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Innovations in the Use of Bentonite in Oenology: Interactions with Grape and Wine Proteins, Colloids, Polyphenols and Aroma Compounds

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Additional information is available at the end of the chapter

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Abstract

Bentonite is used in oenology to improve the limpidity and the stability of wine and to predict the formation of deposits in the bottle. The exchangeable cations in its lamellar structures strongly influence some properties, such as the specific surface, the exchange capacity and the adsorption behavior. The unintended use of bentonite for juice settling and/or for wine fining produces jeopardized effects on colloidal and protein stability, the aroma compounds and sensory profiles. The interactions with haze-forming proteins, other colloids, as well as aroma compounds and phenols would have been to discover as the modulation of wine colloids by an adjuvant severely affects the wine resilience and the sensory profile. This chapter reviews several studies that focus on the impact of commercial bentonite samples used for both juice clarification and wine fining on the colloids, proteins, phenols and aroma compounds of white and red wines. Some parameters of practical value, such as the wine heat stability, the concentrations of total and haze-forming proteins and the content of the most relevant aromas, have been assessed to track the effects of bentonite and to achieve findings that are applicable to the field of oenology.

Keywords: bentonite, colloids, proteins, polyphenols, aroma, wine

1. Bentonite

1.1. Chemical structure

Bentonite is a phyllosilicate of the class of dioctahedral smectites [1] with a general composition of $(M+y \cdot nH_2O) (Al^{3+}_2yMg^{2+y})Si^{4+}_4O_{10}(OH)_2$.

The structures of phyllosilicates are all based on tetrahedral T and octahedral O sheets arranged in either a 1:1 (kaolinite, dickite and nacrite) or 2:1 (smectites, vermiculite, mica and chlorite) ratio to form an anisotropic TO or TOT layer, respectively (Figure 1).

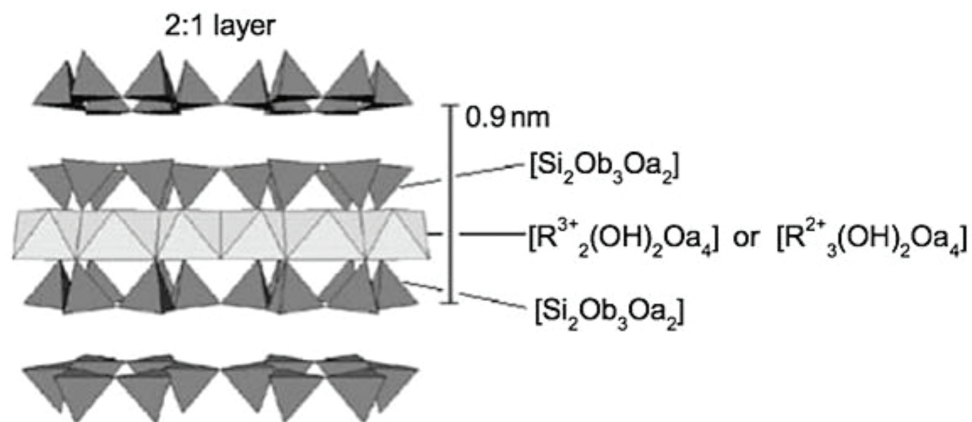


Figure 1. Structure of a phyllosilicate of the class of dioctahedral smectites [1] as bentonite is.

The layers are stacked on top of each other to form what is called a 'particle'. In turn, an assembly of particles is known as an 'aggregate'. Thus, a particle is formed by stacked layers that are separated from each other by an interlayer space. For a single layer, each tetrahedron consists of a cation (Si⁴⁺, Al³⁺ and Fe³⁺ are the most common) coordinated to four oxygen atoms and linked to an adjacent tetrahedra by sharing three basal oxygen atoms to form a two-dimensional 'hexagonal' pattern. However, each octahedron consists of a cation (Al³⁺, Fe³⁺, Mg²⁺, or Fe²⁺) coordinated by six oxygen atoms and linked to a neighbouring octahedra by sharing edges. In the 2:1 structures, such as smectites, one octahedral sheet is sandwiched between two tetrahedral sheets. The surface properties of clay minerals depend on many factors, such as their chemical composition, nature of the surface atoms, extent and type of charge, and type of exchangeable cations. There are two types of surfaces with different properties, the planar surface and the edge surface, which is affected by pH. In a 2:1 clay, such as bentonite, the oxygen atoms of the Si tetrahedron represent the atoms of the basal surface. The charge on the oxygen atoms depends on the difference in electronegativity between oxygen and the other atoms involved in the bond. Thus, the ionicity of the bonds follows the order H-O < Si-O < Al-O < Mg-O < Li-O.

