

COULD BENTONITE PRODUCT CHOICE FIT THE DESIRED WINE STYLE?

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Bentonite fining alters the mineral composition of wines, the concentration of aromatic and phenolic components. Thus, the taste of minerality and wine style are affected. In Tokaj wine region, seven bentonite products were tested on 'Furmint' wine in controlled vinification technology together with an unfinned control. Basic quality parameters and mineral composition were measured. After two years, aromatic components were evaluated. Bentonite treatment significantly increases the Fe content of the wine between 10 and 50 % compared to the unfinned control. In the case of Fe content increase, BW 200, Tükrös and Deriton showed significantly extending values. Bentonite products decrease the presence of Cu in wine, however, a more than 100 % increase for the product Everclar Omega was highlighted. The presented clustering of aromatic components with the heat map suggests methodology to give practical guidelines for wine producers in the selection of the bentonite product corresponding to the desired final wine style. BW 200 and Gélbenton showed the strongest depletion of aromatic components.

Keywords: fining, mineral composition, aromas, clustering, heat map

Kann die Wahl des Bentonitproduktes an die gewünschte Stilistik eines Weines angepasst werden? Die Bentonitschönung verändert die mineralische Zusammensetzung der Weine und die Konzentration der aromatischen und phenolischen Komponenten. Dadurch werden der Geschmack (Mineralität) und der Weinstil beeinflusst. In der Tokajer Weinregion wurden sieben Bentonitprodukte bei Furmint-Weinen nach kontrollierter Weinbereitung zusammen mit einer unbehandelten Kontrolle untersucht. Es wurden allgemeine Qualitätsparameter und die Mineralzusammensetzung gemessen. Nach zwei Jahren wurden die aromatischen Komponenten bewertet. Die Bentonitbehandlung erhöht den Eisengehalt des Weins signifikant zwischen 10 und 50 % im Vergleich zur unbehandelten Kontrolle. Beim Eisengehalt zeigten die Produkte BW 200, Tükrös und Deriton signifikante Erhöhungen. Bentonitprodukte verringern den Cu-Gehalt im Wein, jedoch wurde auch ein Anstieg von mehr als 100 % bei einem Produkt (Everclar Omega) festgestellt. Die vorgestellte Clusterung von aromatischen Komponenten mit einer Heatmap bietet eine Methodik an, die einen praktischen Leitfaden für Weinproduzenten bei der Auswahl des Bentonitproduktes entsprechend dem angestrebten finalen Weinstil bietet. BW 200 und Gélbenton zeigten die stärkste Abnahme der aromatischen Komponenten.

Schlagwörter: Schönung, mineralische Zusammensetzung, Aromen, Clusterung, Heatmap

The mineral composition of wines has often been discussed in previous studies focusing on geographic origin and terroir commonly comprehended to be in correlation with the genuineness of a wine region and the taste of minerality. Apart from the growing site, biological bases (both rootstock and scion) climatic factors (ERDEI and EIFERT, 1985; OZDEN et al., 2010) natural and antropogenic pollution (ESCHNAUER, 1982), grape processing, skin maceration, yeasts, time of racking, clarification with bentonite, contact with pipes, barrels (Al, Cd, Cu, Cr) are also essential factors (KMENT et al., 2005; LARA et al., 2005), which determine the mineral background of the wine. Literature suggests that bottled wine is the most contaminated sample, when analysis aims to characterize a terroir.

From the technological point of view, more attention is to be put on probable changes in hazardous Fe, Cu, Mn contents of wine due to bentonite fining.

A logical supposition rises, namely that aromas partly bound by phenolic components can also undergo considerable depletion, which could be product specific.

