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Preliminary exploration of polysulfide detection in wine using fluorescent probe SSP4 and A-TEEM spectroscopy

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ABSTRACT

Polysulfides have emerged as a novel class of sulfur compounds in wine that continue to be explored and characterised, but analytical challenges still remain. The fluorescent probe SSP4, developed for sulfane sulfur detection in biological systems, was preliminarily evaluated for its potential applicability to wine analysis. Glutathione trisulfide (GS-S-SG) was employed as a standard polysulfide, and the reaction with SSP4 was monitored in phosphate buffer and wine matrices using absorbance-transmittance and fluorescence excitation-emission matrix (A-TEEM) spectroscopy. The SSP4 probe produced a strong fluorescence signal with excitation at 495 nm and emission at 515 nm in buffer solution. Excitation maxima shifts detected in wine were suggestive of solution composition effects upon probe activation. GS-S-SG added to diluted or undiluted wine resulted in variable responses, indicating the presence of endogenous polysulfide or sulfane sulfur intermediates in wine. These initial findings demonstrated the feasibility of SSP4-based fluorescence detection for wine polysulfides, with a need to address matrix-related factors potentially as a consequence of inner filter effects or endogenous polysulfide equilibria. Further investigation of probe activation conditions is therefore warranted to enable quantitative applications in wine.

KEYWORDS: sulfane sulfur, thiol derivative, fluorescence spectroscopy, excitation-emission matrix, wine analysis

INTRODUCTION

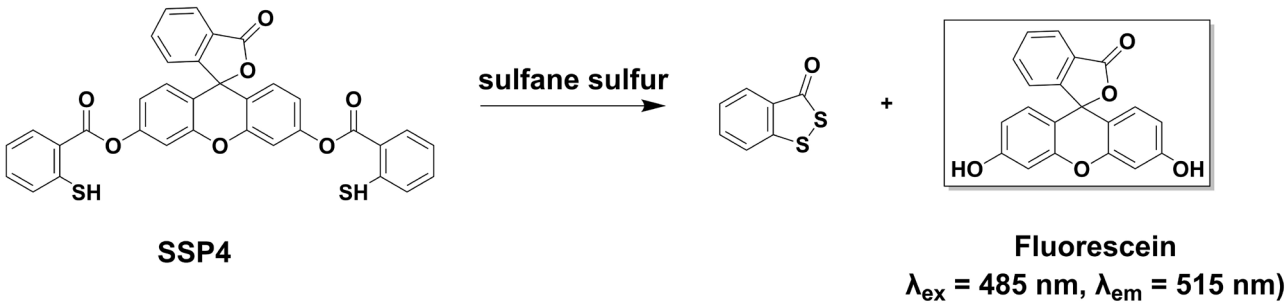
Sulfur chemistry has been investigated in fermented foods and beverages such as wine for decades due to its relevance to flavour perception (Landaud *et al.*, 2008; Mestres *et al.*, 2000; Smith *et al.*, 2015; Swiegers & Pretorius, 2007). Grapes mainly contain inorganic sulfur such as elemental sulfur and sulfate, whereas most organic sulfur forms are produced during fermentation (Waterhouse *et al.*, 2024a). Different volatile sulfur compounds (VSCs) can produce contrary sensory impacts, contributing desirable tropical fruit aromas in the case of certain thiols (Coetzee & du Toit, 2012; Roland *et al.*, 2011) or sulfur-like off-odours (SLOs) considered as wine faults for other sulfhydryl compounds and sulfides (Smith *et al.*, 2015; Waterhouse *et al.*, 2024a). The decomposition of non-volatile sulfur compounds can also contribute to the later occurrence of SLOs during storage (Bekker *et al.*, 2018b; Müller & Rauhut, 2018; Müller *et al.*, 2022). Methods are therefore required to better understand the development of SLOs and to predict wine shelf-life.

Polysulfides (diorganopolysulfanes) of the form RSS_nSR' are a group of sulfane sulfur compounds (*i.e.*, containing sulfur bound to other S-atoms) formed in wine (Müller *et al.*, 2022), especially in the presence of copper added to remediate SLOs (Kreitman *et al.*, 2017; Waterhouse *et al.*, 2024b). They can originate from VSCs bearing a sulfhydryl group (–SH) and non-volatile thiols such as glutathione (GSH) and cysteine (Cys), and occur in symmetrical and asymmetrical forms. Importantly, they may serve as latent sources of VSCs (Bekker *et al.*, 2018a; Hou *et al.*, 2025a), with their degradation during storage potentially contributing to SLO reocurrence (Kreitman *et al.*, 2019). Polysulfides with a sulfur chain length of up to seven sulfur atoms have been reported in wine and wine-like matrices (Bekker *et al.*, 2018a; Dekker *et al.*, 2022a; Dekker *et al.*, 2022b; Dekker *et al.*, 2020; Kreitman *et al.*, 2017; Nardin *et al.*, 2020; Pilkington *et al.*, 2019). Analysis of polysulfides is not a trivial undertaking, however, due to their relatively low abundance and instability. Typically, high-performance liquid chromatography (HPLC) with mass spectrometry (MS) is used, with various methods reported (Bekker *et al.*, 2018a; Dekker *et al.*, 2023; Dekker *et al.*, 2020; Kreitman *et al.*, 2017; van Leeuwen *et al.*, 2020). Recent work characterised the degradation kinetics of wine GSH and Cys polysulfides in aqueous solution (Hou *et al.*, 2025a) and described the occurrence of polysulfides in a selection of commercial fermented beverages, including wine (Hou *et al.*, 2025b).

