, or to induced oxidation leading to an increase in brown pigments (Atanasova *et al.*, 2002). The fact that this effect was not noticed at the highest dose of use could be attributed to the overriding effect of the fining process, removing more phenolic compounds, including anthocyanins, than releasing or oxidising pigments.

It is worth noting that at the same application rate (D1, 20 g/hL), the largest effect was noticed for *gelatine* and *egg albumin*. Besides the intrinsic properties of these fining agents to remove colloids (Kumar & Suhag, 2024), differences in protein content are also likely to have affected the results.

Indeed, it has been demonstrated that the protein content of vegetable proteins used as wine fining agents is slightly lower (80–85 % w/w) compared to gelatine and egg albumin, for which it often exceeds 90 % w/w (Cosme *et al.*, 2007; Shen *et al.*, 2022). However, the analytical differences observed between *vegetable proteins* and *gelatine/egg albumin* might be too substantial to be attributed solely to this relatively modest variation in protein content, and this hypothesis would require experimental confirmation.

It cannot be ruled out that variations, among the tested protein fining agents, in the amino acid residues which may interact with rotundone, have influenced the observed outcomes (Moreira *et al.*, 2007).

As for rotundone, the concentration determined in the control sample was close to the targeted value of 120 ng/L (Figure 2). Overall, fining had a significant but minor effect on rotundone concentration, with none of the agents tested being able to reduce rotundone concentrations below the detection threshold established in red wine at 16 ng/L (Wood *et al.*, 2008). The greatest decrease, –12.1 % in comparison with the *control*, was observed in the sample fined with *vegetable protein (pea protein)* at 40 g/hL, a dosage frequently implemented in the wine industry (Kumar & Suhag, 2024). Despite that this change might not be detectable from a sensory standpoint, this result is not trivial given that such fining proteins are gaining in popularity among winemakers due to the consumers' rising demand for vegan wines and as an alternative to animal proteins, which may have a greater allergenic potential (Marangon *et al.*, 2019).

In comparison with the *control*, the only other significant effect was observed for *egg albumin* used at 20 g/hL, an excessive dosage as typical additions usually vary between

TABLE 1. Impact of fining agents on classical oenological analysis (means, standard deviations and significance). Means followed by different letters within a column are significantly different at $P < 0.05$ by Fisher test.