THE MECHANISM OF BENTONITE-EFFECT

Currently, fining with bentonite is generally used to eliminate the hazardous protein content of wines. Effects of bentonite on protein stability and effects on phenolic and aromatic components, mineral content and tartrate crystal formation are to be studied on the basis of the origin (mining site, depth), formulation and dosage of this silicate. The activity of the bentonite, surface of the particles, adsorption capacity and purity highly depend on the site and depth of excavation (REYNOLDS, 2010). Thus, in the case of bentonite products not only the producer matters in the technology of wine fining but also the lot we buy. It is probable that the behaviour of a product can vary by time, the site and depth of its origin.

Bentonite is a complex hydrated aluminium silicate with exchangeable cation components (Al, Fe, Mg) $\text{Si}_4\text{O}_{10}(\text{OH})_2(\text{Na}, \text{Ca})$ (REYNOLDS, 2010). The most typical component is montmorillonite ($\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$). In wine technology, this form is hydrated to gel or suspension stage with water before dosage, to facilitate the opening of the silica layers and the activation of the binding sites. Depending on the product, the total surface and also internal surface can vary, values of e.g. about $5 \text{ m}^2/\text{g}$ (KÁLLAY, 2010), 13.4 to $38.3 \text{ m}^2/\text{g}$ (CATARINO and MADEIRA, 2008) or 11.2 to $56.7 \text{ m}^2/\text{g}$ (YUKSELEN

and KAYA, 2008) are reported, showing a stable negative charge. Its activation starts when hydrated form meets cations. Hydration with distilled water does not entail flocculation and sedimentation. Flocculation appears only, when cations enter the silica skeleton and cation exchange starts.

Na, Ca and Mg cations are exchanged with other metallic cations. Negatively charged particles flocculate with positively charged metallic cations. At the pH of wine proteins are in most cases positively charged, these can also be bound by particles of the bentonite, forming a certain mutual flocculation, followed by sedimentation. The mechanism of bentonite fining could depend on the quality of the bentonite: the size of the particles and the fraction of clay are more important than its amount (CATARINO et al., 2008; LAMBRI et al., 2010). Literature reports describe a better effect of the bentonite at higher alcohol content, which could be a result of a higher level opening of the layers of montmorillonite. Better clarification is to be experienced at higher pH, which could be a result of lower concentration of H^+ ions in the competition with proteins. LAMBRI et al. (2010) based on works of WATERS et al. (1993) and MARCHAL et al. (1996) compared Chardonnay wines of aged (sur lie) and fresh style, and drive attention to glycosylated proteins of cell wall origin. Removal of these negatively charged proteins by bentonite – abundant in aged wines – is more complicated. Other works distinct proteins of grape origin and yeast origin (SAUVAGE et al., 2009; VINCENZI et al., 2015).

THE EFFECT OF BENTONITE FINING ON MINERAL COMPOSITION

At a dosage of $100 \text{ g}/\text{hl}$ ($1 \text{ g}/\text{litre}$) of bentonite, alteration of ionic composition of the wine earlier in Hungary was reported to be insignificant. KÁLLAY (2010) also drives attention to expectable increasing Na-content, which is a critical parameter in the case of export items. Fining, and mostly bentonite fining increases contamination of Na, Al and Ca (DÍAZ et al., 2003; LARA et al., 2005; MCKINNON, 1997; SAUVAGE et al., 2002; POHL, 2007).

The divergent results of these studies on mineral elements have revealed the need for further specific information concerning the effect of bentonite products on the mineral composition of wines in general, but especially on the effect of bentonite on the most critical elements, like Fe, Cu and Mn.

For the sake of a balanced functioning of the yeasts in fermentation, besides K, Ca, Mg and Na, elements present in minor concentrations, like Cu, Fe, Mn and Zn are also required as prosthetic groups in metallo enzyme formation. However, increasing pH levels in wines enhance Cu and Fe oxidation, in addition Al, Cu and Fe clouding can occur (MCKINNON, 1997; POHL, 2007; RODRIGUEZ et al., 1999). The browning of old wines is closely correlated with the presence of higher levels of Fe³⁺, Cu²⁺ and Mn²⁺. These oxidized metal ions can entail reaction cascades by activating molecular oxygen and formation of hydroxyl radicals. This can induce the oxidation of organic components and further oxidation reactions of organic components to aldehydes and ketones (BENITEZ et al., 2002; DANILEWICZ, 2003, 2007; LARA et al., 2005; POHL, 2007). In addition, Mn supports the formation of the acetaldehyde, which in Fe mediated reaction forms a precipitate with polyphenols (POHL, 2007).

