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

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This work extends the concepts proposed in a previous research paper about the interaction between the aroma of two white wines and bentonites. Such previous results led to hypothesized that some odor-active compounds were removed through direct adsorption mechanism on the clays. As a consequence, this paper examined the adsorption isotherms at 17±1°C of three bentonite samples added in three different amounts to a model white wine without the presence of wine macromolecule. The clays were analyzed for the elemental composition, the surface charge density, and the specific surface area (SSA) and differences were analyzed by Tukey's test. The analysis of variance (ANOVA) was used to demonstrate the significance of the bentonite on the aroma reduction. The Langmuir and the Freundlich models were fitted to the adsorption data. The most experimental adsorption isotherms were robustly fitted by the Freundlich equation indicating that, under the condition of the study, the adsorption process more frequently occurred with a heterogeneous energy distribution of an infinite number of surface active sites. Overall, the modeling prediction ability tested by the error on r^2 in cross validation enhanced differences both among the odor-active compounds and among the clays. 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 by chemical interactions especially for ethyl esters. For the tested odor-active compounds differences in the adsorption intensity and capacity depended mainly on the bentonite characteristics rather than on the properties of the substances.

1. Introduction

Bentonite fining is commonly used by the wine industry as a clarifying technique to remove the proteins that are a potential source of haziness 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. Its exchangeable cations (primarily Ca^{2+} and Na^+) influence the interlayer spacing and the swelling properties of the bentonite, modulating the intercalation of water into the inner layers. Sodium bentonites are preferred for their swelling properties in water, resulting in an effective treatment for wine (Lambri et al. 2012b). In wine, 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 (Blade and Boulton 1988, 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 (Voilley et al. 1990). Wine volatiles have different chemical characteristics, covering a wide range of polarities, solubilities, and volatilities. These compounds include alcohols, esters, aldehydes, ketones, and monoterpenes in addition to various sulfurous and phenolic compounds (Ugliano and Henschke 2009). 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 after multiple clarification/stabilization treatments and the related effects on the

sensory quality of the different types of wine were evaluated (Lambri et al. 2010, Lambri et al. 2012b). Nevertheless, the direct interaction between the bentonite and the wine odor-active compounds has not yet been fully established. This study extended the concepts proposed in a previous research (Lambri et al. 2010) where the direct adsorption of some odor-active compounds onto bentonite during the fining of two white wines was speculated. Under model conditions and in the absence of any wine macromolecule 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) was examined.
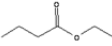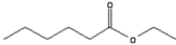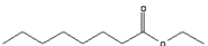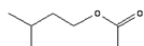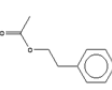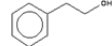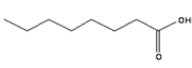
2. Materials and methods

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 (Table 1) 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).

Table 1: Odor-active compounds added to the model white wine.

Molecule	CAS Registry Number	Structure [a]	MW [a] (Da)	Boiling point [a] (°C)	Water solubility [b] (mg/L)	LogP (octanol/ water) [b]	Vapor Pressure [b] (mmHg)
Ethyl butyrate	105-54-4		116.1583	121.5	4900 [*]	1.85	12.8
Ethyl hexanoate	123-66-0		144.2114	167.0	629 [**]	2.83	1.56
Ethyl octanoate	106-32-1		172.2646	208.5	70.1 [**]	3.81	0.211
Isoamyl acetate	123-92-2		130.1849	142.5	2000 [**]	2.25	5.6
β-phenylethyl acetate	103-45-7		164.2011	232.6	711 [**]	2.30	0.0314
β-phenylethanol	60-12-8		122.1644	218.2	$2.22 \cdot 10^4$ [**]	1.36	0.0868
Hexanoic acid	142-62-1		116.1583	205.2	$1.03 \cdot 10^4$ [**]	1.92	0.0435
Octanoic acid	124-07-2		144.2114	239.0	789 [**]	3.05	0.00371

[*] measured at 20°C

[**] measured at 25°C

[a] Data from NIST Chemistry WebBook. NIST National Institute of Standard and Technology.

