# Odor-active Compound Adsorption onto Bentonite in a Model White Wine Solution

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The mechanisms and occurrence of aroma depletion during wine fining is still not clear. Previous results of our group led to hypothesized that some odor-active compounds were removed through direct adsorption mechanism on the clays without the involvement of any wine macromolecules. This paper examined the adsorption isotherms at 17±1 °C of some volatile compounds, principally responsible of the fruity character of white wines, onto three bentonite samples. The bentonites were added in three different amounts to a model white wine "spiked" with eight odor-active compounds and in the absence of wine macromolecule. The elemental composition, the surface charge density, and the SSA of the clays were determined and differences were analyzed by Tukey's test. The Langmuir and the Freundlich models were fitted to the adsorption data. The most experimental adsorption isotherms were robustly fitted by the Freundlich equation and evidenced differences in the adsorption intensity and capacity values for the tested odor-active compounds. The main interaction forces controlling adsorption appeared to be related more to the clay characteristics, than to the compounds properties: samples having a lower SSA value and a greater charge density per surface unit seemed to interact with most of the odor-active compounds primarily through physical mechanisms. Differently, the clay with a large SSA value and a low charge density per surface unit promoted stronger adsorptions that were probably driven also by chemical interactions especially for ethyl esters.

## 1. Introduction

Bentonite fining is currently and commonly used by the wine industry as a clarifying technique to remove proteins that are source of potential haze in wines (Lambri et al., 2012a). Bentonite is a natural clay mineral with a high montmorillonite content. Montmorillonite is a 2:1 dioctahedral smectite that is structurally composed of an octahedral alumina sheet between two tetrahedral silica sheets (Xifang et al., 2007). The adsorption properties of bentonite in wine are chiefly due to the cation exchange action of this clay. Bentonite, carrying a net negative charge at the wine pH, interacts electrostatically with the positively charged wine proteins, producing their flocculation (Blade and Boulton, 1988). The components with effectively no overall positive charge or a net negative charge may be readily exchanged onto bentonite by different mechanisms, including physical or Van der Waals force interactions, hydrogen bonding, ion exchange interactions, coordination, and chemisorptions (Xifang et al., 2007). Through these multiple interactions, the addition of bentonite can reduce both undesirable and desirable components, such as aroma and flavor compounds (Lambri et al., 2010). Aroma compounds are able to interact with different macromolecules such as proteins or polysaccharides through multiple interaction mechanisms (Voilley et al., 1990). Fining agents may indirectly remove molecules contributing to the wine aroma by fixing the substances that act as a support for them. The loss of aroma was studied (Lambri et al., 2010; Lambri et al., 2012b) but the direct interaction between the bentonite and the wine odor-active compounds has not yet been fully established.

This study examined the interactions between the bentonite samples and the odor-active compounds that contribute to the sensory profile of the young white wines (Ugliano and Henschke, 2009) under model

conditions and in the absence of any wine macromolecule, contributing to collect information for predicting the behaviour of the different types of bentonite to be used in wines.

## 2. Materials and methods

### 2.1 Materials

**Chemicals**
The standards for the odor active compounds, including ethyl butyrate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, β-phenylethyl acetate, β-phenylethanol, hexanoic acid, octanoic acid and 1-heptanol were purchased from Fluka–Sigma Aldrich (St. Louis, MO, USA). Absolute ethanol 99.8 % v/v, pentane, dichloromethane, tartaric acid, and potassium hydroxide were provided by Carlo Erba reagents (Milan, Italy).

**Model wine solution**
A buffer solution containing 6 g/L tartaric acid adjusted to pH 3.30 with 1N potassium hydroxide and supplemented with 13 % ethanol (v/v) was prepared. For each of the odor-active compounds a defined volume of 1,000 mg/L stock solution was prepared in absolute ethanol and stored at -28 °C until use. An aliquot was added to a defined volume of the buffer solution to obtain a mixture with the following concentrations: 4,000 µg/L for ethyl butyrate; 1,200 µg/L for ethyl hexanoate; 200 µg/L for ethyl octanoate; 4,500 µg/L for isoamyl acetate; 160 µg/L for β-phenylethyl acetate; 2,000 µg/L for β-phenylethanol; 40 µg/L for hexanoic acid; and 750 µg/L for octanoic acid.

