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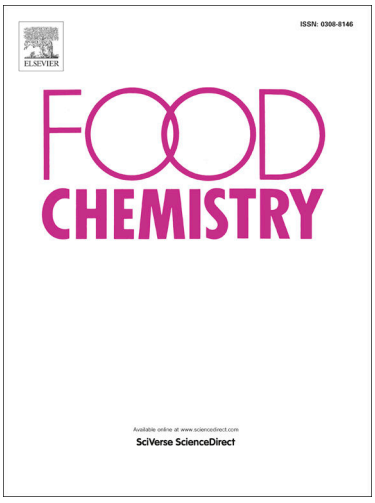


Short communication

# Wine Putrescine Abatement by Bentonites: From Ideal Case to Practice

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# Wine Putrescine Abatement by Bentonites: From Ideal Case to Practice

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**Abstract**

Herein, we demonstrated that bentonites can be incisively used to reduce wine BAs content, especially putrescine molecules. Pioneering kinetic and thermodynamic studies of putrescine adsorption onto two commercially available bentonites (optimal concentration of 0.40 g $dm^{-3}$) were performed resulting in ca. 60% removal by physisorption mechanism. Both bentonites showed also promising results in more complex systems, resulting in a lower putrescine adsorption due to the competition with other molecules (as proteins, polyphenols), typically present in wines. Nonetheless, we managed to reduce the putrescine content below 10 ppm both in red and white wines.

**Keywords**

Bentonites; wine fining; biogenic amines; putrescine; adsorption kinetics.

## 1. Introduction

Biogenic amines (BAs) are non-volatile, low molecular weight organic compounds obtained from the decarboxylation of the corresponding amino acids (Amghouz et al., 2014; Guo et al., 2015; Lonvaud-funel, 2001). They can easily accumulate in food and beverages because of the microbial activity resulting in toxic effects for consumers. In particular, wine is a good substrate for BAs accumulation: its manufacturing process involves available free amino acids, the presence of lactic acid bacteria (considered the microorganisms mainly associated with wine amino acid decarboxylation) (Restuccia et al., 2018; Ribéreau-Gayon et al., 2006), and favourable biological and physicochemical conditions that cannot be easily modified (Costantini et al., 2019; Mir-Cerdà et al., 2021). Several studies reported that histamine, tyramine and putrescine are the most important BAs found in all types of wines: specifically, in white wines, the total BAs content is lower (0-10 mg $dm^{-3}$) than in red ones (0-30 mg $dm^{-3}$) (Costantini et al., 2019; Esposito et al., 2019). Looking over putrescine molecule, it is reported to be the most abundant amine present in wines (Costantini et al., 2013; Mangani et al., 2005) deriving from the degradation of arginine *i.e.*, the α-amino acid of which must is rich (Mira de Orduña et al., 2001; Petrovic et al., 2019). Furthermore, putrescine has been described as toxic, being a precursor of carcinogenic nitrosamines, and also capable of spoiling wine organoleptic profile (Costantini et al., 2013; del Rio et al., 2019; Mangani et al., 2005; Smit et al., 2016). The presence of small amounts of BAs in wine is not usually considered a health risk since, under normal conditions, exogenous amines are quickly detoxified in the organism by amine oxidases (Alvarez & Moreno-Arribas, 2014; Ruiz-Capillas & Herrero, 2019). In addition, since the toxic effect depends on the consumer's sensitivity to BAs, it is hardly difficult to establish maximum limits for these compounds, also because complex interactions may occur between BAs and other substances present in foodstuffs (Mir-Cerdà et al., 2021; Švecová & Janovská, 2021; Vincenzini et al., 2017).