Despite the power of modern chromatographic instrumentation, spectroscopic approaches have attracted attention as complementary tools for wine analysis. Benefits include faster measurement, lower cost, and minimal sample preparation (Basalekou, 2025; Lohumi *et al.*, 2015). Along with the conventional spectroscopic tools such as UV-Vis, Fourier transform infrared (FTIR), near and mid-infrared (NIR and MIR), and nuclear magnetic resonance (NMR) (Ranaweera *et al.*, 2021a), a more recent development is the absorbance-transmittance and fluorescence excitation-emission matrix (A-TEEM™) approach (Quatela *et al.*, 2018). The A-TEEM method simultaneously generates multidimensional spectral data for chromophores and fluorophores in a sample by recording absorbance data along with an excitation-emission matrix (EEM). When coupled with chemometric modelling, it has been utilised in wine research to classify varieties and regions, to quantify phenolic and colour compounds, and for wine authentication purposes (Armstrong *et al.*, 2023; Gilmore *et al.*, 2022; Ranaweera *et al.*, 2021c; Wang & Jeffery, 2024). These studies have demonstrated the potential of A-TEEM to complement chromatographic analyses, yet the application to sulfur chemistry in wine remained unexplored. One potential impediment to be addressed is the lack of chromophoric or fluorophoric moieties within compounds such as polysulfides.

Fluorescent probes offer a promising strategy for detecting sulfane sulfur atoms in biological systems, providing simplicity, high sensitivity and selectivity, and timely analysis without sophisticated sample preparation (Prabakaran & Xiong, 2025; Roy *et al.*, 2023; Zeng *et al.*, 2021). A range of fluorescent probes has been developed and applied for inorganic hydrogen polysulfide (H_2S_n, $n > 1$, also known as polysulfane) detection in studies ranging from living cells to application in food samples (Liu *et al.*, 2022; Takano *et al.*, 2017; Wang *et al.*, 2020; Zhang *et al.*, 2024). A selective probe termed sulfane sulfur probe 4 (SSP4) has been reported to detect GSH- and Cys-derived polysulfides in HEPES buffer and cells (Li *et al.*, 2019; Shieh *et al.*, 2022). Originally developed to identify sulfide metabolites in cells (Sakaguchi *et al.*, 2014), SSP4 remains non-fluorescent until reaction with sulfane sulfur species, which cleaves its protecting groups. This releases fluorescein with a strong fluorescence signal at certain excitation and emission wavelengths (λ_{ex} = 485 nm, λ_{em} = 515 nm) (Scheme 1) (Shieh *et al.*, 2022).

A previous study utilising high-performance liquid chromatograph-tandem mass spectrometry (HPLC-MS/MS) highlighted that wines contain relatively abundant disulfides, whereas the polysulfides reported so far are mainly GSH tri- and tetrasulfides, and mixed Cys/GSH tetrasulfide (Hou *et al.*, 2025b). Accurate quantitative information is critical to better understanding the relationship between polysulfides and SLOs in wine, but timely access to data is also important in an industry context. Seeking a simple and rapid technique that could be suitable for use in a predictive assay, the present study aimed to provide a preliminary exploration of SSP4 as a fluorescent probe for polysulfide detection in wine. Using a synthesised GS-S-SG standard, SSP4 spectroscopic responses were evaluated in buffer and wine matrices through simultaneous absorbance and fluorescence measurements based on A-TEEM. This appeared to be the first attempt to apply a sulfane sulfur probe in wine chemistry, providing initial insights into the feasibility and limitations of this approach for analysis of polysulfides in wine.



SCHEME 1. Principle of SSP4 fluorescence activation upon reaction with sulfane sulfur (Shieh *et al.*, 2022).

MATERIALS AND METHODS

1. Chemicals and wines

Dimethyl sulfoxide (≥99.5 %, DMSO), hexadecyltrimethylammonium bromide (≥96 %, abbreviated as CTAB), sodium chloride (≥99 %, NaCl), potassium chloride (≥99 %, KCl), sodium phosphate dibasic (≥99 %, Na_2HPO_4), and potassium phosphate monobasic (≥99 %, KH_2PO_4) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). SSP4 (≥95 %) was purchased from Sapphire Bioscience (Redfern, NSW, Australia). Laboratory-grade ethanol (≥95 %, EtOH) was obtained from ChemSupply Australia (Gillman, SA, Australia). Water (18.2 MΩ/cm) was produced from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). One 2020 Shiraz (15.7 % *v/v*, McLaren Vale) and one 2023 Chardonnay (13.5 % *v/v*, South Australia) were sourced from a local winery (Belvidere, SA, Australia).