**Bentonites**
Three samples of natural $Ca^{2+}$-bentonite activated with $Na^+$ were purchased from Dal Cin Gildo (Concorezzo, Italy). Two of the samples were powdery clays (A and C) and one of the samples was a granular bentonite (B). The A and B samples came from the same raw montmorillonite and the C bentonite originated from a montmorillonite containing magnesium smectite.

### 2.2 Bentonite analyses
Bentonite samples were analyzed in triplicate. An elemental analysis of the inorganic content was determined with an energy dispersive X-ray detector (EDS-EDAX Genesis, Mahwah, NJ, USA) coupled to a scanning electron microscope (XL30 Esem, Philips, Eindhoven, the Netherlands). The surface charge density was determined with the procedure recommended by Ferrarini et al. (1996). The specific surface area (SSA) was measured using the methylene blue titration method (OIV, 2003). The charge density per surface unit was calculated as the ratio between the surface charge density (meq/100g) and the specific surface area ($m^2$/100g).

### 2.3 Batch experiment procedure
Each bentonite sample was used in three different concentrations (0.2, 0.5 and 1 g/L) to treat the model wine solution. The bentonites were rehydrated in deionized water at a bentonite:water ratio of 10:100 (w/w). For each bentonite sample concentration, three replicates were prepared in order to arrange for three adsorption independent experiments. After 90 min, the resulting slurries were stirred. Each suspension was then added to glass conical flasks containing 500 mL of the model wine solution and thoroughly mixed for 90 s. A sample of the model wine solution without any addition of bentonite was treated under the same conditions and kept in triplicate as a control test. The glass-stoppered flasks (samples and control test) were then placed in a 60 % relative humidity incubator at 17 ± 1 °C in static conditions to simulate the sedimentation occurring during the real use of the bentonite in white winemaking. After 24 h, the limpid liquid phases of both samples and control test were separated and filtered through folded filters (595 ½, Whatman GmbH, Germany). The odor-active compounds were recovered from the entire volume of the filtered limpid liquid phases both in the samples and in the control test following the procedure reported in Lambri et al. (2010). Taking into account the low amounts of odor-active compounds to be detected and the sensitivity of the flame ionization detector (FID), a 500 times concentration of the liquid fraction was applied after the liquid-liquid extraction. Moreover, the same chromatographic conditions as reported in Lambri et al. (2010) were applied, but the quantification of the data was calculated from the internal standard 1-heptanol.

### 2.4 Adsorption isotherms
To ensure equilibrium, the concentration of the adsorbate was measured after 12 and 24 h of contact time between the model wine solution and the bentonite. As there was no further increase in adsorption past the 24-hour mark, a contact time of 24 h was established as the equilibrium time. The concentration of the non-adsorbed odor-active compounds measured after 24 h was assumed to be the equilibrium

concentration ($C_e$). As reported by de Bruijn et al. (2009), $q_e$, i.e. the amount of the solute adsorbed per unit mass of bentonite (µmol/g), was calculated from a mass balance as:

$$q_e = \frac{V(C_0 - C_e)}{M}$$

(1)

where $C_0$ and $C_e$ are the initial and the equilibrium concentrations (µmol/L), respectively, V is the volume of the solution (L) and M is the mass of the adsorbent (g).