Therefore, to the authors' best knowledge, legislation concerning BAs in wine does not exist yet and just some recommendations especially regarding the upper limit for histamine are given, generally around 10 ppm (Guo et al., 2015). In the case of putrescine, its co-occurrence (at around 13 ppm) together with ethanol and histamine in wine may enhance the latter toxicity, exerting adverse effects already at a low histamine concentration (Esposito et al., 2019). In addition, as demonstrated by Martuscelli *et al.* (Martuscelli et al., 2013) in Abruzzo wines, putrescine has a high correlation factor ($\beta$ of 0.95) with the total BAs. Thus, effective

and selective methods to assess their concentration and to further eliminate or reduce their amount are required. With this aim, herein, we exploited two bentonites, already finely investigated in our previous work as promising adsorbent materials for the reduction of wine haze and turbidity caused by high or medium-molecular weight (MW) proteins (Pargoletti et al., 2021). In this respect, our aim was to deeply unveil for the first time putrescine kinetic and thermodynamic adsorption onto bentonites and the possible competitive adsorption mechanisms in presence of other molecules, as proteins and polyphenols, till the investigation of BA/bentonite interactions in real wines.

## 2. Materials and Methods

All the chemicals were of reagent-grade purity and were used without further purification. Two different activated bentonites (purchased from Enolife S.r.l., Paragraph 1 and Table S1 in the Supporting Information) were adopted (Pargoletti et al., 2021). They were labeled as Na/Ca_x, where x stands for the sodium-to-calcium atomic ratios (0.27, 0.49). All the bentonites are extracted from European soils and they fulfill the requirements of the International Oenological Codex (*International Oenological Codex*, 2015). Putrescine and albumin were purchased from Sigma-Aldrich. The adopted polyphenols come from a Chardonnay extract and they comprise grape flavanols and their dimers (Pargoletti et al., 2021). Then, taking into account the extremely wide range of water hardness values, MilliQ water was used for the tests to have rigorous and reproducible methods.

### *2.1 Putrescine adsorption kinetics and thermodynamics onto bentonites*

Adsorption kinetic and thermodynamic isotherms together with the relative fittings are finely described in Paragraph 2 in the Supporting Information file. The putrescine removal was assessed upon its derivatization with *o*-phtalaldehyde and 2-mercaptoethanol (see Paragraph 2 and Figure S1 in the Supporting Information).

### *2.2 Competition tests*

In order to investigate the selective adsorption of putrescine onto bentonites in a complex matrix as wine, competition tests were performed. In particular, two model wine solutions were prepared consisting in 200 ppm albumin/10 ppm of putrescine or 10 ppm polyphenols/5 ppm of putrescine in EtOH/Water 12% v/v,

at pH 3.20. The concentration of adsorbents was fixed at 0.40 g $dm^{-3}$ and the kinetics lasted 120 min, as previously optimized elsewhere (Pargoletti et al., 2021), performing three consecutive samplings for each interval time. In both competitions, albumin or polyphenols concentration was followed through UV/Vis spectroscopy.

*2.3 Real cases*

In order to evaluate the potentialities of the two bentonites towards biogenic amines and, above all, putrescine removal, tests on commercial wines were performed. Specifically, *Chardonnay* white and *Primitivo* red wines were adopted (see physicochemical features in Table S2). As in the case of model wine, bentonites concentration was fixed at 0.40 g $dm^{-3}$ and the adsorption lasted 120 min. Each sampling was centrifuged and analyzed by High-Performance Liquid Chromatography (HPLC) coupled with a diode-array detector to determine both putrescine and total biogenic amines (namely amylamine, cadaverine, phenethylamine, isobutyl amine, histamine, methylamine, n-propylamine, putrescine and tyramine) concentrations. To test the precision of the method, three replicates were performed consecutively, and the computed standard deviations never exceeded the 5% of the corresponding value.

Concomitantly, a set of measurements aimed at evaluating the haze formation in both untreated and bentonite-clarified real wines were carried out. All the samplings were passed through a 0.45 μm filter, heated for 2 h at 80 °C and, then, cooled down to room temperature for other 3 h (according to the protocol reported elsewhere (McRae et al., 2018)). Turbidimetric tests were performed before and after the aforementioned procedure and the results were reported as an average difference (Δ) in Nephelometric Turbidity Unit upon three replicates (NTU; *i.e.* to pass the test ΔNTU should be lower than 2 (McRae et al., 2018)).