2. Polysulfide standard solutions

A polysulfide standard was previously synthesised and predominantly consisted of GS-S-SG (~70 %), with minor proportions of disulfide and other polysulfides according to HPLC-MS/MS analysis (Hou *et al.*, 2025b). Synthesised standard was dissolved in Milli-Q water containing 10 % EtOH *(v/v)* to yield a 1 mM solution of GS-S-SG. This stock solution was spiked into phosphate buffered saline (PBS) and into diluted wines to give 0–50 µM GS-S-SG in the final reaction mixtures. For undiluted wines, GS-S-SG was directly prepared at 2 mM in the Shiraz and Chardonnay wines, sonicated at low temperature for dissolution, and subsequently diluted with 50 % EtOH by 37.5-fold and 12.5-fold, respectively, before reaction with SSP4, resulting in a concentration range under 40 µM.

3. Reaction protocol with SSP4

The treatment of samples with SSP4 followed a previously reported protocol (Shieh *et al.*, 2022), with a doubling of the final probe concentration based on anticipated polysulfide concentrations. The fluorescence responses of SSP4 towards the synthesised polysulfide standard were conducted in 50 mM PBS buffer matrix (pH 7.4) containing 25 µM CTAB, 10 µM SSP4, and sample (1 mL) spiked with GS-S-SG at various concentrations. The final dilution factors of 150-fold for Shiraz and 50-fold for Chardonnay were consistent with the protocol for A-TEEM analysis of wines (Ranaweera *et al.*, 2024). Reactions were gently mixed and then incubated in the dark at room temperature (20 min, 20 °C) before fluorescence spectra were acquired.

4. A-TEEM spectroscopy

Spectral data were recorded with an Aqualog spectrophotometer (UV-800-C, HORIBA Scientific, Quark Photonics, Adelaide, SA, Australia). Fluorescence measurements were performed with an integration time of 0.2 s, using an excitation wavelength range of 240–700 nm (5 nm increments) and emission range of 242–824 nm (4.66 nm increments). Instrument settings included a saturation mask width 10 nm, medium detector gain, and automatic spectral pre-processing with inner filter effect correction and Rayleigh masking. The fluorescence data were normalised against a sealed, high-purity water standard for Raman scattering, and blank EEMs were subtracted according to the reported procedure (Gilmore *et al.*, 2022; Ranaweera *et al.*, 2024).

5. Data analysis

EEM datasets were exported from the Aqualog software (version 4.3, HORIBA Scientific). The maximum intensity at each wavelength was determined using Microsoft Excel Professional Plus 2019, and excitation and emission spectra were visualised using GraphPad Prism (version 10.1.0, GraphPad Software Inc., La Jolla, CA).

RESULTS AND DISCUSSION

1. Initial assessment of SSP4 with polysulfide solution

The fluorescence behaviour of wine-related polysulfides in the presence of SSP4 was first investigated in PBS solution containing increasing concentrations of GS-S-SG (0–50 µM). Distinct fluorescence signals were observed in all spiked samples, with excitation at 485–490 nm and emission at 510–515 nm (Figure 1), whereas the blank (SSP4 reaction mixture in the absence of polysulfide solution) effectively showed no fluorescence response. These outcomes were consistent with the previous work, which reported an excitation wavelength of 482 nm and emission wavelength

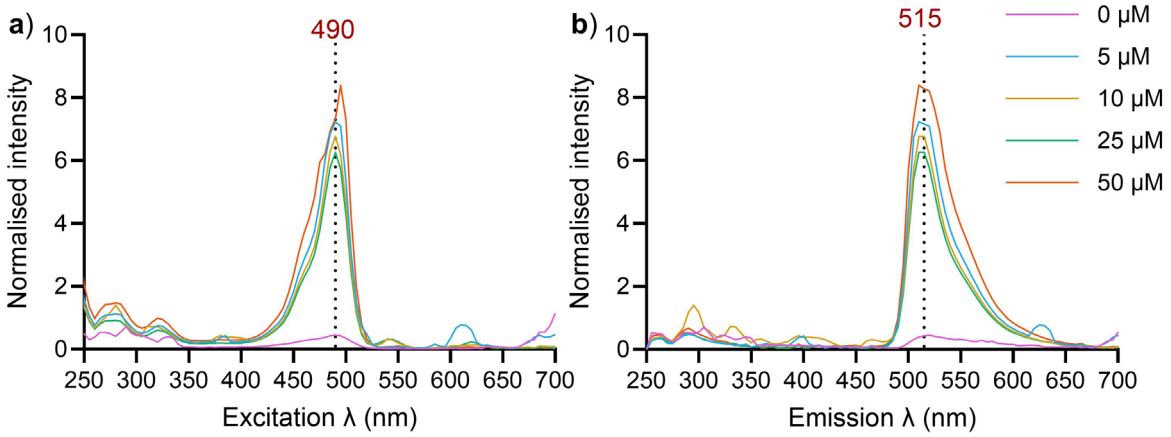


FIGURE 1. Overlay of normalised fluorescence spectra showing a) excitation and b) emission wavelengths at a consistent maxima for 10 µM SSP4 in PBS with increasing concentrations of GS-S-SG (0–50 µM).