### 2.5 Data analysis and statistics.
Among the various models to fit the data of the adsorption at 17±1°C both for the mixture of the odor-active compounds and for the single odorous substances, the Langmuir and the Freundlich equations were chosen. They are commonly used to describe the general adsorption performance of bentonite (Meneguin et al., 2011; Vieira et al., 2009) and the specific adsorption performance of bentonite with proteins (de Bruijn et al., 2009; Dif et al., 2012). In detail, the Langmuir (1916) equation has been used in the following linear form:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{1}{q_m} C_e$$

(2)

where $q_m$ is the maximum adsorption capacity of the monomolecular layer and the Langmuir constant $K_L$ is related to the adsorption energy. The Freundlich (1906) model , that assumes an infinite number of surface adsorption sites with a heterogeneous energy distribution of the active sites (de Bruijn et al., 2009), has been used in its original form:

$$q_e = K_F C_e^{1/n}$$

(3)

where the Freundlich constant $K_F$ and the Freundlich exponent $n$ are related to the adsorption capacity and intensity, respectively. The data from the batch adsorption experiments were analyzed by the curve estimation procedure, generating the related plots for the different regression models. Moreover, the bentonite characteristics were analyzed by a factorial ANOVA using Tukey's test at $p \leq 0.05$. The statistics package IBM SPSS Statistics 19 (IBM Corporation, New York, USA) was used.

## 3. Results and discussion

### 3.1 Physicochemical analysis of bentonite
The characterization of the bentonite samples is shown in Table 1. Differences among the samples can be observed from the ratio of the sodium to the calcium, highlighting the surface cation distribution, a parameter that affects the swelling potential and the ion exchange capacity of the bentonite (Jiang and Zeng, 2003). In samples B, the sodium prevailed over the calcium, increasing the potential adsorption capacity (Blade and Boulton, 1988). For a richer $Na^+$-bentonite with a favorable sodium/calcium ratio that absorbs water in the interlayer space, such as sample B, the double layers that form repel each other leading to disintegration of the montmorillonite into the individual layers or stacks of layers and exposing a wide surface area (405.79 $m^2$/g). For samples A and C, the low sodium/calcium ratio contributed to low SSA values as already observed by Vieira et al. (2009) even if detecting the SSA by means of the BET method. The values of the surface negative charge densities of the tested bentonite samples (Table 1) were consistent with recently reported values in the literature, ranging from 57.0 to 0.4 cmol/kg (Xifang et al., 2007).

*Table 1: Physicochemical analysis of bentonite samples. Values are means ± SD (n = 3). Within each column, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.*

| Bentonite | Na/Ca ratio | Surface charge density (meq/100g) | Specific Surface Area (SSA) ($m^2$/g) | Charge density per surface unit (meq/$m^2$)·$10^{-2}$ |
|---|---|---|---|---|
| A | 1.05 ± 0.09 b | 35.2 ± 4.6 b | 102.25 ± 1.72 b | 0.34 ± 0.04 b |
| B | 1.44 ± 0.12 a | 43.1 ± 5.7 a | 405.79 ± 4.55 a | 0.11 ± 0.01 c |
| C | 1.00 ± 0.10 b | 43.5 ± 4.9 a | 87.97 ± 1.75 c | 0.50 ± 0.04 a |

With the negative electric charge of the bentonite relating to the capacity to remove the proteins from the solution (Ferrarini et al., 1996), the sample C might be the most efficient at removing ionic compounds from the solution.

### 3.2 Adsorption of the odor-active compounds by the bentonites

With the high ratio between the amount of bentonite used and the concentration of the odor-active substances in the model wine solution, the experimental adsorption isotherms were able to describe the early stages of the process. At these conditions, the ability of the different clays to remove both the mixture of the odor-active compounds and the individual substances becomes easier to evaluate. By examining the studied bentonite samples, a determination can be made to describe which clay characteristics affect the adsorption mechanism of the odor-active compounds that form part of the aroma of a white wine. In this regard, the $n$ constant of the Freundlich model (Table 2) acquires a great deal of importance, because it is primarily related to the strength of the adsorption with the adsorbent (Jiang and Zeng, 2003).