[b] Data from SRC. Interactive PhysProp Database Demo, Syracuse Research Corp., Environmental Science CenterSyracuse, NY, www.syrres.com/esc/physdemo.htm.

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 listed in Table 1, a defined volume of 1000 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: 4000 µg/L for ethyl butyrate; 1200 µg/L for ethyl hexanoate; 200 µg/L for ethyl octanoate; 4500 µg/L for isoamyl acetate; 160 µg/L for β-phenylethyl acetate; 2000 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 (m2/100g).

2.3 Batch experiment procedure

Batch adsorption experiments were performed to simulate the bentonite addition to a wine containing some odor-active compounds contributing to the fruity aroma. 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 concentration of bentonite sample, three replicates were prepared in order to arrange 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 hours, the limpid liquid phases of both samples and control test were separated and filtered through folded filters (595 ½, Whatman GmbH, Germany).

2.4 Adsorption isotherms

The adsorption data were expressed by the Langmuir (1916) and Freundlich (1906) models. To ensure equilibrium, the concentration of the adsorbate was measured after 12, 24 and 36 hours 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 hours was established as the equilibrium time. The concentration of the non-adsorbed odor-active compounds measured after 24 hours was assumed to be the equilibrium concentration (C_e).

Determination of the content of the non-adsorbed odor-active compounds

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.

Determination of the content of the adsorbed odor-active compounds

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

$$q_e = \frac{V\,(C_0 - C_e)}{M}$$ (1)

where C_0 and C_e are respectively the initial and the equilibrium concentrations (µmol/L), i.e. the concentration of the non-adsorbed odor-active compounds measured after 24 hours. V is the volume of the solution (1 L) and M is the mass of the adsorbent (0.2, 0.5, 1 g).

2.5 Data analysis and statistics.

The data were analyzed using the statistics package IBM SPSS Statistics 19 (IBM Corporation, New York, USA). The bentonite characteristics and the data from the odor-active compound analysis were analyzed by a factorial ANOVA. Significant differences were also analyzed using Tukey's test at $p \leq 0.05$. The data from the batch adsorption experiments were analyzed by the curve estimation procedure. This procedure is the most appropriate when the relationship between the dependent variable(s) and the independent variable is not necessarily linear (Garson, 2012) and generates for each dependent variable the related plots for 11 different curve estimation regression models. Finally, the risk (error on r^2) and the prediction ability of the best models selected from curve estimation procedure were evaluated by cross validation (CV). CV compared learning algorithms by dividing adsorption data into two segments: one used to learn or train the model and the other used to validate the model (Arlot and Celisse, 2010). In detail, as reported in the CV test set method, the dataset was randomly broken into k=3 partitions: the 33% of the data was chosen as the test set, while the remainder was the training set. The regression was performed on the training set and the future performance

was estimated with the test set. The training and the validation sets have been crossed-over in three successive rounds as indicated for the k-fold CV. Then, the test-set sum of errors on r^2 was founded.

3. Results and discussion

3.1 Physicochemical analysis of bentonite

The characterization of the bentonite samples is shown in Table 2. 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.

Table 2: 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

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 2) were consistent with recently reported values in the literature, ranging from 57.0 to 0.4 cmol/kg (Xifang et al., 2007). 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

The parameters for the Langmuir and Freundlich models resulted from adsorption experiments are reported in Tables 3 and 4.

Between the amounts intended to be added to the model solution (*see 'Material and method section'*) and the concentrations measured in the liquid phase of the control test (Table 3), differences by a factor ranging from 0.01-11% (for ethyl hexanoate and hexanoic acid respectively) to 90-99% (for ethyl octanoate and isoamyl acetate respectively) were detected. The largest differences occurred for molecules such as ethyl butyrate and octanoic acid (79%), ethyl octanoate (90%) and isoamyl acetate (99%) having the highest volatility or the greatest LogP (Table 1). This was expected because of both the loss due to the treatment of the model solutions (settling, storage, filtration) and of the difference between volatility of pure aroma compounds and of aroma compounds in aqueous solution that especially exists for the largest molecules (i.e. ethyl hexanoate and ethyl octanoate) as stated by the research of Tromelin et al. (2010).