Treatment	Alcohol (% v/v)	Titratable acidity (g/L as tartaric acid)	pH	Tartaric acid (g/L)	Volatile acidity (g/L as acetic acid)	Glucose + fructose (g/L)	A420	A520	A620	Colour hue	Modified colour intensity (MCI)	TPI
Control	14.1 ± 0.0 a	5.16 ± 0.01 a	3.78 ± 0.01 a	1.96 ± 0.01 a	0.29 ± 0.01 a	1.13 ± 0.53 a	3.12 ± 0.01 bc	3.73 ± 0.02 b	0.822 ± 0.004 ab	0.838 ± 0.002 a	7.67 ± 0.04 b	57.7 ± 0.05 a
Bentonite 20 g/hL	14.1 ± 0.0 a	5.16 ± 0.00 a	3.76 ± 0.01 a	1.94 ± 0.02 a	0.29 ± 0.01 a	0.93 ± 0.06 a	3.17 ± 0.00 a	3.80 ± 0.01 a	0.823 ± 0.002 ab	0.840± 0.000 a	7.79 ± 0.01 a	57.3 ± 0.6 a
Bentonite 40 g/hL	14.1 ± 0.0 a	5.15 ± 0.01 a	3.76 ± 0.01 a	1.94 ± 0.02 a	0.30 ± 0.01 a	1.00 ± 0.00 a	3.12 ± 0.00 bc	3.72 ± 0.01 b	0.810 ± 0.002 bc	0.840 ± 0.004 a	7.64 ± 0.01 b	57.0 ± 0.0 ab
Egg albumin 10 g/hL	14.1 ± 0.1 a	5.14 ± 0.01 a	3.77 ± 0.01 a	1.95 ± 0.03 a	0.30 ± 0.01 a	1.07 ± 0.15 a	3.09 ± 0.01 c	3.71 ± 0.01 b	0.820 ± 0.002 ab	0.833 ± 0.001 b	7.62 ± 0.01 bc	56.3 ± 0.6 bc
Egg albumin 20 g/hL	14.1 ± 0.1 a	5.15 ± 0.00 a	3.77 ± 0.01 a	1.95 ± 0.03 a	0.30 ± 0.00 a	0.90 ± 0.01 a	3.02 ± 0.03 d	3.64 ± 0.03 c	0.797 ± 0.013 c	0.830 ± 0.001 b	7.46 ± 0.07 de	55.7 ± 0.6 cd
Gelatine 10 g/hL	14.0± 0.1 a	5.16 ± 0.01 a	3.77 ± 0.01 a	1.93 ± 0.01 a	0.32 ± 0.01 a	0.97 ± 0.12 a	3.15 ± 0.01 ab	3.82 ± 0.01 a	0.820 ± 0.003 ab	0.830 ± 0.001 b	7.78 ± 0.02 a	55.3 ± 0.6 de
Gelatine 20 g/hL	14.1 ± 0.0 a	5.16 ± 0.01 a	3.77 ± 0.01 a	1.95 ± 0.01 a	0.29± 0.01 a	1.13 ± 0.15 a	3.01 ± 0.04 d	3.63 ± 0.05 c	0.797 ± 0.024 c	0.833 ± 0.005 b	7.54 ± 0.13 cd	53.7 ± 0.6 f
Vegetable protein (pea protein) 20 g/hL	14.1 ± 0.1 a	5.16 ± 0.01 a	3.77 ± 0.02 a	1.94 ± 0.02 a	0.29 ± 0.01 a	1.03 ± 0.12 a	3.16 ± 0.01 a	3.81 ± 0.01 a	0.827 ± 0.008 a	0.830 ± 0.001 b	7.79 ± 0.02 a	55.7 ± 0.6 cd
Vegetable protein (pea protein) 40 g/hL	14.0 ± 0.0 a	5.15 ± 0.01 a	3.78 ± 0.01 a	1.93 ± 0.01 a	0.29 ± 0.00 a	0.97 ± 0.15 a	3.00 ± 0.05 d	3.63 ± 0.05 c	0.780 ± 0.012 d	0.830 ± 0.002 b	7.42 ± 0.11 e	54.7 ± 0.6 e
Significance (*P*)	0.183	0.147	0.105	0.629	0.494	0.941	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