of 514 nm (Shieh *et al.*, 2022). Fluorescence intensity generally increased with GS-S-SG concentration, with the strongest signal observed at 50 µM. However, the response did not follow a strictly linear concentration-dependent trend, as 5 µM GS-S-SG produced a stronger signal than additions of 10 and 25 µM. This irregular trend might be due to the heterogeneous composition of the polysulfide standard, which contained GSH (very minor component), GSSG, and several polysulfide species, although GS-S-SG was predominant (Hou *et al.*, 2025b). GSH and GSSG were reported to yield no significant fluorescence with SSP4, in accord with the probe's selectivity towards sulfane sulfur (Shieh *et al.*, 2022). Polysulfides varying in chain-length have different reactivity (Hou *et al.*, 2025a) and longer chain polysulfides have multiple S-S groups. As such, the variable amounts of releasable sulfane sulfur due to the somewhat polydisperse mixture of polysulfides may have altered the simple concentration-response relationship, leading to the non-linear fluorescence trends. Further optimisation of the reaction conditions could be undertaken, but the outcome nonetheless demonstrated the fluorescence activation of SSP4 in the presence of the synthesised polysulfide mixture. Attention therefore moved to assessing the fluorescent probe approach for detection of polysulfides in wine.

2. Detection of GS-S-SG in wine matrix

2.1. SSP4 response in diluted wine spiked with GS-S-SG

As popular varietals produced in Australia, a Shiraz and a Chardonnay were selected for the wine trials. Previous work revealed higher GS-S-SG levels in red wines than white wines (median ~0.02 µM *vs* ~0.003 µM, respectively), whereas the mixed Cys GSH tetrasulfide (Cys-S_2-SG) was found at relatively similar levels (median of 0.024 µM as GS-S_2-SG equivalents) across wine types (Hou *et al.*, 2025b). Wines might contain additional polysulfide species beyond those detected so far, although the abundance of GS-S-SG provided a rational basis for probe investigation. Given that SSP4 contains two sulfane sulfur reaction sites (Shieh *et al.*, 2022), 10 µM of SSP4 was used in the PBS trials to ensure an excess of probe for the expected polysulfide concentrations in wine.

Wine requires dilution for A-TEEM analysis to maintain the absorbance at 280 nm within the range of 0.2–1 a.u., thereby minimising inner filter effects and background fluorescence (Ranaweera *et al.*, 2021b). Acidified aqueous EtOH (50 % *v/v*, pH 2) typically used for A-TEEM analysis of wine interfered with the SSP4 reaction with GS-S-SG (data not shown). This effect can be attributed to the presence of uncharged thiophenol groups on SSP4 under acidic conditions, lowering sulfur nucleophilicity (compared to a thiolate anion) and limiting attack on the sulfane sulfur: pH in the range of 6.5–8 was found to work best (Shieh *et al.*, 2022). Therefore, Shiraz and Chardonnay wines were instead diluted with unacidified 50 % aqueous ethanol. After spiking with GS-S-SG at the requisite concentration, the reaction with SSP4 in PBS provided a further 4-fold dilution. The final dilution factors were 150-fold for Shiraz and 50-fold for Chardonnay, which consistently yielded strong and reproducible fluorescence signals.

Fluorescence spectra of wine and PBS matrices were compared at the same GS-S-SG spiking level (0 and 25 µM, Figure 2). In the blank samples (Figures 2a and 2b), noticeable absorbance and fluorescence associated with the probe were only observed in Shiraz, indicating the presence of inherent polysulfides. A minor shift in excitation maxima was observed in Shiraz (495 nm), whereas the reaction of SSP4 in Chardonnay or PBS showed a maxima at 490 nm (Figure 2c). The slight difference is unlikely to affect the overall interpretation when using this fluorescent probe. In all cases, the emission maximum remained consistent at 515 nm (Figure 2d). Additionally, the wine matrices exhibited characteristic UV fluorescence bands (excitation at 265–270 nm, emission at 305–375 nm), which can be attributed to usual wine components such as flavan-3-ols, anthocyanins (in the case of red wine), aromatic amino acids, hydroxybenzoic acids, phenolic acid/aldehydes, and flavonols (Airado-Rodríguez *et al.*, 2011; Fan *et al.*, 2025).