Moreover, under the conditions of the present study, when the headspace of the flasks above the model white wine was created in a very short period of time and followed by agitation, the mass transfer coefficient governs the release of aroma compounds from the liquid to the vapor phase. A great variability in mass transfer coefficient values of aroma compounds between liquid and vapor phase is reported in the literature, emphasizing that diffusivity values are highly dependent on the experimental conditions, which are difficult to control tightly (Cayot et al. 2008).

Besides the convection process, the volatile compound release in the headspace depended mostly on the physicochemical properties of the molecules themselves, where the partitioning (LogP), and the volatility are of crucial importance (Table 1). The differences between the amounts added to the model wine and the concentrations measured in the liquid phase of the test sample (Table 3) showed values also reported by Banavara et al. (2002) by modeling the flavour release from the liquid phase to the headspace.

Table 3: Experimental parameter C_e (µmol/L) of the control test and of the samples for the Langmuir and the Freundlich adsorption isotherms. Values are the means $\pm$ SD (n = 3). Within each row and each bentonite sample, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Odor-active compound	Control	Bentonite A 0.2 g/L	0.5 g/L	1.0 g/L	Bentonite B 0.2 g/L	0.5 g/L	1.0 g/L	Bentonite C 0.2 g/L	0.5 g/L	1.0 g/L
Ethyl butyrate	7.158 ± 0.697	1.730 ± 0.037 a	1.636 ± 0.055 a	1.532 ± 0.037 b	4.408 ± 0.049 a	2.402 ± 0.030 b	1.300 ± 0.043 c	6.878 ± 0.061 a	4.649 ± 0.124 b	4.098 ± 0.155 b
Ethyl hexanoate	8.924 ± 0.044	4.202 ± 0.044 a	2.399 ± 0.039 b	2.101 ± 0.044 b	4.847 ± 0.025 a	1.914 ± 0.054 b	0.589 ± 0.020 c	4.854 ± 0.113 a	4.369 ± 0.015 a	4.216 ± 0.025 a
Ethyl octanoate	0.119 ± 0.016	0.068 ± 0.008 a	0.056 ± 0.008 a	0.045 ± 0.004 b	0.073 ± 0.004 a	0.051 ± 0.001 b	0.034 ± 0.004 c	0.051 ± 0.004 a	0.048 ± 0.004 a	0.045 ± 0.004 a
Isoamyl acetate	0.362 ± 0.026	0.315 ± 0.011 a	0.238 ± 0.005 b	0.161 ± 0.003 c	0.253 ± 0.016 a	0.177 ± 0.016 b	0.131 ± 0.011 c	0.353 ± 0.011 a	0.177 ± 0.027 b	0.092 ± 0.022 c
β-Phenylethyl acetate	0.132 ± 0.006	0.134 ± 0.009 a	0.097 ± 0.004 b	0.079 ± 0.009 c	0.128 ± 0.004 a	0.110 ± 0.004 b	0.073 ± 0.004 c	0.091 ± 0.004 a	0.110 ± 0.009 a	0.110 ± 0.009 a
β-Phenylethanol	3.635 ± 0.090	2.914 ± 0.029 a	2.472 ± 0.237 b	2.169 ± 0.058 b	3.160 ± 0.145 a	3.070 ± 0.162 a	3.029 ± 0.295 a	3.102 ± 0.174 a	3.021 ± 0.046 a	2.922 ± 0.041 a
Hexanoic acid	0.305 ± 0.029	0.284 ± 0.024 a	0.207 ± 0.012 b	0.095 ± 0.014 c	0.207 ± 0.006 a	0.181 ± 0.006 b	0.146 ± 0.012 c	0.146 ± 0.015 a	0.112 ± 0.012 b	0.086 ± 0.006 c
Octanoic acid	1.172 ± 0.134	1.102 ± 0.010 a	0.756 ± 0.029 b	0.520 ± 0.034 c	0.700 ± 0.020 c	0.832 ± 0.010 b	0.992 ± 0.039 a	1.165 ± 0.074 a	0.888 ± 0.020 b	0.693 ± 0.020 c