Thus, in smectites, the planar surfaces are negatively charged, with the magnitude of the layer charge ranging from 0.2 to 0.6 per half-unit cell. The type of metals that are bonded to the oxygen atoms governs the hydrophobicity of the surface. In the absence of isomorphous

substitutions, Si–O bonds prevail in the tetrahedra and Al–O bonds prevail in the octahedral sheets, resulting in a hydrophobic surface. When isomorphous substitutions occur, that is substitution of trivalent cations for Si^{4+} in tetrahedral sites and of divalent cations for Al^{3+} in octahedral sites, hydrophilicity is introduced and exchangeable cations are present [1].

Using the electronegativity equalization method (EEM) [2], beidellite and montmorillonite were shown to be the most electronegative and reactive 2:1 clays. The negative charges that result from isomorphous substitutions are balanced by the presence of exchangeable cations in the interlayer space. Na^+ , Ca^{2+} , Mg^{2+} and K^+ are the most common ions. The mechanisms involved in the cation hydration for smectites are mainly electrostatic interactions that minimize water–water repulsion [3].

The presence of clustered or ‘nanoconfined’ water molecules around the exchangeable cations and the water bound to the clay through hydrogen bonds, charge dipole attractions and van der Waals interactions expands the interlayer space. This expansion or swelling depends on the cations present, the ionic strength of the medium and other factors. During hydration, water molecules orient their negative dipole towards the cations, thus weakening the electrostatic interactions with the charged layer and leading to increased separation between the two successive layers. As a result, the cations on the layer surface can be exchanged with the cations in the external solution and, based on the persistence or absence of significant interactions between two successive layers, this separation is called delamination or exfoliation, respectively [4]. In the first case, the smectite increases its volume to maintain its structure, whereas in the second case, isolated layers from the stacks of layers detach from the structure, becoming independently mobile.

Based on their swelling properties, sodium bentonite is different from calcium bentonite because the former is a high-swelling type of clay, while the latter is a low-swelling type. In fact, when sodium bentonite takes up water, exfoliation is more frequent than in the case of calcium bentonite. This difference is due to the type of ion that is intercalated on the surface of the layers. The amount of Na^+ needed for the compensation of the negative charge on the layers is double that needed for Ca^{2+} because the charge of Na^+ is half that of Ca^{2+} . In addition, based on the hydrated ionic radii of Na^+ and Ca^{2+} , the number of water molecules intercalated in the interlayer space during wetting in the presence of Na^+ is higher than that in the presence of Ca^{2+} .

The other type of surface, the edge surface, is characterized by the presence of hydroxyl groups called terminal OH groups. These hydroxyl groups carry a charge that is dependent on the type of metal ion to which they are bonded in the structure and on the pH of the solution. The charging arises from the adsorption or dissociation of protons. At low pH values, the excess protons in the aqueous medium create positive edge charges, whereas negative charges are produced by the dissociation of silica and alumina groups at high pH values [5, 6].

1.2. Extraction sites

In 2009, the worldwide production of bentonite amounted to 9.66 Mt. Approximately 90% of the world production is concentrated in 13 countries, with 55% of the total production in

Greece, the USA and the Commonwealth of Independent States. The different properties of bentonite reflect its different origins (47 countries contribute to the total production). Bentonite deposits were formed by diagenetic or hydrothermal alteration of volcanic glass and by authogenesis of smectite-rich materials in alkaline continental basins [7]. Dioctahedral smectites first become Fe^{3+} -rich and then subsequently become Al^{3+} -rich (montmorillonite) minerals formed in the absence of Mg^{2+} , and the most important deposits are located in Wyoming, Montana, Arizona (USA) [8], India and Europe (Czech Republic, Denmark, Germany and Greece). Regarding the contents of Na^+ and Ca^{2+} , which are important for the swelling and rheological properties, there is high variability among the clays from different deposits in the same country. Generally, American bentonites are Na^+ rich, while the European bentonites are Ca rich, with few exceptions.