## 3. Results and Discussion

*3.1 Kinetic and thermodynamic adsorption of putrescine onto bentonites*

To the authors' best knowledge, putrescine adsorption onto bentonite materials has never been studied so far. Therefore, prior to assess bentonites efficacy in biogenic amines (especially putrescine) removal, adsorption tests were performed adopting simplified model solutions (*i.e.*, 12%v/v $EtOH/H_2O$, pH 3.2,

presence of tartaric acid). Two different adsorbents concentrations (0.20 and 0.40 g $dm^{-3}$) and putrescine amounts, namely 10 (just below the concentration found by Esposito *et al.* (Esposito et al., 2019)) and 20 ppm were investigated (Figures 1a,b). Both bentonites exhibited the same behaviour resulting in a greater removal of almost 60% with the 0.40 $dm^{-3}$/10 ppm combination already after 160 minutes, as expected from their similar physicochemical features (Table S1). Going deeply into the adsorption kinetics, considering the abovementioned optimal combination, we tried to fit the obtained curves (displayed in Figure 1c) with the most exploited methods, widely described in the literature (*i.e.,* Pseudo-First Order, Pseudo-Second Order, Elovich and Intraparticle Diffusion models; see Paragraph 2.1 in the Supporting Information) (Sahoo & Prelot, 2020). As clearly observable in Figure S2, the PSO model seems to fit the experimental data for both Na/Ca_0.27 and 0.49 slightly better, giving rise to the most optimal linearization ($R^2$ around 0.99, achieving an average kinetic constant of ca. 26 g $mg^{-1}$ $min^{-1}$) with respect to the other reported methods, especially to PFO one (Figure S2c). According to the literature (Sahoo & Prelot, 2020), if one assumes that the attachment of the adsorbate molecules to the adsorbent site is the rate-determining step, PFO should be the best kinetic model since it suggests the occurrence of a physisorption process. However, Hubbe et al. exploited the PSO model (that conversely refers to chemisorption) since it is the most reported fitting method to explain the adsorption of different metal ions, dyes, organic molecules from aqueous solutions onto a wide range of adsorbents classes, from cellulose-based to carbon-based materials (Hubbe et al., 2019). Hence, when unavoidable and sluggish diffusion processes (from the bulk to the surface and through the heterogenous pores of the adsorbents) become the real rate-determining step, adsorption rate slows down. Subsequently, notwithstanding PSO does not correspond to physical adsorbent/adsorbate interactions, it is the most reported model to describe physisorption as an approximation, considering the diffusion-dependent processes which necessarily occur before the mere adsorption. In this respect, here, we may assume that putrescine molecules are physisorbed by bentonites through both $H_3N^+$---OH hydrogen bonds and electrostatic interactions (Lotierzo et al., 2016), since at pH 3.2 putrescine is positively charged ($pK_a$ ca. 10.4) and bentonite exhibits a negative charge on the surface (as schematically reported in Figure 2a).

Once unravelled the adsorption kinetics, thermodynamic studies were carried out to identify the optimal model to fit the experimental data, allowing the final determination of the enthalpy, entropy and Gibbs free energy variations. Hence, always considering the most performing adsorbent concentration of 0.40 g $dm^{-}$