Table 4: Experimental adsorption parameter q_e (µmol/g) for the Langmuir and the Freundlich isotherms. Values are the means + SD (n = 3). Within each row and each bentonite sample, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Odor-active compound	Bentonite A 0.2 g/L	0.5 g/L	1.0 g/L	Bentonite B 0.2 g/L	0.5 g/L	1.0 g/L	Bentonite C 0.2 g/L	0.5 g/L	1.0 g/L
Ethyl butyrate	28.667 ± 0.944 a	11.656 ± 0.341 b	5.931 ± 0.189 c	15.281 ± 1.370 a	6.680 ± 0.390 b	6.164 ± 0.183 c	5.630 ± 0.822 a	3.366 ± 0.499 b	2.927 ± 0.170 c
Ethyl hexanoate	23.611 ± 2.476 a	13.050 ± 0.981 b	6.823 ± 0.495 c	20.387 ± 2.378 a	14.021 ± 1.010 b	8.335 ± 0.471 c	20.352 ± 2.819 a	9.112 ± 0.873 b	4.708 ± 0.476 c
Ethyl octanoate	0.232 ± 0.082 a	0.116 ± 0.033 b	0.070 ± 0.012 c	0.203 ± 0.062 a	0.221 ± 0.016 a	0.081 ± 0.012 b	0.319 ± 0.062 a	0.128 ± 0.025 b	0.070 ± 0.012 c
Isoamyl acetate	0.192 ± 0.001 b	0.384 ± 0.011 a	0.192 ± 0.005 b	0.499 ± 0.136 a	0.353 ± 0.011 b	0.223 ± 0.001 c	0.001 ± 0.001 c	0.353 ± 0.033 a	0.261 ± 0.011 b
β-Phenylethyl acetate	0.076 ± 0.008 a	0.037 ± 0.007 c	0.055 ± 0.004 b	0.030 ± 0.016 b	0.049 ± 0.017 b	0.061 ± 0.017 a	0.213 ± 0.043 a	0.049 ± 0.009 b	0.024 ± 0.004 c
β-Phenylethanol	3.684 ± 0.605 a	1.866 ± 0.625 b	1.482 ± 0.492 b	2.456 ± 0.026 a	1.212 ± 0.076 b	0.622 ± 0.055 c	2.742 ± 0.881 a	1.424 ± 0.207 b	0.729 ± 0.109 c
Hexanoic acid	0.043 ± 0.030 c	0.344 ± 0.012 a	0.198 ± 0.006 b	0.430 ± 0.061 a	0.275 ± 0.024 b	0.146 ± 0.006 c	0.732 ± 0.030 a	0.362 ± 0.012 b	0.207 ± 0.022 c
Octanoic acid	0.312 ± 0.070 b	0.125 ± 0.069 c	0.645 ± 0.029 a	0.936 ± 0.117 a	0.388 ± 0.147 b	0.173 ± 0.025 c	0.001 ± 0.003 b	0.416 ± 0.088 a	0.472 ± 0.044 a

By taking these factors into consideration, the results from the batch adsorption experiments at 17±1°C however indicated significantly different C_e and q_e values (Tables 3 and 4) within each clay according to the dose employed showing that in the model white wine without additional macromolecules, the direct adsorption of the odor-active substances onto the bentonite sheets was significantly enhanced and related to the amount of the clay used (Tables 3 and 4). Compared with the observations in real wine (Lambri et al. 2010), these data support the "protective role" played by the wine macromolecules towards the aroma compounds during the fining process (Lambri et al. 2012b). Moreover, these outcomes confirm the porous nature of montmorillonite clay as favorable property for adsorption (Zaghouane-Boudiaf and Boutahala 2011).