1.3. Industrial activation treatments

To modify the properties of bentonite, industrial activation processes are performed on bentonites that do not present the optimal characteristics for the use for which they are designed. There are two types of modification processes, mineral activation and organic modification.

In the mineral activation process, wet mud is treated at 80°C in the presence of Na_2CO_3 . During the process, Ca^{2+} ions are precipitated as CaCO_3 . Consequently, the Na/Ca ratio of the clay is modified by substituting the interlayer and surface Ca^{2+} ions for Na^+ ions. The treatment improves the swelling ability of the slurries and enhances the adsorption of wine proteins [9]. Significant changes in the rheological characteristics (viscosity), pH, cationic exchange capacity (CEC), and structure of bentonite could also be produced after alkaline magnesium activation [10]. Another treatment that is used to improve the adsorbent performance of bentonite is the acid activation treatment with HCl and H_2SO_4 , in which the main task is to increase the specific surface area (SSA) and the porosity by degrading the structure of the clay due to the leaching of Al^{3+} , Fe^{2+} and Mg^{2+} . In this case, the exchangeable cations are replaced by H^+ . Organic modification is widely performed to improve the ability of the clay to remove heavy metals and organic compounds from water [11]; it reduces the total number of interlayer ions (Na, Ca, Mg) by replacing them with organic cations. Two categories of modified bentonite are produced, organoclays [12] and oxide pillared clays [13]. The main modifications induced by these processes are to the surface properties of the clays, which transition from hydrophilic to hydrophobic surfaces. This improves the ability of the modified bentonite to remove organic contaminants, such as phenols, from water [14].

2. Bentonite applications

2.1. Uses in non-food fields

Based on its properties and its relatively low cost of production, bentonite use has attracted interest from researchers in several fields, particularly those in the industrial and environmental fields [15]. Due to its viscosity, thixotropy, plastering ability and plasticity, bentonite is

used to grout cracks in rocks, for soil injections, as a thickener in paints and as an additive in ceramics [16]. To reduce gas release from landfill decomposition and to impede water percolation across the landfill, clay liners have been used as substitutes for the compacted soil components normally used [17]. In the context of disposal programmes, bentonite is projected to be used in engineered barriers for high-level waste and spent nuclear fuels [18]. A significant amount of the global bentonite production is destined to iron ore pelletizing, foundry moulding and oil-well drilling processes [19]. Bentonite is also used in animal feed, as a litter adsorbent and as an oil and grease adsorbent. Its importance in animal feed is derived from its ability to adsorb mycotoxins, such as aflatoxins [20]. Bentonite plays an important role in the development of health products [21], such as cosmetics and pharmaceuticals [22]. Moreover, it has been shown to be useful in different medical treatments [23]. Bentonite is also applied to the production of sunscreen lotions, as it acts as physical barriers against UV radiation [24] as well as a thickener in paste masks, eyeliners, nail lacquers, shampoos and toothpastes [25]. Other, less important applications include the production of additives for cement and mortar [26] and the cleaning of wastewaters. Recently, bentonites have been used for enzyme immobilization [27] and as bactericidal materials [28].

2.2. Uses in syrup and juice production

The application of bentonite's characteristics has been investigated in different juice and syrup production processes. Fruit juices are naturally cloudy due to the presence of polysaccharides (pectin, cellulose, hemicellulose, lignin and starch), proteins, tannins and metals [29]. Other molecules, such as flavonoids, polyphenols and organic acids, contribute to the dark colour of sugarcane and other juices. Because these components are present in varying amounts, clarification is one of the key steps in juice processing. The commonly used clarification methods include the use of enzymes, ultrafiltration, gelatine, bentonite, PVPP [30], lime, active carbon [31] and ion exchange resins [32]. Bentonite is cheap, does not pose environmental threats and represents one of the most suitable alternatives for the other methods. In particular, activated Na–Ca bentonite is the type of clay used in beverage technology [32]. It is used as the main clarifying agent or in preclarification steps [33]. In the clarifying step for concentrated must production, the basic treatment is based on the addition of bentonite and gelatine for various times of contact (usually 24 or 48 h). In fruit juice production, bentonite can be added to reduce the brown pigments resulting from enzymatic reactions: positive results concerning the browning index and the haze potential have also been attained for banana juice [34]. Finally, bentonite has been evaluated for use as a moisture-regulating adjuvant in food technology [35].

