Research Article
Using Zeolites to Cold Stabilize White Wines

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1.Introduction

Cold stabilisation is a process of removing excessive L-tartaric acid natural ionic salts (potassium bitartrate: KHT, and to a lesser extent, calcium tartrate: CaT) from the wine. After fermentation but before bottling, cold stabilisation is routinely carried out to prevent the wine salt KHT from precipitating out of the wine during storage or cooling in bottle.

Traditional cold stabilisation methods involve cooling the wine to a temperature just above freezing and keeping it at this temperature for weeks or even months. Chilling the wine reduces the solubility of KHT and facilitates its crystallisation and removal through precipitation. During cold storage, precipitation of KHT occurs rapidly in the initial phase and slows down over time as the saturation level of KHT decreases. The temperature and storage time required to stabilize a wine depends on wine composition [1]. Wines with higher sugar and alcohol content require a lower storage temperature than dry wines with 11-12% alcohol [2]. The stability and precipitation of KHT are also influenced by other factors such as the concentration of acids, anions,
cations, the pH of the wine, and various complexing agents [2]. To achieve reproducible KHT precipitation, the wine must be clarified and filtered before refrigeration and cold storage. As mentioned previously, storing a wine at near-freezing temperatures is sufficient to remove excess KHT. To prevent the redissolution of the KHT, the wine must also be cold filtered after cold storage to remove the crystalline KHT precipitate. Wine stabilisation by cooling is widely used in the wine industry. The process is time-consuming and energy-intensive [1] and involves a filtration step to remove the sediment. According to the South Australian Wine Industry Association, refrigeration consumes between 50 and 70% of the electricity used in a typical winery. Other technical solutions to prevent precipitation of KHT after bottling are reverse osmosis [3], ion exchange [4], electrodialysis [5], and inhibition methods involving additives such as carboxymethyl cellulose (CMC) [1, 6], meta tartaric acid [1, 7], mannanproteins [1, 8], or potassium polyaspartate [9]. Processes such as ion exchange and electrodialysis require significant capital investment and considerable expertise to be used effectively. In addition, the cost of water and maintenance can also be high. Tartrate stabilisation has recently focused on the development of compounds capable of inhibiting tartrate crystallisation. However, the possibilities of achieving long-term stability in wines without compromising colour and favourable organoleptic properties are limited. Moreover, the addition of additives in winemaking contradicts the modern trend towards organic and additive-free winemaking envisaged by consumers.

Given these shortcomings of current practices, we tried to find a new solution to cold stabilisation, which could be performed at higher temperatures.

Zeolites [10, 11] are hydrated aluminosilicates of sodium, potassium, calcium, or other heteroatoms [12]. These materials are formed naturally by volcanic activity (natural zeolites) but can also be synthesized in the laboratory (synthetic zeolites) [12]. Zeolites are extensively used in various technological applications, e.g., as catalysts [13, 14] and molecular sieves [10], to separate and sort molecules [15], to dehydrate [16, 17], to purify water [18] and air [19–21], and to remove radioactive contaminants [22]. In this work, we explored the potential of zeolites for the cold stabilisation of white wines.

The rationale for applying natural zeolites in cold stabilisation of white wines was based on our previous research [23] and the knowledge that the selective removal of potassium ions from wine down to, i.e., 10–30% of the initial potassium amount, likely inhibits the precipitation of potassium bitartrate salts [24]. We therefore hypothesised that natural zeolites could be a material with a promise to be successfully used in cold stabilisation of white wines.

2. Materials and Methods

2.1. Natural Zeolites. Samples used for the present studies came from Australian zeolite distributors such as ZeoNatural (Z1), Orku (Z2), and Nikita Naturals (Z3) and an Indonesian mining company (CV Mountain Stones) in micronised powder form (Z4).

The fifth zeolite sample came from Enfield Produce in a granular form and was micronized by Bureau Veritas Minerals for our purposes, referred to in the text as grind zeolite (Z5). Natural zeolite samples were used after three hours of rehydrating in Milli-Q water at a zeolite : water ratio of 1 : 10. The wines were transferred to 50 ml centrifuge tubes and treated with the zeolite suspension by stirring on a rotary mixer for three hours. The zeolite was then separated from the wine by centrifugation (3,750 rpm, 10 min) as previously specified in the optimisation procedure [23]. The zeolite treatment for Z1, Z2, Z3, and Z5 included a preliminary test with a range of zeolite doses (from 4 g/L to 10 g/L, increasing by 0.5 g/L) to determine the optimum dose to achieve protein stability. The same optimum zeolite dose was also used in the cold stability testing. A higher zeolite dose range was investigated for Z4 (from 8 g/L to 18 g/L) with an increase of 1 g/L. As it was not possible to achieve protein stability of the wines with Z4, the 10 g/L dose was used for SAB and CHA wines and the 16 g/L dose was used for Muscat Gordo wine in the cold stability test.