Models selected for fitting the adsorption data of the wine odor-active compounds onto bentonite
Under the conditions of the present study, it was also necessary to considered that all the odor-active compounds became simultaneously in contact with the bentonite with the same extent for all the adsorption tests. Moreover, because of the dilution factor the possible interactions in solution between the molecules of the mixture were reasonably negligible. The Langmuir (1916) and the Freundlich (1906) models were selected by the curve estimation procedure as the best ones fitting the data of the adsorption at 17±1°C both for the mixture of the odor-active compounds and for the single odorous substances (Tables 5 and 6). Among the various models, the Langmuir and the Freundlich equations are commonly used to describe the general adsorption performance of bentonite (Akçay and Akçay 2004, Jiang and Zeng 2003, Maarof et al. 2004, Zaghouane-Boudiaf and Boutahala 2011) and the specific adsorption performance of bentonite with proteins (Blade and Boulton 1988; de Bruijn et al. 2009, Xifang et al. 2007). The Langmuir equation (1916)

$$q_e = \frac{q_m \, K_L \, C_e}{1 + K_L \, C_e} \tag{2}$$

has been used in the following linear form:

$$\frac{C_e}{q_e} = \frac{1}{q_m \, K_L} + \frac{1}{q_m} \, C_e \tag{3}$$

where q_m (µmol/g) is the maximum adsorption capacity of the monomolecular layer obtained from the isotherm model, C_e and q_e are the experiment equilibrium concentrations (µmol/L) and the experiment equilibrium capacity (µmol/g), respectively, and the Langmuir adsorption equilibrium constant K_L (L/µmol) is related both to the adsorption grade and energy.
The Freundlich model (1906), an empirical and exponential equation that assumes an infinite number of surface adsorption sites with a heterogeneous energy distribution of the active sites, has been used in its original form:

$$q_e = K_F \, C_e^{1/n} \tag{4}$$

where the Freundlich constant K_F and the Freundlich exponent n are related to the adsorption capacity and intensity, respectively. K_F is the adsorption value, the amount adsorbed at unit concentration, that is, at 1 µmol/L. It is characteristic for the adsorbent and the adsorbate adsorbed (de Bruijn et al. 2009, Zaghouane-Boudiaf and Boutahala 2011).
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 curves (Figure 1 and Table 5), indicating that this approach may be useful in fitting the data 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 6) 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 (Langmuir 1916), 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 6) than in the Freundlich model (Table 5) indicated that the process did not follow the assumption of a heterogeneous energy distribution of an infinite number of surface active sites.
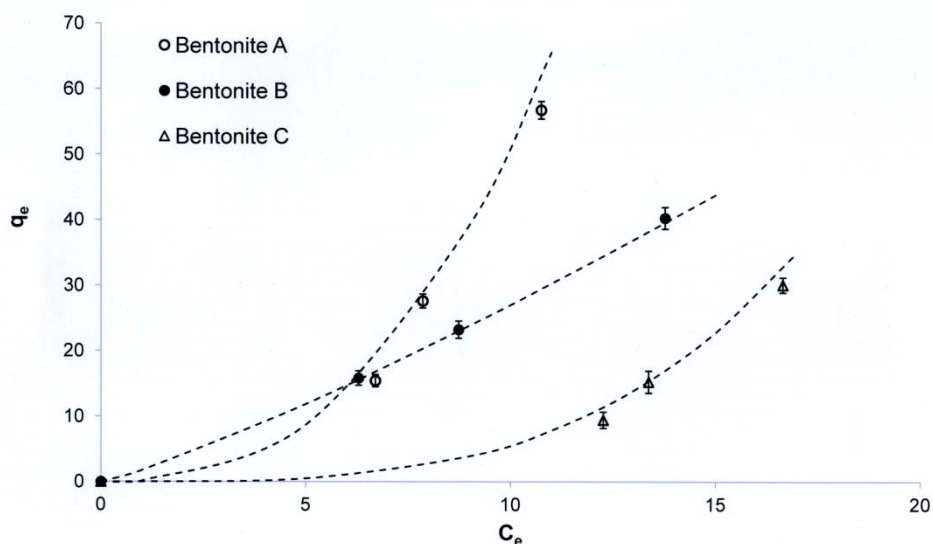
Table 5: Constants from the Freundlich model for the adsorption of the odor-active compounds on the three bentonite samples at 17 ± 1 °C.