Constant values of $n > 1$ indicate a favorable adsorption isotherm, values of $n = 1$ indicate a linear relationship between the amount adsorbed ($q_e$) and the equilibrium concentration ($C_e$), and values of $0 < n < 1$ indicate an unfavorable adsorption isotherm (de Brujin et al., 2009). Given a fixed $C_e$ and $K_F$, the higher values of the $n$ constant indicate a stronger adsorption bond.

*Table 2: Constants from the Freundlich model for the adsorption of the odor-active compounds on the three bentonite samples at 17 ± 1 °C.*

| Bentonite | | Mixture | Ethyl butyrate | Ethyl hexanoate | Ethyl octanoate | Isoamyl acetate | β-phenylethyl acetate | β-phenyl ethanol | Hexanoic acid | Octanoic acid |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $K_F$ | 0.14 | 0.01 | 3.17 | 1130 | 0.29 | $1.27 \cdot 10^{-5}$ | 0.09 | 0.11 | 0.17 |
| | n | 0.39 | 0.07 | 0.71 | 0.32 | 10.2 | 0.30 | 0.29 | -0.31 | -0.51 |
| | $r^2$ | 0.993 | 0.992 | 0.933 | 0.990 | 0.007 | 0.901 | 0.975 | 0.068 | 0.175 |
| B | $K_F$ | 1.74 | 3.66 | 10.6 | 2.06 | 2.44 | 0.01 | $1.66 \cdot 10^{-14}$ | 61.8 | 0.16 |
| | n | 0.84 | 1.06 | 2.39 | 1.18 | 0.87 | 1.03 | 0.04 | 0.32 | 0.20 |
| | $r^2$ | 0.999 | 0.900 | 0.999 | 0.881 | 0.985 | 0.815 | 0.983 | 0.999 | 0.999 |
| C | $K_F$ | $2.00 \cdot 10^{-3}$ | 0.47 | $1.59 \cdot 10^{-6}$ | $3.44 \cdot 10^{11}$ | 0.06 | $4.41 \cdot 10^{-11}$ | $1.04 \cdot 10^{-11}$ | 89.5 | 0.20 |
| | n | 0.29 | 0.78 | 0.11 | 0.11 | -1.44 | 0.11 | 0.04 | 0.39 | -0.39 |
| | $r^2$ | 0.990 | 0.999 | 0.981 | 0.923 | 0.379 | 0.985 | 0.998 | 0.998 | 0.594 |

Among the bentonite samples the adsorption process for the mixture of the odor-active compounds was more favored on clay B. Both the adsorption intensity $n$ and the adsorption capacity $K_F$ showed decreasing values moving from the bentonite B, to the clay A and then to the C. The greater adsorption on clay B may be due to its large SSA and to the high sodium/calcium ratio (Table 1). This result can be explained by the increase of the electrostatic forces as also observed by Dif et al. (2012) for adsorption of casein onto Na-bentonite at acidic pHs. The lower adsorption capacity of bentonite C depended on the low values of both the parameters. This confirms that these clay characteristics are two of the main drivers for adsorption (Blade and Boulton, 1988). In detail, for the most odor-active compounds and the mixture (Table 2), the adsorption intensity $n < 1$ indicated that the bentonite had a slightly unfavorable adsorption profile that was mostly driven by physical interactions. Differently, the adsorption isotherms having an intensity value $n > 1$ suggested that the adsorption was favorable and mediated by chemical interactions (Jiang and Zeng, 2003). $n < 1$ values were observed for the adsorption of o-cresol on a modified montmorillonite (Maarof et al., 2004), whereas for the adsorption of other phenolic compounds (Akçay and Akçay, 2004) and proteins (Blade and Boulton, 1988) the Freundlich constant $n$ was >1, indicating that the predominant interactions between the adsorbent and the adsorbate were chemical in nature.