To reduce aluminium leaching into wine after treatment with the zeolites, they were also subjected to a calcination process. In the text, these zeolites are referred to as calcinated zeolites. During calcination, zeolites were placed in an oven at 400°C for two hours.

In addition, two other zeolite samples, Z6 and Z7, were dealuminated using 3N HCl by soaking for 24 hours, followed by neutralization by washing them several times in water and finally drying at 100°C. The dealuminated zeolites are referred to in the text as Z6 dealuminated and Z7 dealuminated. Like Z4, samples Z6 and Z7 were also supplied in the form of micronised powder by CV Mountain Stones, an Indonesian mining company.

2.2. White Wines. Unstable Muscat Gordo, Sauvignon Blanc, Chardonnay, and Pinot Grigio wines expected to give tartrate and protein precipitates when bottled without further treatment were used in this study. All wines were donated by commercial producers, produced according to standard and commercial-scale winemaking processes, filtered (0.45 μm filters), and stored at cellar conditions (15°C) before conducting the experiments. The basic chemistry of wines was studied, and the results are presented in S1 (Supplementary Materials). The wines, which were fined with zeolites to test cold stability, were stored at 15°C in the cellar for nine months.

2.3. X-Ray Diffraction (XRD). Powder X-ray diffraction patterns were recorded at room temperature using a PANalytical X’Pert Pro MPD diffractometer in the Bragg–Brentano reflection configuration. Copper Cu Kα radiation from a sealed tube was used. Data were collected in the 2θ range of 5–90° with a step of 0.0167° and exposure per step of 27 s. Since the raw diffraction data contained some noise, the background during the analysis was subtracted using the algorithm of Sonneveld and Visser [25]. The data were then smoothed with a cubic polynomial function.
2.4. Fourier Transform Infrared Spectroscopy (FTIR). An IRTracer-100 FTIR spectrometer (Shimadzu), equipped with a liquid nitrogen-cooled MCT detector, was used for all measurements. Measurements were performed using the Quest Single Reflection ATR Accessory (Specac), equipped with a diamond ATR crystal. In all cases, 128 scans were performed with a resolution of 4 cm\(^{-1}\) to achieve a satisfactory signal-to-noise ratio. The ATR effect and atmospheric contributions from carbon dioxide and water vapor were corrected by the background performed on an empty ATR device.

2.5. X-Ray Photoelectron Spectroscopy (XPS). To determine the surface composition of the five zeolites used, XPS analysis was performed. The XPS spectra were obtained with a Specs SAGE XPS spectrometer using an Al Ka radiation source (h\(_\text{υ}\) = 1253.6 eV) at 10 kV and 20 mA. Elements present on the sample surface were identified from the survey spectrum recorded in the energy range 0–1,000 eV with a pass energy of 100 eV and a resolution of 0.5 eV. The areas under the selected photoelectronic peaks in a broad scan spectrum were used to calculate the percentage of atomic concentrations. High-energy-resolution spectra (0.1 eV) were then recorded for the relevant photoelectronic peaks at passing energy of 20 eV to identify the possible chemical bonding environments for each element. All binding energies were referred to the neutral carbon C1s peak at 285 eV to compensate for the effect of surface charge. Data processing and curve fitting were performed with the Casa XPS software.

2.6. Scanning Electron Microscopy/Energy Dispersive X-Ray (SEM/EDX). SEM was used to determine the morphology of the natural zeolite samples, while the elemental composition of the zeolites was determined with EDX. An FEI Quanta 450 FEG-ESEM with an EDAX Apollo X Energy Dispersive X-ray (EDX) spectrometer was used for the analysis.

2.7. Specific Surface Area and Porosity. The specific surface area of the natural zeolite samples was determined using the Brunauer, Emmett, and Teller adsorption technique (BET). Pore size distribution was determined using the BJH method of Barrett et al. [26]. Measurements were performed on the Micrometrics ASAP 2020 adsorption instrument (Surface Area and Porosity Analyzer, Micromeritics Instrument Corporation, Norcross, GA, USA).