3. Bentonite in oenology

The addition of bentonite to musts and/or wines is aimed at reducing the protein contents, thus reducing the haze potential of the wine (fining). Fining is defined as the process of addition of substances that induce the precipitation of particles in suspension by promoting their sedimentation.

In oenology, bentonite could also be applied to the must to improve the precipitation of the suspended solids [36]. Clarification results in the variable reductions in the protein contents in the juice, but this aspect is not always directly related to the haze potential of the final wines because other wine components, such as phenolic compounds and non-proteinaceous colloids, and the storage temperature significantly affect the colloidal stability of the final wine [37].

Bentonite is mainly used on white wines, whereas in red wines, the aim of fining is softening, that is the removal of some of the tannins and polyphenols to improve the astringency of the product, and it is performed by applying fining agents other than bentonite (gelatine, albumin, isinglass, skim milk, casein and potassium caseinate) [38].

The main advantages of bentonite are its low cost, effectiveness and availability. Moreover, it is easily removed via sedimentation. Alternative treatments and adjuvants have been sought in oenology because of the drawbacks of bentonite, such as its direct and indirect costs. Indeed, the separation of the lees requires specific plants and is accompanied by the retention of variable quantities of wine (3–10%). The disposal of bentonite represents a limitation exacerbated by the fact that bentonite is not reusable [39], due to the lack of processes that promote the desorption of the adsorbed proteins.

Alternative treatments and adjuvants have been sought in oenology because of the drawbacks of bentonite, such as its direct and indirect costs. Indeed, the separation of the lees requires specific plants and is accompanied by the retention of variable quantities of wine (3–10%). Another concern regards the environmental impact of bentonite wastes, a problem that is exacerbated by the fact that bentonite is not reusable [39]: the enrichment of metals and important alterations of pH have been observed above certain levels of addition of bentonite in poor soils [40]. Even though the enrichment of organic matter by spent bentonite could be promising for poor soils and for applications in new vineyards, the disposal of big volumes bentonite should be, thus, carefully studied.

3.1. International regulations on oenological use of bentonite

According to the EU regulations, there is no upper limit for the addition of bentonite during the winemaking process. On the other hand, The International Oenological Codex [41] establishes the properties of the bentonites that are useful in oenology. The following three classes of bentonite are useful: calcium bentonite, sodium bentonite and activated bentonite, all of which are capable of swelling to different extents and must be dried at 80–90°C after grinding and before their commercialization.

According to the Resolution Oeno 11/2003 [42], the quality control of each bentonite should be based on 5 different test trials prior its application as an adjuvant. Mould contamination is not accepted, and as a result, no odour should be perceived. For the release of cations, the extraction trial should be performed in a tartaric acid solution (5 g/L, pH = 3). This recommendation seems to be insufficient to characterize the safety of a bentonite because the release of cations in wine has been shown to be more intense than extraction with tartaric acid [43].

The metals that are covered by the Resolution Oeno 11/2003 are Pb, Hg, As, Fe, Al, Ca, Mg and Na, with appropriate concentration limits for each metal. However, other metals can be part of the elemental composition of a bentonite, which include significant amounts of Li, Be, Sc, V, Co, Ni, Ga, Ge, As, Sr, Y, Zr, Nb, Mo, Cd, Sn, Sb, Ba, W, Tl and Bi [44]. The enrichment of cations from bentonite in wine partially results from the cation exchange mechanisms on which the adsorption of proteins is based [45] but also from the physicochemical conditions of the medium (pH, ethanol content and presence of other cations). Because the concentration of each cation in wine is important in the context of food safety and for the technological stability [46] and the release of metals from bentonite has been shown to be increased over the last few years [44], the control of the release phenomena has gained importance [45]. Other parameters that are controlled are the presence of large particles, the de-acidification test, and the protein adsorption test [42].