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

Table 6: 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

Figure 1: Freundlich isotherms for the mixture of the odor-active compounds on the three samples of bentonite at 17±1°C according to the following model: $q_e = K_F\, C_e^{1/n}$. Each point represents the mean value of three independent adsorption experiments. The bar on each point indicate the standard deviation (SD).

Despite the high correlation coefficients (r^2), the Langmuir model evidenced a low prediction ability with estimated model errors ranging from 28% of r^2 for octanoic acid to 52% for isoamyl acetate both on bentonite C. The best prediction ability (6% error on r^2) of the Langmuir model was showed for the adsorption of isoamyl acetate on bentonite A, but on the same clay the model evidenced a 46% error for

hexanoic acid. On the other hand, excepted for the adsorption of hexanoic acid on bentonite A (Table 6), the constants of the Langmuir model could not provide a simple and unequivocal interpretation of the data. Finally, the adsorption processes of β-phenylethyl acetate, ethyl hexanoate, β-phenylethanol and octanoic acid on bentonite B, were well described by both the models (Tables 5 and 6) with similar percentages of errors on r^2 respectively for the Langmuir and the Freundlich regression plots: 3.3% and 2.7% for ethyl hexanoate; 72% and 63% for β-phenylethanol; 44% and 53% for β-phenylethyl acetate; 12% and 15% for octanoic acid. For these compounds both the homogeneous and the heterogeneous adsorption energy may be supposed to occur during the process of adsorption onto clay B.

The two models produced, instead, highly different estimated errors in the case of the adsorption process of β-phenylethyl acetate onto bentonite A: 1.8% error on r^2 with Langmuir and 28% with Freundlich plotting. As a consequence, a prevailing mechanism of a monolayer adsorption onto a completely homogeneous surface with a finite number of identical sites may be considered for the adsorption of β-phenylethyl acetate onto bentonite A. Conversely, the adsorption isotherm of octanoic acid on bentonite A badly fitted by both the (3) and the (4) equations, and probably necessitated to be explained by further models.

Bentonite adsorption intensity and capacity toward the wine odor-active compounds
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 (Figure 1). 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. In this regard, the n constant of the Freundlich model 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 that the adsorption was favorable and mediated by chemical interactions (Jiang and Zeng 2003), 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 that the bentonite had a slightly unfavorable adsorption profile (de Brujin et al. 2009) that was mostly driven by physical interactions (Jiang and Zeng 2003). Given a fixed C_e and K_F, the higher values of the n constant indicate a stronger adsorption bond.

Among the bentonite samples the adsorption process for the mixture of the odor-active compounds was more favored on clay B (Figure 1). Both the adsorption intensity n and the adsorption capacity K_F (Table 5) showed decreasing values moving from the bentonite B, to the clay A and then to the C with very low percentage errors on r^2 which are decreasing from the bentonite B (0.02%), to C (0.09%) and then to A (0.41%). The greater adsorption on clay B may be due to its large SSA and to the high sodium/calcium ratio (Table 2). 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; Alther 2004).