2.8. Quantification of Tartaric Acid in Wine by HPLC. Wine samples were diluted with water and injected onto an Agilent Technologies Hi-Plex H column (300 mm × 7.7 mm). Separation was performed with an isocratic 10 mm sulfuric acid/water solvent at a flow rate of 0.6 mL/min. Tartaric acid was detected at 210 nm and quantified against a standard curve of known tartaric acid concentration.

2.9. Crystal Quantification by HPLC. For the crystal quantification method, 10 mL of filtered wine was cooled to −4°C and kept at this temperature for 72 hours. After returning to the room temperature, the sample was centrifuged to ensure that all loose crystals were collected at the bottom of the tube. The supernatant was decanted, and the remaining sample was washed with 1 mL of absolute ethanol. After another centrifugation, the supernatant was decanted again, and the sample was dried. Dilute hydrochloric acid was then added to dissolve any tartrate crystals before the solution was analyzed for tartaric acid by HPLC. Quantification was performed using a tartaric acid standard curve.

2.10. Protein Quantification in Wine by HPLC. The concentration of wine proteins was measured by HPLC (Agilent Technologies) according to the previously published method [27]. Briefly, filtered wine samples were injected onto an Agilent 1.260 UHPLC with a Prozap C18 column. Separation was performed using 0.1% trifluoroacetic acid (TFA)/H\(_2\)O and 0.1%TFA/ACN at 0.75 mL/min. Protein detection was achieved by diode array monitoring at 210 nm. Protein identification was obtained by comparing the retention times of the peaks of the samples with those of the isolated standards, and quantification was performed by comparing the areas of the peaks with those of a thaumatin standard curve.

2.11. Metal Analysis. Metals content in wine was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) performed by Affinity Labs.

2.12. Analysis of the Si/Al and Ca Content of Indonesian Natural Zeolite. XRF was applied to determine the bulk composition of Indonesian natural zeolite. The micronized natural zeolite was prepared as a pellet by high-pressure technique. The XRF analysis was conducted using PAN-analytical Epsilon4 at BRIN Laboratory, Yogyakarta.

2.13. Statistical Analysis. Data significance was assessed by Student’s t-test. The mean values with a different letter were significantly different (p < 0.05). Figures were prepared using Origin 6.0 and CorelDRAW 11 software.

3. Results and Discussion

3.1. Characterization of Natural Zeolites. The surface properties and structure of the natural zeolites used in this work are listed in Table 1. In addition, S2 (Supplementary Materials) shows the X-ray diffraction patterns of the five zeolites studied. According to the X-ray diffraction studies, the five zeolites (Z1 to Z5) have a distinct crystalline structure with the characteristic peaks of clinoptilolite [28] ((Na\(_4\)K\(_4\)) (Al\(_8\)Si\(_{40}\)O\(_{96}\))·24 H\(_2\)O) and mordenite [29] (Na\(_8\) (Al\(_8\)Si\(_{40}\)O\(_{96}\)). Interestingly, besides clinoptilolite and mordenite, Z4 is the only one with calcite Ca (CO\(_3\)) structure, which confirms previous studies [30–32].
Table 1: Characterisation of natural zeolites.

<table>
<thead>
<tr>
<th>Nos.</th>
<th>Zeolites</th>
<th>BET surface areas (m²/g)</th>
<th>Pore volumes (cm³/g)</th>
<th>Pore diameters (nm)</th>
<th>Structure by XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1</td>
<td>ZeoNatural</td>
<td>17.4</td>
<td>0.05</td>
<td>11</td>
<td>Mordenite and clinoptilolite</td>
</tr>
<tr>
<td>Z2</td>
<td>Orku zeolite</td>
<td>18.2</td>
<td>0.06</td>
<td>14</td>
<td>Mordenite and clinoptilolite</td>
</tr>
<tr>
<td>Z3</td>
<td>Nikita zeolite</td>
<td>26.0</td>
<td>0.04</td>
<td>7</td>
<td>Mordenite and clinoptilolite</td>
</tr>
<tr>
<td>Z4</td>
<td>Indonesian zeolite</td>
<td>60.0</td>
<td>0.07</td>
<td>5</td>
<td>Calcite, mordenite, and clinoptilolite</td>
</tr>
<tr>
<td>Z5</td>
<td>Grind zeolite</td>
<td>17.2</td>
<td>0.06</td>
<td>14</td>
<td>Mordenite and clinoptilolite</td>
</tr>
</tbody>
</table>

The average pore diameter, surface area, and pore volume are important physical properties that influence the quality and utility of zeolites and are determined using the BET adsorption technique. The average pore size of the Z4 and Z3 was smaller (5 and 7 nm, respectively) than that of Z5, Z2, and Z1 (14, 14, and 11 nm, respectively). The BET surface area of the Z4 was the highest at 60 m²/g, resulting in a pore volume of 0.07 cm³/g. In contrast, the lowest surface area was measured for Z2, Z5, and Z1, with BET surface areas of 18.2, 17.2, and 17.4 m²/g, and pore volumes of 0.06, 0.06, and 0.05 cm³/g, respectively. The Z3 had a surface area of 26 m²/g and the lowest pore volume of 0.04 m³/g.