$^3$, tests were performed varying the putrescine amount (6-30 ppm) at three different temperatures (298, 303 and 308 K; Figures 2b,d). Then, among the most used thermodynamic models (Paragraph 2.2, in Supporting Information) and their relative linear forms, Langmuir's method seems to be the best one, since the corresponding $R^2$ values are the highest ones, always above 0.96 (see Figures 2c,e and Figure S3). In this respect, we may assume that putrescine adsorption leads to the formation of a monolayer until reaching the complete saturation of the surface active sites. From the elaboration of these data, ΔG values were also determined. For both the adsorbents, negative values were obtained (a little more negative trend for Na/Ca_0.27 material) (Figure 2f), confirming the process spontaneity. In addition, plotting ΔG as a function of the three operating temperatures, ΔH (considered approximately constant in this small T range) and ΔS were computed. From the inset of Figure 2f, similar negative data were obtained for both bentonites: specifically, a ΔH of ca. -47 kJ $mol^{-1}$ and ΔS of about -40 J $K^{-1}$ $mol^{-1}$ were calculated. Concerning the former, negative values stand for exothermic processes corresponding to condensation (Erkey & Türk, 2021). Usually in physisorption, ΔH values are quite low (around 20-40 kJ $mol^{-1}$) in line with our results (Lian et al., 2009), thus corroborating once again that putrescine adsorption is physical in nature. Conversely, a negative value of ΔS corroborates the molecules adsorption onto substrates (Lian et al., 2009), confirming the reduction of freedom degrees.

*3.2 An outlook on competitive adsorption and tests on real wines*

In order to evaluate the bentonites efficacy, competitive putrescine adsorption tests, firstly in presence of albumin or polyphenols and, secondly, in real commercial wines, were performed. Regarding the former, albumin was chosen since it mimics medium-high molecular weight proteins that must be removed during wine clarification (*International Oenological Codex*, 2015; Sun et al., 2007). On the contrary, polyphenols as tannins have a double role since they have significant impact on wine flavor preservation enhancing esters volatility (Mitropoulou et al., 2011), and they can mitigate the light-struck taste of bottled white wines thanks to their interaction with the photosensitizer riboflavin (Fracassetti et al., 2019). Figure S4 shows the main results: in both cases, albumin or polyphenols adsorption was followed and Na/Ca_0.49 was adopted as representative adsorbent material being the two bentonites very similar in behavior. Specifically, in the albumin/putrescine system, a ca. 10% reduction of albumin adsorption in presence of putrescine was observed

(Fig. S4a); contrarily, a more significant decrease (~50%) was obtained for polyphenols (Fig. S4b). Indeed, from our previous work (Pargoletti et al., 2021), the ΔG of polyphenol adsorption (-21 kJ $mol^{-1}$) is lower than the albumin one (-25 kJ $mol^{-1}$) at 298 K, highlighting different affinity towards bentonite. Thus, taking into account the computed ΔG value for putrescine (ca. -35 kJ $mol^{-1}$, Fig. 2f), this latter molecule may be more favourably adsorbed especially in the presence of polyphenols. Moreover, due to the high amount of albumin alongside with its intrinsic steric hindrance, putrescine/bentonite interaction is hindered resulting in a less appreciable competition. Therefore, we can conclude that Na/Ca_0.49 could be a promising adsorbent material to be adopted during the clarification process (Pargoletti et al., 2021) to reduce the unwanted putrescine content.

To corroborate this inference, tests on real wines were carried out. Specifically, *Chardonnay* white and *Primitivo* red wines were adopted. In both cases, total biogenic amines and putrescine removals were determined. As clearly visible in Table 1, white wines as *Chardonnay* have a lower content of BAs (ca. 5 ppm and 3 ppm of putrescine) with respect to *Primitivo* red (ca. 40 ppm and 14 ppm of putrescine). As far as it concerns the former, both Na/Ca_0.27 and 0.49 removed around 25-30% of putrescine and ca. 25% of total BAs, already after 120 min (Table 1a). Furthermore, in order to stress the system and to evaluate the subsequent performances of the two bentonites, ca. 7 ppm of pure putrescine were added to the *Chardonnay* wine, resulting in a total of 10 ppm (being very close to the threshold value, as adopted in our previous simplified tests). Notably, putrescine and total BAs amounts were removed of about 20% by both adsorbents (Table 1b), still resulting efficient to reduce their wine content below the allowed limits. Table 1c, instead, reports the results obtained with *Primitivo* red wine. By adopting the same bentonites concentration of 0.40 g $dm^{-3}$ and since putrescine and BAs are higher in content, a removal of ca. 15% was reached revealing insufficient putrescine removal capability (reached value of ~12 ppm). Therefore, we adopted a two-step procedure in which a first adsorption process (that lasted 120 min) was followed by adsorbents elimination and a second addition always of 0.40 g $dm^{-3}$, for other 120 min. In this case, a greater removal of both putrescine (25%) and total BAs (ca. 23%) was achieved, thus taking the content of putrescine down to at least the allowed value (< 10.4 ppm).