3.2. Physicochemical characteristics of oenological bentonite

To optimize the performances of the fining of wines, the choice of the best bentonite is based on the characterization of its structural properties. The parameters that are considered are as follows:

- the surface charge density (SCD), expressed as meq 100 g⁻¹ of bentonite [47];
- the specific surface area (SSA) (m² g⁻¹), whose determination by methylene blue titration is reported by Resolution Oeno 11/2003 [42];
- the charge density per surface unit (CDSU) (meq m⁻²), derived by calculating the ratio between the SCD and the SSA;
- the natural pH, expressed as the pH of the supernatant of a 20% water suspension (w/v) after 1 h of contact;
- the swell index (SI) (mL g⁻¹), expressed as the difference in the volumes of dried bentonite and hydrated bentonite after 24 h of contact with demineralized water (2 g in 100 mL of water).

The SCD is an expression of the substitution of ions into the bentonite structure, which was described in Section 1.1. Because the adsorption of proteins on bentonite is mainly driven by cation exchange [48], the higher the negative charge of the clay particles, the higher the removal of proteins, when all other parameters are equal. However, these data alone are not sufficient to describe the complete performance of a bentonite in wines. Although a correlation between the net charge of the bentonite and the removal of proteins in white wines was observed [49], other factors affect the ability of charged bentonite to remove positively charged colloids [50].

The SSA is an indirect measure of the medium-sized bentonite particles and depends on the grinding process. The ratio between the SCD and the SSA results in the most significant parameter, the CDSU, and does not take into account the dose of the bentonite added to the wine and is only related to the surface of the clay. As shown for the SCD, the CDSU could sort the bentonites in the order of their charges.

Calcium bentonite is usually more acidic than an activated bentonite, which ranges from pH 6.5 to 8.5 and from pH 8.5 to 10.0, respectively, whereas natural sodium bentonite shows a quite wide range of pH values (4.5–10.0) [42]. The natural pH of a bentonite is strongly associated with its rheological properties, as the distribution of charges on the surface and along the edges of the of the platelets results in the organization of the platelets into a structure that confers viscosity to the medium. Currently, there is no global consensus on the type of structure formed, as some researchers [51] support the edge-to-face attraction mode, whereas other authors suggest the formation of a gel produced by long-range electrostatic double-layer repulsion both at the edges and at the faces [52]. The ionic strength and the pH should be considered when ascertaining the predominant type of interaction [53], particularly when the pH is lower than 4.5 [54], as in wine. Below pH 7, the edges of the platelets should be positively charged, allowing edge-to-face attractions, but very acidic pH values also increase the ionic strength of the medium [55].

Therefore, below pH 4.0, the acidic conditions could be so extreme that Al^{3+} and Mg^{2+} are dissolved and the clay structure is subsequently decomposed. In the same range of pH values, the high ionic strength of the medium could result in the compression of the double layers, thus reducing the edge-to-face attraction intensities [56]. The remaining card-house structure is thought to be an important property for the removal of proteins [45]. The capacity of the clay to maintain a residual three-dimensional structure is affected by the pH of the wine and by the natural pH of the bentonite.

4. Bentonite and wine colloids

4.1. Must and wine colloids

The colloidal phenomena are articulated in two steps, which include the formation of small, invisible colloidal particles and their subsequent aggregation and formation of large particles that are able to diffuse light and to precipitate when their size exceeds a certain limit.

In oenology [38], two groups of colloids may be distinguished.

- Associated colloids are charged particles composed of small molecules that are bound by weak bonds (Van der Waals, hydrogen bonds, hydrophobic interactions, etc.). This type of colloid is naturally present in wine as condensed phenols and colloidal colouring matter.
- Macromolecular colloids, such as polysaccharides and proteins, have high molecular masses and can form covalent bonds.

In addition, polysaccharides could also function as protective colloids by coating the associated colloids and preventing their precipitation. The coating action that is typical of protecting colloids is exerted over a range of concentrations, below which a destabilizing effect takes over. The protective colloids are important in stabilizing different colloidal phenomena in wine, such as the precipitation of ferric phosphate and tartrate.

Colloids are also important for the quality properties required in specific products, such as the foamability of sparkling wines resulting from the presence of hydrophilic and hydrophobic domains in proteins that act as surfactants for the bubble film [57]. Further evidence was provided [58] in Prosecco wine, as high molecular weight glycoconpounds showed a high capacity to foam.