Figure 1 presents the SEM images of the natural zeolites. The SEM images indicate that the zeolites are crystalline, which is also confirmed by XRD. Overall, the images depicted zeolite crystals with smooth surfaces and a flake-like crystal configuration. The chemical composition of the zeolites in bulk and at the surface was determined by EDX and XPS measurements, respectively (see Table 2). All zeolites had a Si:Al ratio of nearly 4:1, which classifies these zeolites as intermediate Si/Al zeolites. However, it is noteworthy that the Z4 had the highest sodium and calcium contents as measured by EDX. The XPS analysis showed that the Z4 did not contain sodium ions on the surface.

FTIR was performed to determine the chemical functional groups of the zeolites Z1–Z5 (Figure 2). The bands in the range 650–745 cm⁻¹ were assigned to the symmetrical T-O-T vibrations in the framework of the zeolites. A dip also appeared at 1,000 cm⁻¹, which is a characteristic band for asymmetric Si-O-Si and Si-O-Al stretching vibrations in most zeolitic materials. Another small shoulder peak at 1,075 cm⁻¹ is due to symmetric Si-O-Al stretching. As can be seen from the figure, Z1, Z2, Z3, and Z5 have similar fingerprints, while the Z4 zeolite shows differences in peaks and transmission intensities. Furthermore, the spectrum of the Z4 shows a hydrogen bonding peak at water at the 2,490 cm⁻¹, which is absent in the other zeolites.

3.2. Exchange of Zeolite Sodium and Calcium Ions with Wine Potassium Ions. Zeolites are widely used in various fields of science and industrial applications mainly because of their ion-exchange properties, which are amongst the most important parameters characterising their sorption and technological properties. The ion exchange property of natural zeolites depends on several factors, including ion shape, size, charge density of the mineral network, framework structure, ion charge, and concentration of ions in the external solution [33].

Metal analysis was carried out to investigate the effects of zeolite treatment on the metal content of the treated wines and to understand which metals are involved in the ion-exchange process. Figure 3 shows the changes in potassium, sodium, and calcium concentrations after treatment of wine with natural zeolites. The changes in metal concentrations indicate that an ion exchange mechanism is taking place. It is known that potassium ions have the highest ion exchange potential compared to other metal cations (Ca²⁺, Mn²⁺, Fe²⁺, or Mg²⁺). Therefore, potassium ions in wine can be exchanged with Na⁺ and Ca²⁺ ions in zeolites [34, 35]. This explains the decrease in the concentration of potassium ions and the increase in the concentration of sodium (Figures 3(a) and 3(b) and in Supplementary Materials S3A–S3D) and calcium ions in the wine after treatment with zeolites Z1–Z5. In Z1, Z2, Z3, and Z4, the reduction of potassium ions in the treated wines was predominantly due to the cation exchange of potassium ions with sodium ions, hence the increase of sodium ions in the wine after treatment with these zeolites. As they are located at the surface, sodium ions can be exchanged with potassium [36]. In the case of the Z4, mainly an exchange of Ca²⁺ with K⁺ occurs as this zeolite does not have sodium ions on the surface, but has 4.8% calcium, which was determined by XPS.

The calcium content in Muscat Gordo wine after treatment with Z6 increased considerably as compared to Z1, Z2, Z3, and Z4 (Figure 3(c)). It is important to note that a high Ca²⁺ content in wine is highly undesirable, as calcium-induced instabilities are a major cause of problems in bottled wines.

A possible ion exchange mechanism is depicted in Figure 4.

As shown in Figure 3(a), for all zeolites tested, the potassium content in Muscat Gordo wine was reduced by 47–60% after treatment, resulting in reduced crystallisation and precipitation of KHT. At the same time, the sodium concentration in wine increased to an average of 130 mg/L.