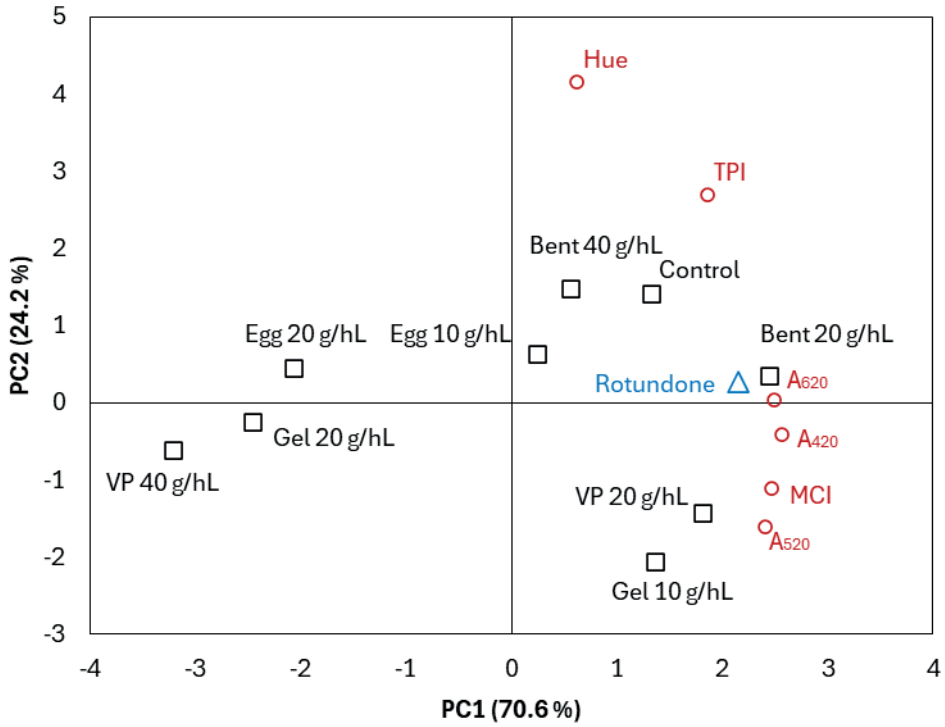


FIGURE 1. Factor loadings and scores for a principal component analysis (PCA) performed on classical oenological analysis data, using only significant variables ($P < 0.05$) and rotundone concentration as an additional data point (triangle marker) for the 9 studied treatments. Bent, Egg, Gel and VP stand for *bentonite*, *egg albumin*, *gelatine* and *vegetable protein (pea protein)*, respectively.

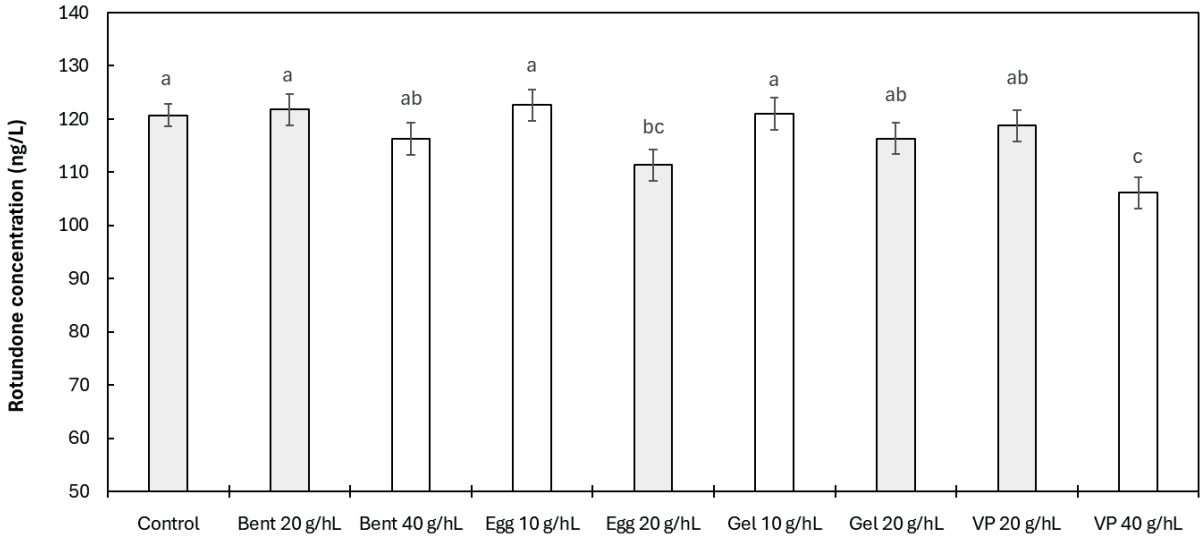


FIGURE 2. Impact of fining agents on rotundone concentration. Bent, Egg, Gel, and VP stand for *bentonite*, *egg albumin*, *gelatine*, and *vegetable protein (pea protein)*, respectively. The control and fining agents used at D1 (20 g/ hL) are highlighted in grey. The initial target concentration of rotundone in the base wine, prior to conducting the trial, was set at 120 ng/L. Different letters indicate means significantly different at $P < 0.05$ by Fisher test. Error bars are 95 % confidence intervals.

5 and 15 g/hL (Kumar & Suhag, 2024). Nevertheless, at the same usage concentration of 20 g/hL, such a fining agent had the largest effect on rotundone in trend, which could be due to the highest surface charge densities and lower degree of hydrolysis previously described (Cosme *et al.*, 2007).

It is noteworthy that the use of bentonite at 40 g/hL, the highest dosage applied only in our study for this agent and *vegetable protein (pea protein)*, exerted a limited effect on rotundone concentration in wine, suggesting minimal interaction between bentonite and this compound.

This experimental conclusion is surprising given previous work in quantum chemistry conducted by our research group on the interactions between organic molecules and clays. Indeed, the cation of bentonite coordinates two basic centres of the organic molecule (Mignon *et al.*, 2009), generally resulting in strong interactions, such as the –27.4 kcal/mol (–114.6 kJ/mol) calculated between atrazine and bentonite (Belzunces *et al.*, 2017). Atrazine is an herbicide banned in Europe since 2003, but still frequently detected in drinking water. This continued presence is largely attributed to atrazine's strong adsorption onto soil clays like bentonite, followed by its gradual release into the soil solution, a process influenced by pH and several physical and chemical factors.