Finally, difference in Nephelometric Turbidity Unit (ΔNTU) was also assessed to investigate the haze formation before and after the bentonites treatment. In all the investigated cases, ΔNTU was below the

threshold value of 2 (Table 1), thus resulting optimal to prevent haze formation in bottled wines (McRae et al., 2018), especially when two different additions of bentonite were performed in *Primitivo*, as expected. Notably, from the latter case, we may assess that with the first bentonite treatment the adsorbent removes more easily medium-high MW compounds like proteins, being thermodynamically more favored, as demonstrated by Pargoletti *et al.* (Pargoletti et al., 2021). Contrarily, with the second addition, putrescine and generally BAs are more prone to be adsorbed by bentonite.

Hence, we demonstrated that bentonites are efficient adsorbents to be used for both wine fining processes and putrescine concentration reduction, having shown that these processes favorably occur on both kinetic and thermodynamic points of view.

## 4. Conclusions

BAs removal from wine is becoming an increasingly debated issue since they can easily concentrate in foodstuff, alter the wine organoleptic profile and be toxic in elevated concentrations. Therefore, we demonstrated for the first time how bentonites, already adopted for wine clarification, can be incisively used also for reducing wine BAs content, especially with a focus on putrescine molecules. A cutting-edge study about kinetics and thermodynamics of putrescine adsorption by two commercially available bentonites was performed resulting in the biogenic amine removal by physisorption mechanism. Both bentonites (at the optimal concentration of 0.40 g $dm^{-3}$) showed also promising results in terms of putrescine adsorption in more complex systems *i.e.*, in simple putrescine/albumin or putrescine/polyphenols systems followed by real wines (*Chardonnay* and *Primitivo*). Specifically, in the case of white wine, which already possesses a lower content in biogenic amines (ca. 5 ppm), ~25-30% of total BAs was removed within 120 min. Conversely, in *Primitivo*, due to its intrinsic higher amount of BAs (around 40 ppm and ca. 14 of putrescine) a two-step treatment with bentonites revealed fundamental to decrease the putrescine content below the limit of 10 ppm and that of total BAs down to 30 ppm. In all the investigated cases, we observed that other molecules, as albumin and in general proteins or polyphenols, can compete with putrescine or other BAs, however, the bentonites efficacy in reducing the amines content is still preserved. Hence, for the first time, we deeply unveiled bentonite behaviour towards putrescine removal and, to a larger extent, to the other BAs present in wines.

Conflict of Interest: The authors declare that they have no conflict of interest.

**Supporting Information description**

Paragraph 1: Bentonites characterization; Paragraph 2: putrescine adsorption onto bentonites; Paragraph 2.1: Kinetic models; Paragraph 2.2: Thermodynamic models.

Table S1: Bentonites physicochemical features; Table S2: *Chardonnay* and *Primitivo* wines features; Figure S1: Putrescine derivatization and relative calibration curve; Figure S2: Different kinetic fitting models relative to 10 ppm putrescine adsorption by 0.40 g $dm^{-3}$ of (a) Na/Ca_0.27 and (b) Na/Ca_0.49 within 120 min. (c) PFO linearizations; Figure S3: Thermodynamic linearization curves; Figure S4: Comparison of competitive adsorptions between (a) albumin (200 ppm)/putrescine (10 ppm), and (b) polyphenols (10 ppm)/putrescine (5 ppm).