In Sauvignon Blanc, the initial potassium concentration in the wine was approximately 750 mg/L and decreased to 650 and 515 mg/L, representing a 13 to 30% decrease in potassium concentration after treatment (Supplementary Materials S3A). The Chardonnay control wine had a higher potassium concentration of 920 mg/L. When treated with Z1–Z5, this concentration decreased by 18 to 28% (Supplementary Materials S3B). The sodium concentration in these wines increased to similar levels after treatment, with a maximum sodium concentration of 70 mg/L, as shown in Supplementary Materials S3C and S3D.
Although with decrease in the potassium content of tested Muscat Gordo, SAB and CHA, the sodium content has increased, the concentrations of these metals were within the range common for wines. Few wines generally contain more than 200 mg/L of sodium, and most have less than 100 mg/L. Most wines contain between 500 mg/L and 1,300 mg/L potassium. Foods with less than 140 milligrams of sodium per serving are considered low in sodium.

The calcium concentration in wine after treatment with zeolites Z1, Z2, Z3, and Z5 increased on average from approximately 40 mg/L to approximately 110 mg/L in Muscat Gordo (Figure 3(c)) and from approximately 60 mg/L to approximately 85 mg/L in SAB and CHA wines (Supplementary Materials S3E and S3F), which is still within the typical range for wines averaging 90 mg/L. However, the increase in calcium content in these wines after treatment with Z4 is four to five times the average concentration of white wines. This indicates that zeolites with lower calcium content or low calcium leaching properties should be selected for wine treatment, as presence of calcium may cause instability problems (as calcium tartrate deposits) [37]. Therefore, chemical analysis of calcium is critical to avoid the precipitation of calcium DL tartrate in the bottle.

<table>
<thead>
<tr>
<th>Zeolites</th>
<th>Analysis</th>
<th>C</th>
<th>O</th>
<th>Si</th>
<th>Al</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>K</th>
<th>Na</th>
<th>SiO₂/Al₂O₃</th>
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<tbody>
<tr>
<td>Z1</td>
<td>XPS</td>
<td>16.3</td>
<td>54.7</td>
<td>17.2</td>
<td>4.9</td>
<td>1.1</td>
<td>0.6</td>
<td>0.2</td>
<td>4.1</td>
<td>0.9</td>
<td>4.01</td>
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<tr>
<td>EDX</td>
<td>2.9</td>
<td>52.4</td>
<td>30.4</td>
<td>8.5</td>
<td>2.5</td>
<td>1.0</td>
<td>0.6</td>
<td>0.6</td>
<td>1.1</td>
<td>1.1</td>
<td>4.08</td>
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<tr>
<td>Z2</td>
<td>XPS</td>
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<td>51.6</td>
<td>15.7</td>
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<td>1.1</td>
<td>0.3</td>
<td>0.2</td>
<td>5.3</td>
<td>0.7</td>
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<td>18.1</td>
<td>5.7</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>3.68</td>
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<tr>
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<td>XPS</td>
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<td>23.5</td>
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<td>0.3</td>
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<tr>
<td>Z4</td>
<td>XPS</td>
<td>21.0</td>
<td>57.1</td>
<td>12.9</td>
<td>3.6</td>
<td>4.8</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
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<tr>
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<td>1.1</td>
<td>1.8</td>
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<tr>
<td>Z5</td>
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<td>55.3</td>
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<td>0.7</td>
<td>0.4</td>
<td>3.98</td>
<td></td>
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</table>

**Figure 1:** SEM images of (a) Z1, (b) Z2, (c) Z3, (d) Z4, and (e) Z5 zeolite.

**Table 2:** The bulk and surface elemental composition of natural zeolite samples.

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3.3. Testing Cold Stability of Wine. A refrigeration/brine test was conducted to determine the cold stability of wines after treatment with zeolites, which is commonly referred to as “crystal quantification.” The weight of precipitated tartaric acid crystals from wine without and with zeolite addition was measured immediately after zeolite treatment and after three and six months of wine storage in the cellar at 15°C. Figure 5 shows the results for highly cold-unstable Muscat Gordo wine. As it can be seen from Figure 5(a), Z4 was found to be the most effective in removing tartaric acid crystals from the wine. Z4 reduced the concentration of tartaric acid crystals in the wine by 99.4, 99.8, and 100% after treatment and after three and six months of wine storage. Z1, Z2, Z3, and Z5 zeolites were also highly effective. These zeolites reduced the concentration of tartaric acid crystals by an average of 95.5, 98, and 99% after treatment and after three and six months of wine storage. Simultaneously, tartaric acid concentrations in Muscat Gordo wine decreased after treatment with Z1, Z2, Z3, and Z5 zeolites due to reduced KHT crystallisation and precipitation (Figure 5(b)). At the same time, the pH value of the wine changed only minimally. The situation was different when...
Muscat Gordo wine was treated with Z4 zeolite. In this case, the tartaric acid concentration decreased so drastically after treatment that the pH of the wine increased from pH 3.6 to pH 5.2. The same high pH was measured for SAB and CHA wines treated with Z4 zeolite. No pH change was observed in these wines after treatment with Z1, Z2, Z3, and Z5 zeolites. An 8% reduction in tartaric acid was observed for SAB and CHA wines when Z1, Z2, Z3, and Z5 zeolites were used for treatment (Supplementary Materials S4C and S4D). However, when SAB and CHA wine were treated with Z4 zeolite, the tartaric acid was almost completely removed (only 2% left), which explains why the pH of these wines increased after treatment.