Although there is some controversy about the degree of glycosylation of wine proteins [59], a variable fraction of the colloids from red wines, ranging from 4.3 to 5.2%, is composed of arabinogalactans [60], which have a protein content of <10%. These molecules are considered polysaccharides and are not enlisted in the class of wine proteins.

4.2. Must and wine proteins

Among the colloids that are present in musts and wines, proteins are the most important because they have a central role in haze formation.

Previous analyses have identified the most heat-unstable proteins in white wines, such as grape class IV chitinases, β -glucanases and a fraction of thaumatin-like (TL) proteins [61, 62]. These proteins are able to persist throughout the winemaking process because they are resistant to proteolysis and are stable at an acidic pH [63]. Conversely, yeast- and grape-derived high-molecular mass glycoproteins have been found to exert a stabilizing effect in white wines and are considered 'haze-protective factors' [39]. In recent years, several reports have attempted to characterize the factors affecting protein instability and haze formation in white wines. Because wines with different haze potentials typically contain very similar protein fractions [49], one or more non-proteinaceous wine components (sulphate anions, ionic strength, phenolic compounds, organic acids and pH values) are thought to have an impact on haze formation [39].

4.3. Interactions of bentonite with grape and wine proteins

The factors that can affect the capacity of a bentonite to bind proteins are its physical properties, the temperature, the pH and the ethanol content of the medium.

High temperature results in higher adsorption [48, 50], perhaps due to the conformational changes that occur in the proteins at low temperatures.

pH has a greater importance in the mechanisms of protein adsorption because it influences the charges of the proteins and, thus, the efficacy of the cation exchange onto bentonite [45, 49, 50].

The ethanol content of the wine influences the adsorption capacity of bentonite because ethanol molecules could displace water from the platelets and induce considerable swelling. In turn, increased swelling should increase the number of exchangeable cations, resulting in an increased adsorption capacity. Ethanol concentrations >10% in wine model solutions result in a considerable increase in the adsorption capacity [48]. However, the type of protein considered could influence this result because the separation of the clay platelets induced by ethanol

molecules is finite. Under these conditions, the adsorption could only be attributed to those proteins that are small enough to enter the clay structure [64].

Various factors must be considered when the physical properties of bentonite are examined. CEC, SI and SSA are indeed directly correlated to the removal of proteins [50]. From a practical point of view, the choice of the correct contact time is important for effective fining operations. The general approach is to use a long period of contact time between the wine and bentonite, which is on the order of days [38]. In literature, the optimal contact time is reported to be shorter [48, 50]: a plateau trend is observed after a few minutes of contact between bentonite and wine, indicating that no further adsorption of proteins occurs.

The total protein content in wine is a useful, but it is not the definitive information that determines the risk of haze formation in a wine. Indeed, the types of proteins and their relative concentrations are more important than the total protein content [49].

An optimal performance by bentonite comprises the removal of those fractions that are heat unstable and responsible for the colloidal casse. Chitinases, β -glucanases and some TL protein isoforms are greatly affected by bentonite, but the more stable invertases require a very large amount of bentonite for removal [61, 65]. Extensive investigations [45, 49] on the effect of different bentonite labels on the removal of specific proteins in Chardonnay, Sauvignon and Erbaluce wines showed that bentonite could selectively remove specific protein bands. As a result, the resistance of the wine to haze formation after bentonite fining resulted in a distributed low haze up to 50°C and high cloudiness in the range of 60–80°C. When the distribution of the proteins was investigated with SDS-PAGE, a substantial but not total reduction in the concentration of the TL proteins was observed for all the fined wines. Small peptides were almost completely removed by all the labels tested, but some bentonites were able to remove some of the invertase bands.

The amount of protein removed by bentonite changes was affected by both the bentonite characteristics and the wine pH [45] over a typical oenological range (3.00–3.60). Low molecular mass proteins are efficiently removed by different Na-bentonites, regardless of the pH, but fewer high and medium molecular mass proteins are removed to different extents, based on the wine pH. Reductions in the amount of the vacuolar invertase (GIN1) and VVTL1 fractions of the TL-proteins are induced by bentonites with natural pH values of <10 and are affected, to a lesser extent, by the negative effect of acidic pH on the card-house-like structure. Moreover, at higher pH values, the poor removal of glycoproteins (YGP1 and Hmp1) contributed to the increased thermal stability of the wine. As a matter of fact, the modifications in the protein profile of a white wine in the pH range of 3.00–3.60 resulted in increased quantities of glycoproteins from grape and yeasts [66]. Minor pH-driven conformational changes are sufficient to weaken the interactions between the glycoproteins and the other wine macromolecules, especially tannins, resulting in increased release of hydrophobic glycoproteins. As a result, the temperature at which wine begins to show turbidity is higher.