To get preliminary answers on rotundone-bentonite complexation, the interactions between rotundone and a bentonite model were investigated in the framework of planewave periodic DFT (PBE functional plus dispersion corrections: PBE-D2/pw). The geometry of a complex between rotundone and bentonite was first determined (Figure 3). In this complex, the rotundone molecule was spread over the bentonite surface, and the interaction site involved the oxygen atom of the rotundone carbonyl group and one of the cations present at the bentonite surface. The High-Performance Computing (HPC) resources of CALMIP supercomputing centre (Contract grant number: P1222) were required using 4 nodes and 18 tasks per node, accounting finally for about 800 hours of Central Processing Unit (CPU) time. It must be highlighted that the bentonite model had previously been investigated within our research group, which may have contributed to accelerating the current calculations. Based on the computed energies, the complexation energy between rotundone and bentonite for this complex was determined to be –31.2 kcal/mol (–129.7 kJ/mol), with 60 % of this energy coming from dispersion phenomena linked to the size of the adsorbed molecule on bentonite. High absolute values of complexation energy are indicative of strong molecular interactions, and this greater energy than the one observed for atrazine rules out the hypothesis of minimal interaction.

However, these unexpected findings should be interpreted with caution and considered preliminary, as they were obtained using a simplified model containing only bentonite and rotundone, without accounting for wine macromolecules. Nevertheless, they open an interesting avenue for applying theoretical chemistry approaches to help interpret experimental results in wine chemistry. Indeed, considering the other macromolecular constituents of wine, such as polysaccharides, flavonoids and other phenolic compounds, is essential to understanding the competition for adsorption on bentonite. It is likely that these molecules, which are larger and have more potential interaction sites, will lead to much higher complexation energies with bentonite (in particular, due to dispersion effects), thus taking the place of rotundone. In the longer term, to cover all the fining agents used in this article, calculations involving *vegetable protein (pea protein)*, *gelatine* and *egg albumin* need to be carried out. For these fining agents and the wine components mentioned above, one difficulty arises from the varied 3D structures of these natural compounds, which are also difficult to access experimentally. However, the resolution of these structures is essential for fruitful theory/experiment collaboration. Moreover, additional investigations would also be necessary to get insight into the role of the solvent (a mix of ethanol/water) in the adsorption process of these molecules.

A significant correlation at $P < 0.05$ ($R^2 = 0.85$; n = 9) was observed between rotundone and A_{620} (Figure 4) for the studied treatments using mean data across replicates which suggests that this proxy can be used by winemakers, when performing fining trials on Tardif red wines, to predict the impact of this winemaking operation on the pepper aroma compound. Interestingly, although the relationship remained statistically significant ($P < 0.05$), it was weaker when replicate data were used ($R^2 = 0.42$; n = 27), potentially indicating limited robustness of the association. Even though no specific research has been conducted, it can be assumed that polymeric pigments sharing a similar affinity to rotundone for binding to fining agents and absorbing specifically at 620 nm might be involved. These findings are

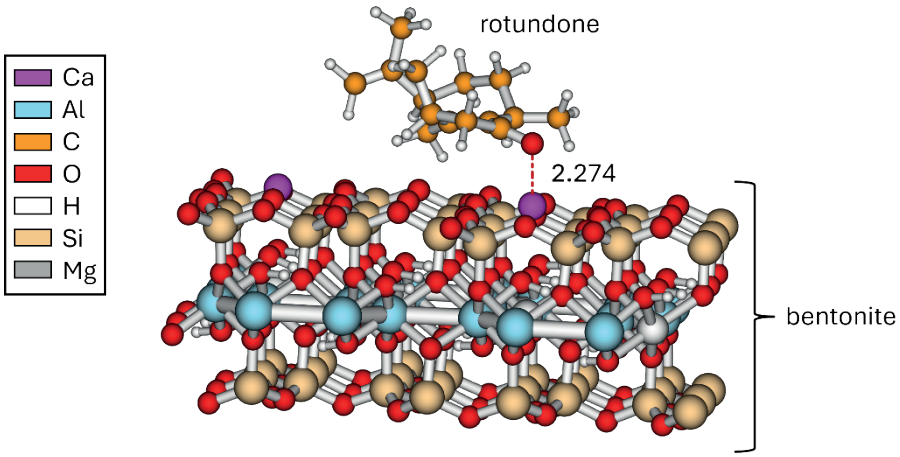


FIGURE 3. Geometry of the studied complex between rotundone and bentonite at the PBE-D2/pw level of theory. The O-cation distance is given in Å.