Sauvignon Blanc was initially cold unstable and remained unstable until three months of storage in the cellar (Supplementary Materials S4A). After treatment with natural zeolite, the concentration of tartaric acid crystals in this wine initially decreased by an average of 92% and by 98% after three months of storage in the cellar. After six months of storage, the wine was almost cold stable even without zeolite addition. Chardonnay was cold stable almost from the beginning, indicating that no treatment was required to cold stabilize the wine (Supplementary Materials S4B).

### 3.4. Effect of Zeolites on Aluminium Content in Treated Wines

Metal analysis of the treated wines revealed that the aluminium concentration was drastically increased, particularly, after treatment with Z1, Z2, Z3, and Z5 zeolites. As presented in Figure 6(a), the concentration of aluminium in control Muscat Gordo wine was 0.2 mg/L. After treatment, the aluminium concentration increased to 9 mg/L for Z3, Z2, and Z1, to 11 mg/L for Z5 zeolite, and to 2 mg/L for Z4 zeolite. After six months of wine storage in the cellar, these aluminium concentrations dropped to around 7 mg/L for all Australian zeolites. However, the levels were still high.

A high aluminium concentration was also found in SAB and CHA wines after treatment with Z1, Z2, Z3, and Z5 (Supplementary Materials S3G and S3H). At the beginning, the aluminium content was 16 mg/L for Z5. After three and six months of storage, the aluminium content decreased significantly but remained high, between 10 and 5 mg/L after treatment with Z1, Z2, Z3, and Z5 zeolites. A moderate increase was observed for all treated wines after treatment with Z4 zeolite (approximately 1.5-1.6 mg/L). The increase in aluminium content was no surprise, since it is well-documented that, for example, the addition of aluminosilicate minerals such as bentonite contributes to the aluminium content in wines [38]. What was surprising was the magnitude of this increase, which was between twenty-five and thirty-five-fold. After the addition of bentonite, the aluminium content can increase about two-fold [38].

Two processes were used to reduce aluminium release/leaching from zeolites, namely, zeolite dealumination and calcination. Although dealumination is known to reduce aluminium content in zeolites [30, 32, 39, 40], the process was not entirely successful in reducing the aluminium concentration in Muscat Gordo wine after treatment with Z6 dealuminated and Z7 dealuminated (Supplementary Materials S5). Nevertheless, dealuminated zeolites significantly decreased the leaching behaviour of calcium, as shown in Supplementary Materials S5. Contrary to the dealumination process, calcination of zeolite resulted in a substantial decrease in aluminium, as shown in Figures 6(b) and 6(c) for Muscat Gordo and Pinot Grigio wines, respectively. It is known that the calcination process of zeolites stabilises the structure and functional surface when carried out at an appropriate temperature [31, 32, 41]. The calcination treatment significantly reduced aluminium content, bringing it to the acceptable level for wine with lower dose of zeolite required.
Aluminium is the most abundant metal and the third most common element in the Earth’s crust. It is found in relatively high concentrations in drinking water, pharmaceutical products, and processed foods. However, only traces of aluminium are found in the human body, as the body does not appear to use aluminium for any biological purpose. The small amount of aluminium that is absorbed by the body is normally excreted by the kidneys in the urine.

The total body burden of aluminium in healthy individuals is 30–50 mg because the skin, lungs, and gastrointestinal tract greatly limit the absorption of aluminium from environmental sources. Concern about the toxicology of aluminium has led to many studies on the analysis of aluminium in foods and beverages and on the relationship between aluminium intake and the onset of body disorders or malfunctions [42]. Research has shown that there is a huge difference between oral exposure to aluminium, and injected aluminium. For example, less than 1% of ingested aluminium is absorbed through the gastrointestinal tract (between 0.1% and 0.3% according to most studies), whereas 100% of injected aluminium is absorbed over a period of time that can vary depending on the individual’s state of health.