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**Table 1.** Total Biogenic Amines (BAs) and putrescine removal by both Na/Ca_0.27 and Na/Ca_0.49 bentonites in white *Chardonnay* and red *Primitivo* wines. For each test: $c_{bentonite}$ = 0.40 g $dm^{-3}$, kinetics time = 120 min. ΔNTU = average difference in Nephelometric Turbidity Unit computed on three different replicates. In Table 1c: second addition of 0.40 g $dm^{-3}$ bentonite immediately after 120 min from the first one. Uncertainty values related to putrescine and total BA removal were assessed to be <0.1 and 0.1 ppm, upon three replicates each.

**Table 1a.**

| ***Chardonnay* white wine** | | | | | |
|---|---|---|---|---|---|
| | **Putrescine** | | **Total BA** | | **ΔNTU** |
| | ppm | Removal % | ppm | Removal % | |
| **Starting** | 2.9 | – | 4.8 | – | 22.8 ± 0.5 |
| **Na/Ca_0.27** | 2.1 | 31 | 3.6 | 26 | 1.7 ± 0.3 |
| **Na/Ca_0.49** | 2.2 | 24 | 3.8 | 22 | 1.8 ± 0.3 |

**Table 1b.**

| ***Chardonnay* white wine + ca. 7 ppm pure putrescine addition** | | | | | |
|---|---|---|---|---|---|
| | **Putrescine** | | **Total BA** | | **ΔNTU** |
| | ppm | Removal % | ppm | Removal % | |
| **Starting** | 10.0 | – | 12.0 | – | 22.8 ± 0.3 |
| **Na/Ca_0.27** | 8.0 | 20 | 9.4 | 19 | 1.8 ± 0.1 |
| **Na/Ca_0.49** | 7.9 | 21 | 9.4 | 19 | 1.8 ± 0.1 |

**Table 1c.**

| ***Primitivo* red wine** |
|---|

| | | Putrescine | | Total BA | | ΔNTU |
|---|---|---|---|---|---|---|
| | | ppm | Removal % | ppm | Removal % | |
| **Starting** | | 14.1 | – | 39.7 | – | 4.8 ± 0.5 |
| **Na/Ca_0.27** | I addition | 12.0 | 15 | 35.0 | 12 | 1.8 ± 0.1 |
| | II addition | 10.3 | 27 | 30.8 | 22 | <1.0 ± 0.1 |
| **Na/Ca_0.49** | I addition | 11.9 | 16 | 34.7 | 13 | 1.0 ± 0.1 |
| | II addition | 10.4 | 27 | 30.1 | 24 | <1.0 ± 0.1 |

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**Figure captions**

**Figure 1.** Putrescine removal percentages changing the putrescine concentration (10 or 20 ppm) and adsorbents amount (0.20 or 0.40 g $dm^{-3}$) in the case of (a) Na/Ca_0.27 and (b) Na/Ca_0.49. Kinetic tests were carried out using 12%v/v EtOH/Milli Q water, at pH 3.2, kinetics time = 160 min, T = (25 ± 1) °C and constant stirring at 600 rpm. (c) Adsorption kinetics of 10 ppm of putrescine by 0.40 g $dm^{-3}$ of Na/Ca_0.27 and Na/Ca_0.49 bentonites. Each point is an average value upon three different replicates and is reported together with the corresponding standard deviation. All error bars are within the 3-5% of the corresponding $q_t$ value. (d) Pseudo-Second Order (PSO) linearization of the kinetic curves reported in Figure 1c.

**Figure 2.** (a) Schematic representation of putrescine adsorption onto negatively charged bentonite, at pH 3.2 (red dotted line: hydrogen bond; blue dotted line: electrostatic interaction). Adsorption capacity values at equilibrium ($q_e$) by increasing the concentration of putrescine for both (b) Na/Ca_0.27 and (d) Na/Ca_0.49 bentonites at 298, 303 and 308 K; (c,e) relative Langmuir thermodynamic linearizations. (f) Free Gibbs energy variation with the rise of temperature (inset: Table displaying the computed ΔH and ΔS for both adsorbents).