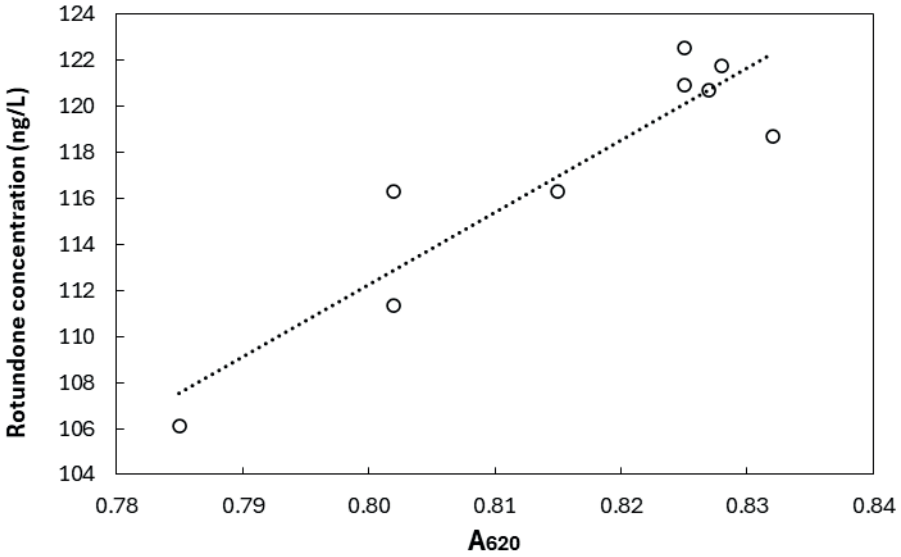


FIGURE 4. Relationship between rotundone concentration and A_{620} measured on wine. Mean values of 3 replicates per treatment (n = 9). Linear model gave y = 314.1x - 137.44; R^2 = 0.85.

only valid for Tardif red wine and might not be transferable to other wines in which rotundone has been previously identified, such as those made from Syrah, Duras or Gamay (Geffroy *et al.*, 2020).

It is also worth mentioning that the dosages tested in our work covered the usual range of oenological applications, particularly for *gelatine*, *egg albumin* and *vegetable protein (pea protein)*. However, though the use of bentonite for red wine fining is less frequent, the dosage can go up to 100 g/ hL for this agent. The impact of other fining agents, such as polyvinylpolypyrrolidone (PVPP), a synthetic agent that has a strong affinity with phenolic compounds frequently used on white musts, would deserve to be investigated. Nevertheless, PVPP is less popular in the industry for red wines due to its higher cost of implementation and its plastic nature (G. Nogues, unpublished).

CONCLUSION

For the first time, our study identified a significant impact of fining on the varietal aroma of a red wine made from *Vitis vinifera* L. cv. Tardif. Overall, this oenological practice led to a modest reduction in rotundone concentrations, which may not be perceptible from a sensory standpoint. The greatest decrease observed was approximately 12.1 % when vegetable protein (pea protein) was applied at 40 g/hL.

A significant correlation was also observed between rotundone concentration and A_{620} (R^2 = 0.85) based on mean values from three replicates, suggesting that A_{620} could potentially serve as a proxy in fining trials to estimate the impact on rotundone in Tardif red wines. However, as these results are preliminary and specific to a single cultivar, further studies, including those incorporating computational chemistry, will be necessary to confirm this relationship, extend the findings to additional fining agents and elucidate the underlying mechanisms.

Overall, these results provide reassurance to winemakers, particularly those working with Tardif, that the use of fining agents is unlikely to cause substantial losses of rotundone.

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