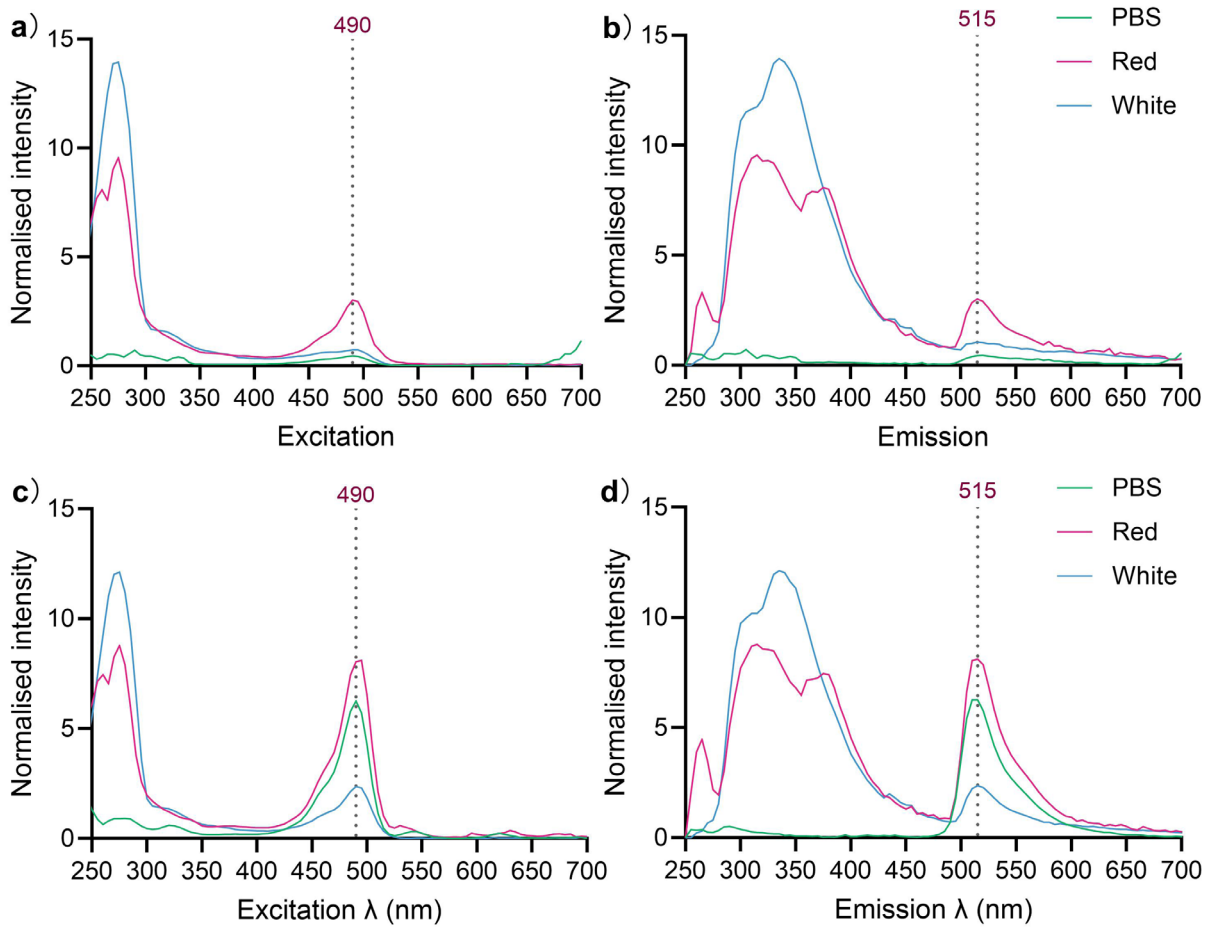


FIGURE 2. Overlay of normalised fluorescence spectra showing a) excitation and b) emission wavelength maxima for 10 µM SSP4 in blank PBS, Shiraz, and Chardonnay matrices; c) excitation and d) emission wavelength maxima of 10 µM SSP4 with 25 µM GS-S-SG spiked in PBS, Shiraz, and Chardonnay matrices.

A series of GS-S-SG spiking experiments were conducted in diluted Shiraz and Chardonnay to further evaluate the probe response. EEMs were acquired for both wines at different spiking levels (0–50 µM, Figure 3). The untreated Shiraz (Figure 3a) and Chardonnay (Figure 3b) reflected their inherent polysulfide levels (or lack of). The results were somewhat indicative of previous findings showing that red wine generally contained higher levels than white wine (Hou *et al.*, 2025b). For each sample, fluorescence intensities for excitation at 490–495 nm and emission at 515 nm were extracted from the EEMs (Figures 3c–3j), and mean values from duplicates were used to generate concentration-response plots (data not shown). The regression for Shiraz yielded an R^2 of 0.958 and for Chardonnay, R^2 was 0.978 after excluding the anomalous 50 µM point (Figure 3j). The pronounced fluorescence intensity observed at 50 µM possibly reflected a matrix-specific effect. Owing to lower dilution applied to Chardonnay compared with diluted Shiraz (12.5× *vs* 37.5×), higher residual levels of endogenous components may interfere with GS-S-SG, especially at lower concentrations of the latter. At 50 µM, the active wine compounds were likely depleted via reaction, although the resulting fluorescence intensity of the Chardonnay in this case (~6.06 a.u.) was still only comparable to that observed in Shiraz with 10 µM GS-S-SG (~5.25 a.u.). Further improvement is therefore required and could be envisaged upon optimising the method for quantitative purposes. Notably, the additional spectral data available from EEMs could be useful for future multivariate data modelling compared to the single wavelength maxima that was extracted in the present work.