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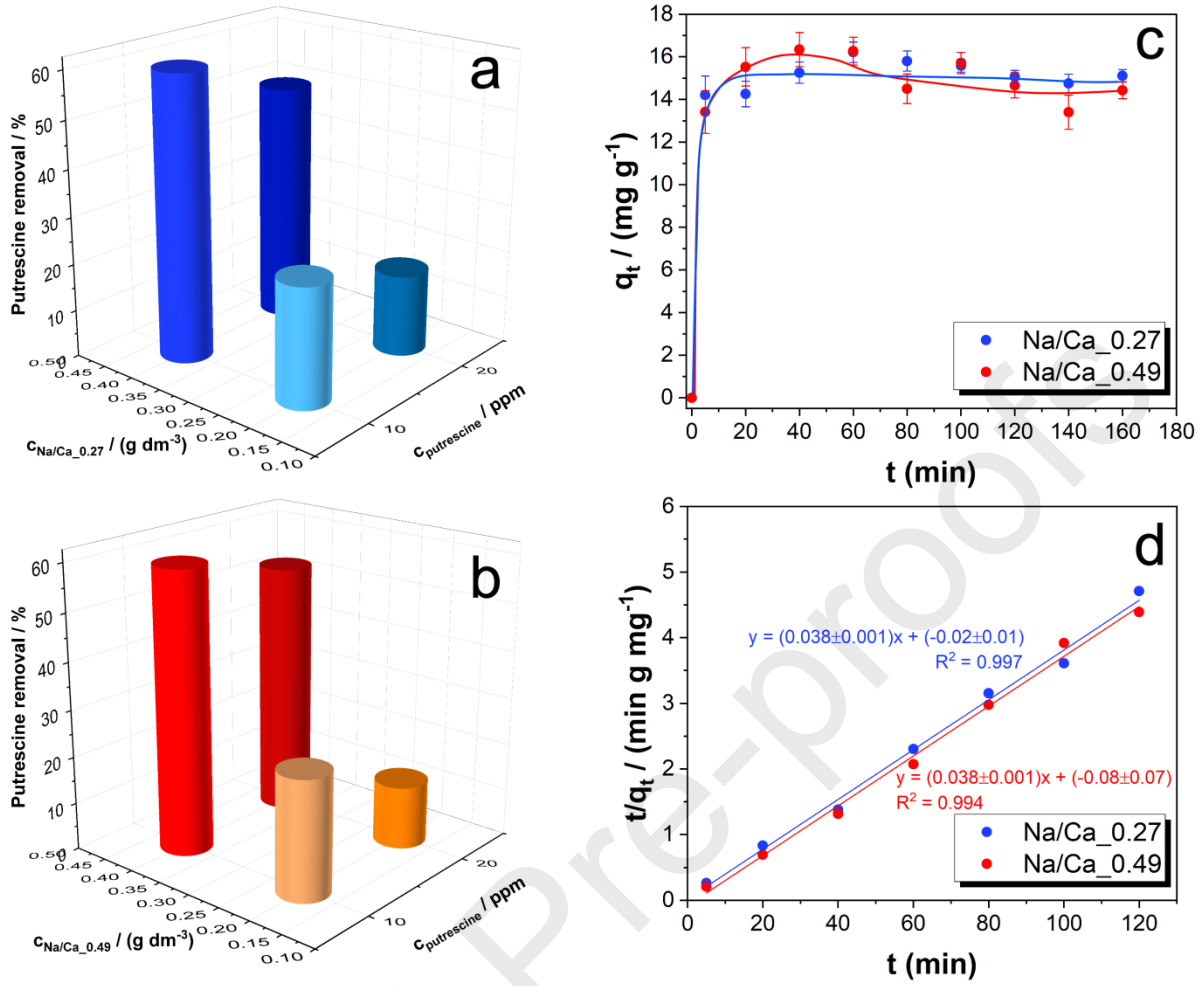