In addition to the toxicity of aluminium, the contamination of wine with this metal (>10 mg/L) can lead to spoilage through haze formation and the generation of undesirable flavours [43]. Several elements, including aluminium (Al), copper (Cu), iron (Fe), manganese (Mn), 

![Figure 6: (a) Aluminum concentration in Muscat Gordo wine measured initially after zeolite treatment and after three and six months of wine storage in the cellar. Comparison of aluminum content in wines after treatment with zeolites and zeolites calcinated for two hours at 400°C for (b) Muscat Gordo (16 g/L zeolite dose) and (c) pinot grigio (5 g/L zeolite dose). (d) Comparison of calcium content in Muscat Gordo wine after treatment with zeolites and calcinated zeolites.](image-url)
nickel (Ni), and zinc (Zn), contribute to the haze formation and the change of colour as they tend to form complexes with anthocyanins and tannins [44]. The average aluminium level in wine, based on a simple classification as red, white, sparkling, or fortified is given in Table 3. The suggested maximum aluminium concentration for wine stability is 3 mg/L [43]. In addition, the calcination process also reduced calcium content in the wine after treatment, as presented in Figure 6(d). The decrease in calcium content in Muscat Gordo wine from an average of 120 mg/L for wine treated with zeolite to an average of 54 mg/L for wine treated with calcinated zeolite is a significant improvement. This is because wines with calcium levels above 70–80 mg/L are considered to be at risk of instability [37]. Lowering the Ca$^{2+}$ content in wine has eliminated the risk of calcium instability in these wines.

3.5. Protein Concentration in Zeolite Treated Wines. As demonstrated in our previous work [23], Indonesian zeolite can protein-stabilize white wines. In this work, we were also able to protein-stabilize Muscat Gordo wines using Z1, Z2, Z3, and Z5 zeolites. Different zeolite doses were required to achieve protein stabilisation. Protein stabilisation was
performed with 8 g/L of Z2 and Z5 zeolite and 10 g/L of Z3 and Z1 zeolite. As shown in Figure 7(a), the protein concentration in the zeolite-treated Muscat Gordo wine was reduced by 90, 94, 94, and 95% after treatment with Z3, Z1, Z5, and Z2 zeolite, respectively, and these wines became heat stable after treatment. SAB and CHA wines could also be made protein stable with Z1, Z2, Z3, and Z5 zeolites. Protein stabilisation of SAB required 6 g/L of Z3 zeolite, 7 g/L of Z5 zeolite, 5 g/L of Z1, and 5 g/L of Z2 zeolites. For protein stabilisation of CHA, the same dose of Z3 and Z5 zeolites was used as for SAB. For Z1 4.5 g/L and for Z2 5.5 g/L to accomplish this task (Supplementary Materials S4E and S4F).

Interestingly, we could not protein-stabilize Muscat Gordo wine with Z4 zeolite, even when 16 g/L zeolite was added to the wine. As can be seen in Figure 7(a), 16 g/L of Z4 zeolite removed only 53% of the proteins. This result was unexpected, as in our previous work, Z4 zeolite protein-stabilized wines with similar protein content to Muscat Gordo (183 mg/L). For example, 6 g/L of Z4 zeolite protein-stabilized Sauvignon Blac wine with an initial protein content of 182 mg/L or 4 g/L of Z4 zeolite stabilized Semillon wine with 165 g/L initial protein content [23]. A similar, unexpected result was obtained for SAB and CHA. After treatment with 10 g/L Z4 zeolite, the protein concentration in the wines was reduced by 34 and 16% for SAB and CHA wines, respectively, and the wines remained heat unstable (Supplementary Materials S4C and S4D). Interestingly, in our earlier work, we succeeded in making SAB and CHA heat stable after adding 6 g/L of Z4 zeolite [23]. This led us to conclude that even though the two Z4 zeolites used where from the same Indonesian mining company, they must have some significant differences. The differences between the two Z4 zeolite samples were the Ca content and the Si/Al ratio. It is worth noticing that the two samples came from the same Indonesian mining site but from two different batches. In our previously published study, the Ca concentration of the Z4 zeolite measured by EDX was 3.5%. In the current work, the concentration of Ca ions was 11.2%, also measured by EDX.