2.2. SSP4 response in wine spiked with GS-S-SG and subsequently diluted

The experiments on wine dilution prior to addition of the probe were used to test for an appropriate dilution factor and to yield an obvious result by minimising possible background interferences. Simulating a more practical analytical workflow, GS-S-SG was first spiked into Shiraz and Chardonnay, after which the wines were subsequently diluted with 50 % aqueous EtOH and reacted with SSP4 as before to achieve the same final dilution factors. This approach was intended to evaluate the feasibility of a more usual procedure and to verify the ability of SSP4 to detect the polysulfides pre-existing in an undiluted wine matrix.

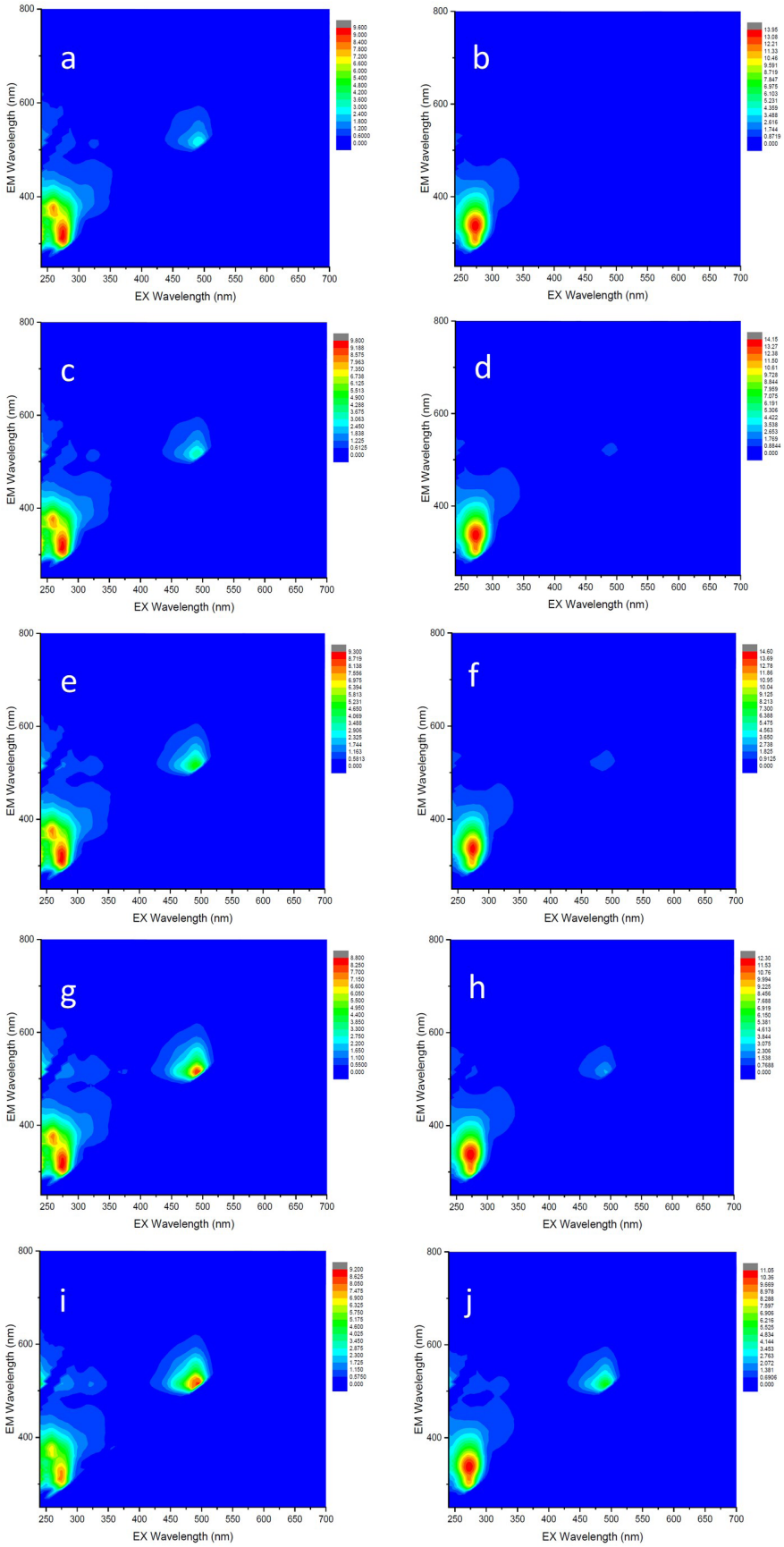


FIGURE 3. Fluorescence excitation and emission matrix (EEM) contour plots of 10 µM SSP4 reaction with increasing concentrations of GS-S-SG spiked in diluted Shiraz (a, c, e, g, i) and Chardonnay (b, d, f, h, j) wines. For each variety, the GS-S-SG concentration increases down the column as follows: 0 µM (a, b), 5 µM (c, d), 10 µM (e, f), 25 µM (g, h), 50 µM (i, j).