**FIGURE 1**

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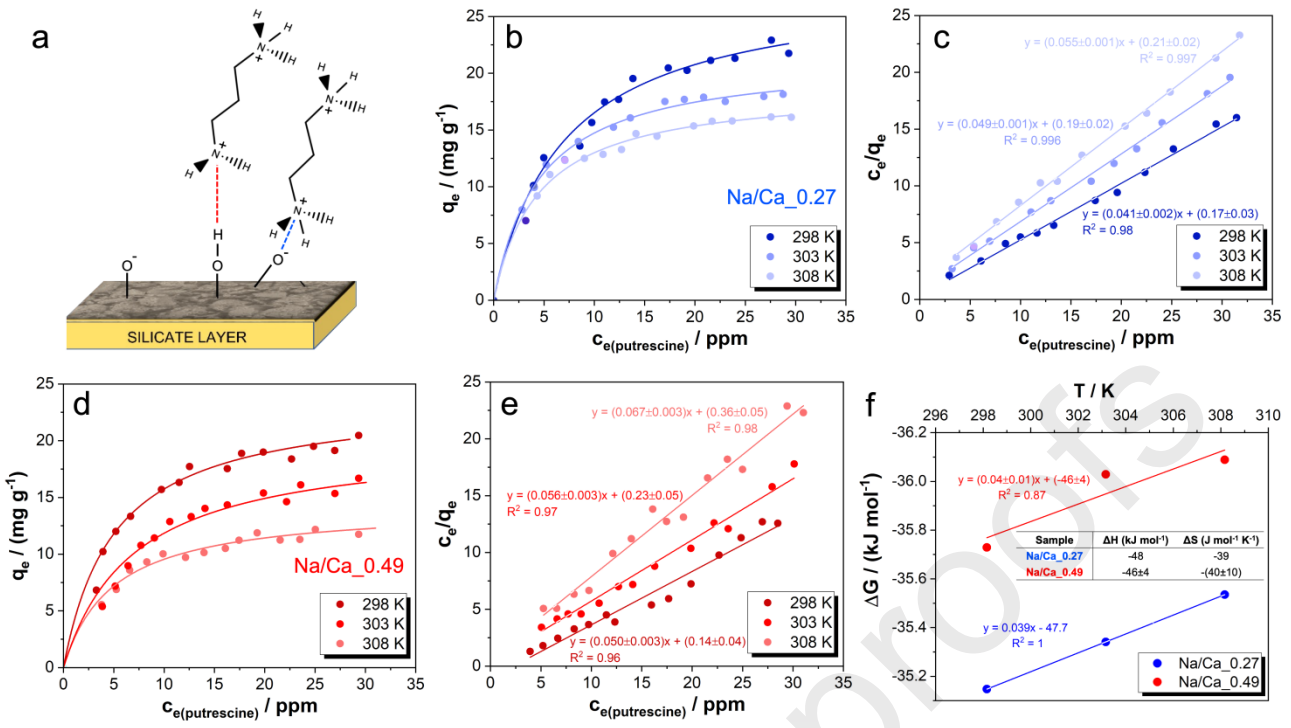


| Sample | ΔH (kJ mol$^{-1}$) | ΔS (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|
| Na/Ca_0.27 | -48 | -39 |
| Na/Ca_0.49 | -46±4 | -(40±10) |

**FIGURE 2**

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**HIGHLIGHTS**

- Bentonites as efficient adsorbents for wine putrescine removal
- In simple system, removal of 60% with the 40 g $dm^{-3}$/10 ppm combination after 120 min
- Kinetic and thermodynamic assessment of putrescine physisorption onto bentonites
- Promising results in white *Chardonnay* and red *Primitivo* wines

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**Credit Author Statement**

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