4.4. Interactions of bentonite with polysaccharides and tannins

It has been shown that bentonite is capable of removing large phenolic compounds, such as anthocyanin, and phenolic compounds that are bound to proteins [67]. Consequently, bentonite may be responsible for modifying wine colour and astringency. The results of the fining of red wines [68] have shown that the high SCD of the bentonite could reduce the tannin levels to some extent, which is accompanied by a relatively high reduction in the anthocyanin levels. It is likely that the absorbance of positively charged anthocyanin induces a significant change in the properties of the bentonite surface such that tannins could be adsorbed via hydrogen bonds and π - π stacking. On the other hand, no effect of bentonites on the removal of tannins in red wines was observed [69], regardless of the molecular weight of the molecules, although the specific physical properties of the bentonite used were not reported in the study.

5. Bentonite and wine aroma compounds

5.1. Varietal odour-active compounds

Grape aroma has an important contribution to wine flavour and characteristics, particularly in the aromatic cultivars, and it evolves during berry development according to the cultivar. Terpenes, which represent the main family of compounds and are present both as free and as glycosylated terpenoids, are mainly present in the grape skin and are present in high amounts in Muscat grape varieties [70]. Variable concentrations of norisoprenoids, that is C-13 norisoprenoids, such as β -damascenone, can improve the fruity note of wines, even at low concentrations [71], and are responsible for the aroma characteristics of Cabernet Sauvignon [72] and other non-floral grapes [73]. In rare cases, methoxypyrazines and sulphur compounds with thiol functional groups have been identified among the aroma compounds of wines. Methoxypyrazines are often associated with 'green' or 'herbaceous' aromas in Cabernet Sauvignon, Sauvignon Blanc, Cabernet Franc and Merlot Noir, among other varieties [74].

5.2. Fermentative odour-active compounds

The aroma complexity dramatically increases during alcoholic fermentation as a result of the synthesis of important volatile compounds and the release of some varietal aroma precursors [75]. The nature and amount of the synthesized volatile compounds depend on multiple factors, such as the nitrogen content of the must, the temperature of fermentation and the yeast strain. They consist of compounds with a wide range of polarity, solubility and volatility. The volatile compounds synthesized by wine yeasts include higher alcohols, medium- and long-chain volatile acids, acetate esters and ethyl esters and aldehydes, among others [76]. Aroma compounds interact with different macromolecules such as proteins or polysaccharides [77], so fining agents may fix substances that act as support for aromatic components [78].

5.3. Impact of bentonite on varietal odour-active compounds

Protein stability and the presence of intense fine aromas are two important requirements for aromatic white wines. Bentonite treatment is typically performed on wine, but it can be applied to increase the rate of juice settling and to facilitate the precipitation of suspended solids [36].

The use of fining agents to clarify juice can positively or negatively affect the composition of the derived must. The must clarification prior to the onset of alcoholic fermentation improves the sensory characteristics of white wines [79]. The removal of grape solids from must enhances the production of ethyl esters and acetates and limits the release of fusel alcohols during alcoholic fermentation, which results in a global increase in wine aroma quality [36]. Nevertheless, a certain amount of colloids must be present because they confer structure and volume, contribute to the fixation of aromatic compounds and produce bubbles in sparkling wines as a result of their tensioactive properties [64]. Conversely, excessive turbidity in musts induces the presence of an herbaceous aroma in the resulting wines, which promotes an evolution towards reduction. Finally, excessive limpidity can cause a retardation or cessation of the fermentation process [79].

A report [37] studied the fates of proteins and terpenols during the processing of Chambave Muscat grapes. The experiments included bentonite addition to must only (100 g hl), to wine only (100 g hl) and double bentonite addition on must and wine in two vintages (2006 and 2007). The results of the experiments demonstrated that a reduced removal of free terpenols was observed in the samples from the double treatment (must + wine) compared with the wines that were only fined with bentonite after alcoholic fermentation. In general [80], bentonite alone has a small effect on the loss of terpenes, but it removed ethyl esters and fatty acids.