Dissolving GS-S-SG directly in wine was challenging, even with sonication. Warming the solution may have assisted but polysulfides are more unstable at elevated temperatures (Hou *et al.*, 2025a). A 2 mM GS-S-SG stock solution in each wine was prepared after several attempts, giving final concentration ranges of 0.17–13.33 µM in Shiraz and 0.1–40 µM in Chardonnay (lower dilution applied) after dilution with the matching wine.

Fluorescence spectra of both wines (Figure 4) showed a shift in excitation maxima from 490–495 nm to 500–505 nm when final GS-S-SG levels exceeded 3.3 µM in Shiraz and 5 µM in Chardonnay, whereas emission at 515 nm was consistent. The results suggested that the same fluorophore (*i.e.*, from the probe) was responsible for the response, and the emissive fluorescein state remained unchanged compared to the earlier trials (Lakowicz, 2006a). The shift in excitation was potentially driven by matrix-dependent inner filter or microenvironmental effects occurring upon SSP4 activation, since the final dilution factors (and therefore concentration of natural matrix components) were identical for a given wine. It was unlikely that the small difference in ethanol concentration in the final diluted samples (~0.5 % *v/v*) would cause such an effect, although that would need to be tested.

A concentration-dependent excitation shift phenomenon has been investigated by Divya and Mishra (2008), who indicated that the inner filter effect can induce an apparent shift, especially under right-angle detection geometry as employed with A-TEEM. However, no such shift was evident in wine matrices that were diluted before GS-S-SG addition, suggesting a microenvironmental effect may have contributed. When GS-S-SG was added into undiluted wines, intrinsic wine constituents such as SO_2, GSH, or trace metals (*e.g.*, Cu^{2+}) could influence polysulfide profiles via sulfitolysis (Bekker *et al.*, 2018a; Hou *et al.*, 2025b) or thiol-related formation of persulfides (Cerda *et al.*, 2017; Hou *et al.*, 2025a). Cu^{2+} was reported to exert a significant inhibitory effect on SSP4 (Shieh *et al.*, 2022), which needs to be considered in any future work with wine. Moreover, in the present case, the concentration of copper species in the wine was the same across the different levels of GS-S-SG, making it an unlikely cause of the observed excitation shift. Localised variations in polarity and solvent relaxation within the wine matrix could further stabilise the ground or excited states of molecules (fluorescein in this case), lowering the excitation energy and leading to a shift to longer wavelengths (Lakowicz, 2006b).