To examine the role of Ca ions on the protein removal capacity of zeolites, we have used four Indonesian zeolites (Z6, Z6 dealuminated, Z7, and Z7 dealuminated) with different Ca content. The lowest Ca content was 3%, and the highest was 28%. The Si/Al ratio was also different in these samples as presented in Supplementary Material S6. Based on structure-activity studies, as presented in Figures 7(b) and 7(c), we found that zeolites with a lower calcium content (6%) had a better capacity to remove haze-forming proteins from wines than zeolites with higher (14 and 28%) or very low (3%) Ca content. Furthermore, the zeolite with a very high Si/Al ratio (8.4) was less effective in removing haze-forming proteins from wines than the zeolite with a lower Si/Al ratio (6.5). The most effective in removing haze-forming proteins from Muscat Gordo wine was zeolite Z7. This zeolite had a Ca content of 5.8%, and the ratio of Si to Al for this zeolite was 6.5.

The alumina-silicate lattice in the zeolite has an overall negative charge with either sodium (Australian zeolites) or calcium (Indonesian zeolites) ions occupying the interlattice space. Protein removal takes place by ion exchange. Haze-forming proteins have a net positive charge at wine pH [45–47]. Thus, positively charged proteins can replace sodium or calcium ions and once mixed and settled, the zeolite-protein solids can be removed. Since zeolites can also remove potassium ions through the ion exchange, competition occurs between proteins and potassium ions. Due to its high calcium content, it appears that the Indonesian zeolite removes potassium cations first. Once calcium ions are realised into the treated wine, complexes between protein and calcium might be formed. Proteins bind calcium via the carboxyl groups of glutamic and aspartic acid residues. These complexes present a steric hindrance. As a result, only some proteins and a quarter to half of the protein in these experiments could be removed through zeolite addition. Australian zeolites have sodium ions instead of calcium ions on their surface. It seems that the removal of potassium ions and proteins occurs simultaneously for Australian zeolites.

4. Conclusions

This work demonstrates that zeolites can remove excessive haze proteins and tartaric acid in a single treatment to achieve heat and cold stability, which is a considerable advantage over conventional bentonite fining and refrigeration. One of the main disadvantages of zeolite treatment appears to be relatively high dosage levels, aluminum leaching into a wine after treatment, and that some zeolites are less effective than others at protein removal. However, this work identifies means to address these shortcomings. Unlike bentonites, zeolites can be regenerated for example by treatment with (10%) NaCl solution, which results in a much more sustainable process. Furthermore, calcinating the zeolite before adding it to the wine is an effective method to reduce undesired aluminium leaching. The calcination process also reduces calcium content in the wine after treatment with zeolite, thus eliminating the risk of calcium instability. Based on structure-activity studies, we found that natural zeolites with low calcite content had a better ability to remove haze-forming proteins in wines, making these zeolites more attractive for use as they might induce both heat and cold stability in a single treatment. In addition, zeolites with low calcium content are a lower risk to produce calcium instability in the bottle. Preliminary results presented in this work indicate that zeolites can/may be an effective way to achieve wine cold and heat stability. Further testing and sensory evaluation will be required in the near future to fully validate the usefulness of these products in winemaking.

Data Availability

The data supporting the findings of this study are available within the paper and Supplementary Materials. The data can also be available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
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Supplementary Materials

Supplementary 1: The following are available online at the publisher’s website: https://wiley.com/doi. Supplementary 2: S1: Basic chemical analysis of the wines used in this study. Supplementary 3: S2: XRD of natural zeolite samples. Supplementary 4: S3: Potassium concentration in control and zeolite treated A SAB and B CHA wines as a function of time. Sodium concentration in control and zeolite-treated C SAB and D CHA wines as a function of time. Calcium concentration in control and zeolite-treated E SAB and F CHA wines as a function of time. Aluminum concentration in G SAB and H CHA wine measured initially after zeolite treatment and after three and six months of wine storage in the cellar. Supplementary 5: S4: Concentration of tartaric acid crystals in control wine and wine treated with natural zeolites, A SAB, and B CHA. Tartaric acid content in C SAB and D CHA measured directly after treatment and three and six months later after storage in the cellar. Protein concentration in control wine and wine after treatment with five different natural zeolites, E SAB, and F CHA. Supplementary 6: S5: Aluminium and calcium concentration in control and zeolite-treated wines including dealuminated zeolites. Supplementary 7: S6: Si/Al ratio and Ca content in Indonesian zeolites. (Supplementary Materials)

References