In detail, for the most odor-active compounds and the mixture (Figure 1 and Table 5), 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 $n>1$ (Table 5) 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, Zaghouane-Boudiaf and Boutahala, 2011) and proteins (Blade and Boulton 1988, de Brujin et al. 2009) 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 5) 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. CV estimation of model prediction ability evidenced similar error percentages on r^2 for the adsorption onto clay C of β-phenylethanol (39%) and of β-phenylethyl acetate (36%). The adsorption of β-phenylethanol onto bentonite B showed a 63% error, while that of ethyl butyrate onto clay A was of 12%. Differently from the process observed for bentonite A, the adsorption isotherms of ethyl butyrate on bentonite B and C were more favored with errors of 3.7% and of 7.1% respectively, 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. The estimated error of Freundlich model prediction ability for the adsorption of ethyl hexanoate increased from bentonite

A (1.2%), to C (1.9%) and then to B (3.7%), while ethyl octanoate showed adsorption plots better predicted for bentonite A (2.2%), than for clays B (17%) and C (35%). 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 Furuya et al. (1997), indicating that in an aqueous solution, hydrophobic compounds, having low solubility, tend to be 'pushed' to the adsorbent surface. Thus the affinity between the adsorbate and the adsorbent can increase.

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 ($n>1$). For this bentonite, the Freundlich model prediction ability showed errors on r^2 lower for β-phenylethyl acetate (53%) than for β-phenylethanol (63%). Besides, the adsorption of β-phenylethanol, although unfavorable, was promoted more on clay A (i.e. the Freundlich model showed both greater n intensity and K_F capacity and the best prediction ability with a 13% error on r^2) than on clays B and C for which model errors of 63% and 39% were respectively observed. In spite of literature reports for the phenolic compounds (Akçay and Akçay 2004, Maarof et al. 2004, Zaghouane-Boudiaf and Boutahala 2011), 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 (Table 1); 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 2).

The remaining odor-active compounds (isoamyl acetate, hexanoic and octanoic acid) featured a differentiated adsorption behavior depending on the type of bentonite sample. As previously stated, the adsorption of isoamyl acetate well fitted with the Freundlich model only on bentonite B (Table 5) with a 17% error on r^2. On the other clays better correlation coefficients (r^2) were observed in the Langmuir model (Table 6) with highly different errors: 6% and 52% for bentonites A and C respectively. Moreover, the related Langmuir constants left doubts for a simple and unequivocal interpretation of the data. The hexanoic acid had similar both unfavorable adsorption intensities n and error percentages on r^2 of 4.4% and 6.1% on bentonite B and C respectively, with high K_F values on both the clays (Table 5). 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 showed a $n<1$ adsorption intensity on bentonite B and an estimated 15% error on r^2, indicating unfavorable adsorption and prevailing physical interactions between the adsorbate and the adsorbent. As observed with isoamyl acetate, the adsorption isotherms of octanoic acid on clays A and C were not adequately described by the Freundlich model (Table 5). The parameters derived from the Langmuir model (Table 6) for octanoic acid adsorption on bentonites B and C and producing a good correlation coefficient (r^2), could not provide a simple and unequivocal interpretation of the data taking also into account the 12% and 28% error on r^2 observed for clays B and C respectively.

4. Conclusion

This study further characterized the effect of the bentonite on some odor-active compounds that contribute to the sensory profile of the young white wines. The same doses of three bentonite samples having SSA similar to the clays already tested on Chardonnay wines (Lambri et al. 2010) were added to a model white wine. 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 (ethyl hexanoate, ethyl octanoate, isoamyl acetate), and compounds with phenolic ring (β-phenylethyl acetate, β-phenylethanol) which were fitted with the lowest risk error in the case of bentonite A either in the Langmuir or in the Freundlich model. Differently, the adsorption data modeling of these compounds showed on the clay C or the highest error or an intermediate behavior between the other bentonite samples. With larger SSA and lower charge density per surface unit, clay B promoted stronger adsorptions especially for ethyl esters. On this clay rather than on other bentonite samples, either the Langmuir or the Freundlich modeling evidenced better prediction ability for the adsorption data of ethyl butyrate, hexanoic acid, octanoic acid, and the mixture. Therefore, the main interaction forces controlling adsorption appeared to be related more to the clay characteristics, than to the compound properties.

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