Among the isotherms characterized by Freundlich $n < 1$ values (Table 2) the adsorptions of ethyl butyrate on clay A, of β-phenylethanol on clays B and C, and of β-phenylethyl acetate on clay C, showed the lowest $n$ intensities jointly to negligible $K_F$ capacity values. This indicates a very low affinity between these adsorbents and these adsorbates, rendering the related adsorptions strongly unfavorable. This was probably due to the exclusion of the electrostatic interaction with Na-bentonite as in the case of soluble lactose reported by Dif et al. (2012). Differently from the process observed for bentonite A, the adsorption isotherms of ethyl butyrate on bentonite B and C were more favored, reaching an intensity $n > 1$ and the greatest $K_F$ capacity on clay B. This compound appeared to create chemical interactions with the bentonite B, and to promote physical bonds with clay C. In the adsorption of the other two ethyl esters (ethyl hexanoate and ethyl octanoate) on the three bentonite samples, the intensities $n$ were quite similar to $n$ value for ethyl butyrate. The $n$ constants of ethyl hexanoate and ethyl octanoate increased in order from

bentonites C to A and then to B. Unfavorable adsorptions ($n < 1$) seemed to occur on bentonite samples A and C characterized by predominantly physical interactions, while both the molecules showed to be favorable adsorbed on clay B by stronger bonds ($n > 1$). In terms of adsorption capacity $K_F$, on clay B the most hydrophobic molecule (i.e. ethyl octanoate) was adsorbed less than ethyl hexanoate, while on both the other clays ethyl octanoate showed a very high and greater $K_F$ value than ethyl hexanoate. This was in agreement to the observations of Dif et al. (2012), indicating that in an aqueous solution, hydrophobic compounds, having low solubility, i.e. triglycerides, tend to be 'pushed' to Na-bentonite, i.e. to the adsorbent surface. As regard the aromatics, β-phenylethyl acetate interacted more favorably than β-phenylethanol with the tested bentonite samples, in particular with clay B on which the adsorption appeared to be driven by chemical bonds ($n > 1$). Besides, the adsorption of β-phenylethanol, although unfavorable, was promoted more on clay A (i.e. showing a greater $n$ intensity and $K_F$ capacity) than on clays B and C. In spite of literature reports for the phenolic compounds (Akçay and Akçay, 2004; Maarof et al., 2004), the adsorption on montmorillonite of molecules analogous for the phenolic ring appears here to be related on clay properties. Despite the structural similarities, the β-phenylethyl acetate molecule is more hydrophobic and larger than the β-phenylethanol molecule; these compound properties could explain the more favorable adsorption isotherm of the β-phenylethyl acetate especially on a clay B having the lowest negative charge per surface unit and the largest SSA (Table 1).The hexanoic acid compound had a similar unfavorable adsorption intensities $n$ on bentonite B and C with high $K_F$ values on both the clays (Table 2). Despite having the same molecular weight as ethyl butyrate, hexanoic acid possesses a longer alkyl chain. It is conceivable that this molecule was adsorbed primarily through a physical mechanism and with a larger capacity than ethyl butyrate. The octanoic acid molecule showed a $n < 1$ adsorption intensity on bentonite B, indicating unfavorable adsorption and prevailing physical interactions between the adsorbate and the adsorbent.