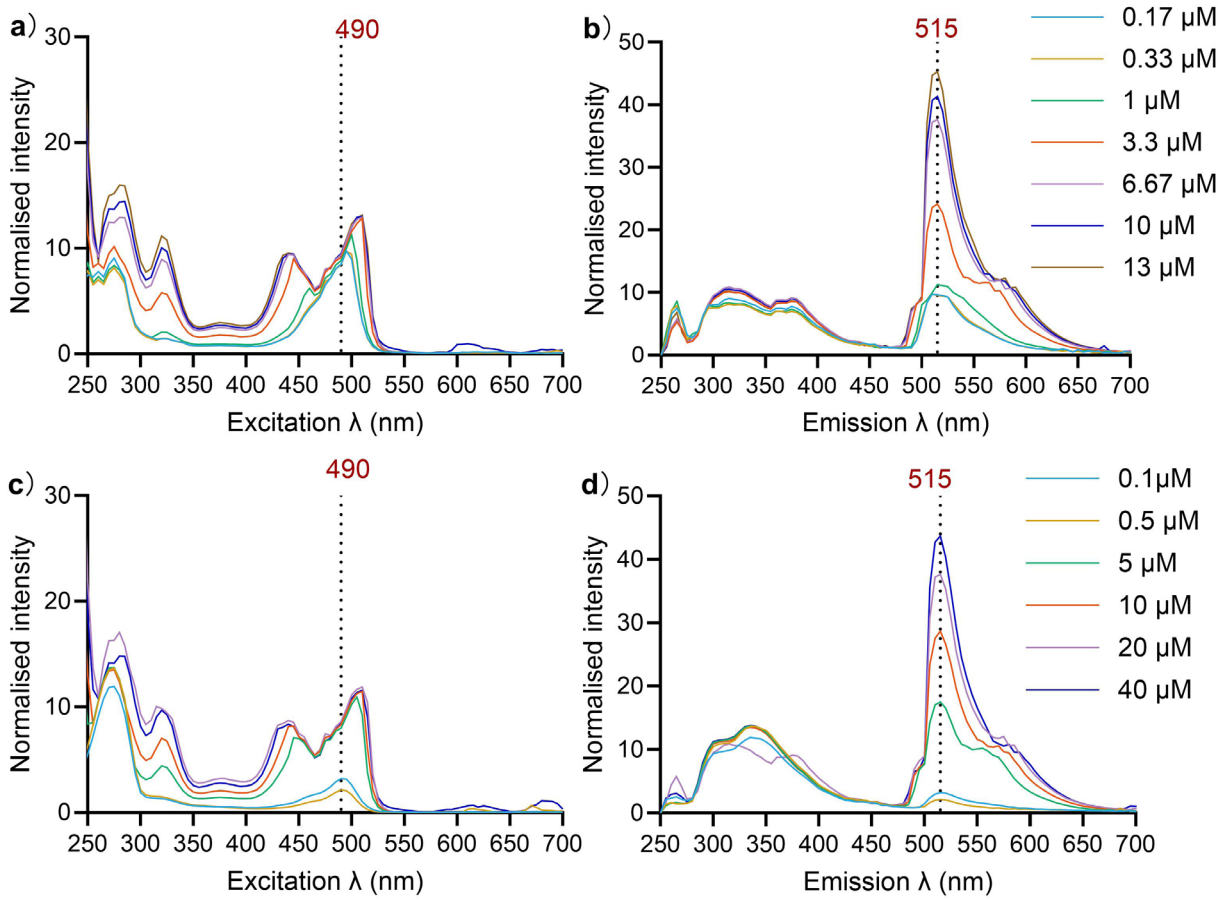


FIGURE 4. Overlay of normalised fluorescence spectra showing a) excitation and b) emission spectra for Shiraz and c) excitation and d) emission spectra for Chardonnay with 10 µM SSP4 reacting with increasing concentrations of GS-S-SG added prior to dilution of wines with 50 % aqueous ethanol. Final concentration range: Shiraz, 0.17–13.3 µM; Chardonnay, 0.1–40 µM.

Within each wine matrix, normalised intensity at excitation 495–500 nm/emission 515 nm was extracted from the EEMs, and mean values from duplicates were used to generate concentration-response plots (data not shown). Both Shiraz and Chardonnay exhibited an increase followed by a plateau in fluorescence intensity with the increasing GS-S-SG concentration, with R^2 values of 0.82–0.84. The behaviour was somewhat reminiscent of the study by Shieh *et al.* (2022), who reported a linear response of 5 µM SSP4 reacting with Na_2S_2 (a model sulfane sulfur source) at up to 15 µM before reaching a plateau. In aqueous solution, Na_2S_2 undergoes rapid hydrolysis and disproportionation to yield hydrogen persulfide (Cuevasanta *et al.*, 2017; Xu *et al.*, 2020), however, which may account for the response reported by Shieh *et al.* (2022) and could suggest that SSP4 reacts more efficiently with persulfide than GS-S-SG due to variable sulfane sulfur reactivity. The observed patterns with plateau occurring much earlier (at 1 µM in Shiraz and 5 µM in Chardonnay) in the present case might instead reflect a reactant-limited process in which SSP4 was fully consumed and converted to its fluorescent form regardless of the matrix. That would indicate the presence of abundant pre-existing sulfane sulfur containing species in wine (including hydrogen persulfide), which potentially contributed to SSP4 consumption, although their identities remained unclear.

Notably, the overall fluorescence responses that reached a plateau in Figure 4 (~11.5 a.u. in Shiraz and ~10 a.u. in Chardonnay) were higher than those observed in Figure 3 (below 9 a.u.), despite the identical final dilution factors for respective varieties. This enhancement suggested that the pre-addition of GS-S-SG at high concentration (2 mM) may have perturbed the polysulfide equilibrium in the wine matrix, generating more reactive sulfane sulfur species (*e.g.*, H_2S_2) or intermediates with more sites available for reaction with SSP4. Whatever the case, the proof of concept for using this fluorescent probe to detect wine polysulfides was successful, although probe concentration could be optimised in future work.

CONCLUSION

The chemistry of polysulfides in wine is gradually being understood, but a gap remains in the ability to predict wine shelf-life with respect to the reocurrence of SLOs. This study investigated the feasibility of combining sulfane sulfur probe SSP4 with A-TEEM spectroscopy for detecting polysulfides in wine via fluorescence. The work preliminarily established that SSP4 can be used to detect a GSH polysulfide within complex wine matrices. A-TEEM was demonstrated as sensitive and information-rich and may serve as a rapid quantitative tool when coupled with a fluorescent probe such as SSP4, providing simultaneous capture of fluorescence dynamics and concentration-dependent responses. The findings emphasised the importance of understanding probe interactions, however, when adapting biological fluorogenic systems for oenological applications. Further investigation is warranted to improve SSP4 performance (or that of another probe) through optimised reaction conditions (*e.g.*, assessment of dilution or interferences) and calibration strategies (*e.g.*, using EEM variables and machine learning regression) across different wines, ultimately leading to a validated quantitative measure of wine sulfane sulfur compounds. Overall, this work offers a new perspective for polysulfide detection in wine. It provides a foundation for future development of rapid, fluorescence-based approaches that complement an evaluation of wine sulfur chemistry in relation to SLOs.

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