Our unpublished data attempted to optimize the clarification of Muscat Blanc must with Ca or Na bentonite or related mixes in a dose range of 10–100 g hL⁻¹ through a central composite design (CCD). Response surface methodology (RSM) estimated the combined effect of the bentonite type and dose on the removal of aglycones and glycosylated aroma compounds. The RSM surface plots predicted the removal of the aglycones from aroma compounds by low doses of Na-bentonite and Ca-bentonite, regardless of the dose. Furthermore, the model estimated the removal of glycosylated aroma compounds, particularly by Ca-bentonite at 50 g hL⁻¹.

5.4. Impact of bentonite on fermentative odour-active compounds

The effects of the clarification/stabilization treatments on the sensory qualities and aroma [36, 78] of wine have been studied, but the origin of these phenomena has rarely been explained.

The effect of the dosing time of bentonite on the aroma profile at both industrial and pilot scales has been evaluated [81]. The results seemed to indicate that the addition of bentonite not only affects the wine aroma by the adsorption of compounds but also by the production of these compounds during fermentation. Bentonite treatments at different stages of fermentation generally affected the production of volatile fermentative compounds. In addition to the possible loss of volatile compounds due to adsorption on bentonite, the effects

on the production of fermentative compounds could be related to the variations in the nitrogen composition and other nutrients of musts and wines that are removed by the bentonite [82]. In a model solution, the presence of total and purified proteins (TL proteins and chitinase) and bentonite tended to increase the loss of esters with the longest carbon chains that is ethyl octanoate and ethyl decanoate [80], showing that hydrophobicity can be one of the driving forces involved in the interaction of aroma compounds with both bentonite and proteins.

As a matter of fact, it was demonstrated [83] that the effect of bentonite treatments on the aroma substances in white wine depended on the chemical nature and initial concentration of the volatile compounds and on the abundance and nature of proteins in the wine. In general, when low bentonite concentrations (20 g/hl) are used, the concentrations of most aromatic substances are not significantly affected. Most aroma compounds are removed as an indirect effect of deproteinization; some hydrophilic odour-active compounds undergo weak hydrogen binding with protein surfaces, whereas the more hydrophobic aromatic molecules can bind to interior protein sites with a stronger affinity for hydrophobic substances. Only a few odour-active compounds are directly adsorbed by the bentonite through an adsorption process, which is robustly fitted by the Freundlich equation, with a heterogeneous energy distribution of an infinite number of surface-active sites [84]. Bentonites with a lower SSA value and a greater CDSU value seemed to primarily interact with most of the odour-active compounds through physical mechanisms. In contrast, the clay with a large SSA value and a low CDSU value promoted stronger adsorptions that were probably driven by chemical interactions, particularly for the ethyl esters. For the fermentative odour-active compounds, the differences in the adsorption intensity and capacity mainly depended on the characteristics of the bentonite than on the properties of the substances. When yeast-derived material represents an important fraction of the wine macromolecules, colloids that favour aroma inclusion are held in suspension. In this situation, there may be fewer opportunities for the odour-active substances to be directly adsorbed onto the bentonite sheets [83].

6. Conclusions

Oenologists do not really know which parameters they must focus on to choose the bentonite that will obtain both the desired degree of limpidity and colloidal stability while avoiding undesirable side effects on the phenolic and aroma compounds.

This chapter detailed the structure, composition and non-food uses of bentonite and summarized the most recent scientific research that detected:

- the proteins targeted by the bentonite;
- the effect of the protein content and pH towards bentonite fining;
- the bentonite characteristics that affect juice clarifying and wine fining;
- the side effects of bentonite on polyphenols and colour;

- the interactions of bentonite with the free- and glycosylated-varietal aroma compounds;
- the interactions of bentonite with fermentative aroma by indirect and direct removal.

As wine haze formation is a product of different matrix parameters, including the wine pH and the concentration of different wine components, a detailed knowledge of the relationships between the adjuvant that is most commonly used to attain colloidal stability and the oenological matter is fundamental for process optimization and to increase wine resilience.

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