Taking into account the validity of the model parameters and comparing the values of the average percentage error, the Freundlich isotherm robustly fitted most experimental adsorption data (Table 2), indicating that this approach may be useful in fitting the results from the batch aqueous adsorption experiments (Baláž et al. 2005). Despite some high values of the correlation coefficients ($r^2$), the general inadequacy of the Langmuir model (Table 3) to explain most of the adsorption processes was highlighted by the negative values of the constant $q_m$, similar to the observations reported by Maarof et al. (2004). Consequently, according to the assumptions of the Langmuir model, the adsorption of most of the odor-active compounds used in the study did not appear to follow the mechanism of a monolayer adsorption onto a completely homogeneous surface with a finite number of identical sites. For a few cases, as isoamyl acetate on bentonite A and C, hexanoic acid on bentonite A, and octanoic acid on bentonite C, the better correlation coefficients ($r^2$) observed in the Langmuir model (Table 3) than in the Freundlich model (Table 2) indicated that the process did not follow the assumption of a heterogeneous energy distribution of an infinite number of surface active sites. On the other hand, excepted for the adsorption of hexanoic acid on bentonite A, the constants of the Langmuir model (Table 3) could not provide a simple and unequivocal interpretation of the data probably due to the fact that our investigated range for the wine odor-active compounds is the beginning of the adsorption isotherm. Meneguin et al. (2011) founded similar results for the Langmuir modeling of lead and Cd(II) adsorption data. Finally, the adsorption processes of β-phenylethyl acetate on bentonite A and B, and of ethyl hexanoate, β-phenylethanol and octanoic acid on bentonite B, were well described by both the Langmuir and the Freundlich models (Tables 2 and 3).

*Table 3: Constants from the linear form of the Langmuir model for the adsorption of the odor-active compounds on the three bentonite samples at 17 ± 1 °C.*

| Bentonite | | Mixture | Ethyl butyrate | Ethyl hexanoate | Ethyl octanoate | Isoamyl acetate | β-phenylethyl acetate | β-phenyl ethanol | Hexanoic acid | Octanoic acid |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $K_L$ | -0.07 | -0.56 | -0.12 | -11.6 | -25.2 | -12.8 | -0.26 | 9.08 | 8.21 |
| | $q_{max}$ | -18.0 | -0.99 | -24.2 | -0.06 | 0.19 | 0.01 | -1.08 | 0.03 | 0.26 |
| | $r^2$ | 0.869 | 0.993 | 0.410 | 0.997 | 0.555 | 0.943 | 0.953 | 0.671 | 0.179 |
| B | $K_L$ | -0.02 | 0.07 | 0.71 | -2.61 | -0.36 | -18.7 | 0.02 | -0.02 | -1.48 |
| | $q_{max}$ | -137 | 56.8 | 25.9 | -0.98 | -4.39 | 0.02 | 0.67 | -18.8 | 0.06 |
| | $r^2$ | 0.995 | 0.139 | 0.991 | 0.044 | 0.538 | 0.875 | 0.858 | 0.989 | 0.963 |
| C | $K_L$ | -0.05 | -0.04 | -0.19 | -19.1 | -8.10 | -11.3 | -0.08 | -0.12 | -1.32 |
| | $q_{max}$ | -6.46 | -15.1 | -1.15 | -0.01 | 0.01 | 0.01 | -0.25 | -11.6 | 0.01 |
| | $r^2$ | 0.879 | 0.992 | 0.815 | 0.996 | 0.902 | 0.696 | 0.984 | 0.994 | 0.836 |

For these compounds both the homogeneous and the heterogeneous adsorption energy may be supposed to occur during the process. Conversely, the adsorption isotherm of octanoic acid on bentonite A badly fitted by both the Eq(2) and the Eq(3), and probably necessitated to be explained by further models.

## 4. Conclusion

This study characterized the effect of the bentonite on some odor-active compounds that contribute to the sensory profile of the young white wines. Under the circumstances of the study, i.e. with a high adsorbent/adsorbate ratio, the possible differences among the bentonite samples were enhanced and allowed to evaluate the clay characteristics that mainly affected the adsorption mechanisms. Having a lower SSA and a greater charge density per surface unit, A and C samples slightly interacted through physical mechanisms with most of the odor-active compounds. They showed high adsorption capacity especially with hydrophobic molecules. With larger SSA and lower charge density per surface unit, clay B promoted stronger adsorptions especially for ethyl esters. Therefore, the main interaction forces controlling adsorption appeared to be related more to the clay characteristics, than to the compounds properties.

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