The iron content of wines is generally around 4 to 15 mg/l, but in certain cases it can be much lower (about 0.06 to 2.7 g/l) or higher (0.06 mg/l in Australia to 23.7 mg/l in Hungary) (MCKINNON, 1997; MURÁNYI and PAPP, 1998) respectively. Higher concentration entails the spoilage of the wine through the formation and precipitation of ferric phosphate (white casse) or ferric tannates (black casse) when Fe³⁺ forms precipitate with colour compounds. This can occur at a much lower 2 to 3 mg/l iron level already (WÜRDIG et al., 1989). The technological problem could be eliminated, if high Fe²⁺ concentration would occur simultaneously with low Cu levels, thus preventing the oxidation of iron (GONZÁLEZ et al., 1996). Copper content in wines is normally between 0.1 to 0.5 mg/l (GREEN et al., 1997; SUTUROVIĆ and MARJANOVIĆ, 1998). Red wines usually contain more than white wines (MARIN and OSTAPCZUK, 1992). A high amount can originate from the copper fungicides used for plant protection, but this fraction is normally reduced by fermentation and sedimentation of the yeast (FRENCZI, 1966).

Manganese in wines can reach a level of 0.5 to 5 mg/l. POHL (2007) summarized the broadest range for Spanish (0.1 to 5.5 mg/l), and the most narrow range for French (0.63 to 0.96 g/l) wines. According to literature reports, technological treatments do not lower its concentration (FERENCZI-LÁSZLÓ, 1978).

VOLATILE COMPONENTS AFFECTED BY BENTONITE

Odor-active components in wine have been broadly studied (BURIN et al., 2016; LAMBRI et al., 2010; LIRA et al., 2014; PUIG-DEU et al., 2016; VINCENZI et al., 2015). The studies focused on the mechanism of the depletion activity of bentonite in model solutions, modified wines and in real wines depending on the focus of the research. Bentonite can also get in connection with phenolic and aromatic components by different types of bindings (Van der Waals hydrogene bonds).

Thus, previous results demonstrated that the depletion activity of bentonite affects the wine style, however, wine style could be approached from the opposite side also: there is a possibility that the selection of bentonite product could be a tool to facilitate the evolution of the desired wine style.

This study presents the possible effects of bentonite products on mineral and aromatic composition of 'Furmint' wines in a two-year stability trial. Data in respect to seven commercially available bentonite products in Hungary are presented.

MATERIALS AND METHODS

FIELD EXPERIMENTS

Grapes for the wines included in the trial originated from the plantation in Szerencs, a little town in Tokaj wine region, on fertile brown forest soil formed on volcanic subsoil. The plantation was established in 1960 by small producers (0.1 to 0.6 ha) with 1.2 m between stock spacing in head training. In 1980 each second vine row had been cut out, which resulted in a 2.4 m row spacing. Vines are treated in single cordon Lenz Moser system. Varieties produced are 'Furmint' (90 %), 'Hárslevelű' and 'Muscat lunel' (altogether 10 %). Grapes from the vintage 2013 were included in the experiment.

Average number of sun hours: 1900 h; yearly mid temperature: 9,5 °C; mid temperature in vegetation: 17 °C; yearly precipitation: 574 mm, dominantly in June.

The two-years before the experiments (2011, 2012) were hotter with less rainfall, 484 mm and 519 mm yearly precipitation, respectively. (Table 1).

Table 1: Meteorological data of the experimental year and previous years (KSH, 2019)

Reference point: Miskolc (20 km from the experimental site)						
	2010	2011	2012	2013	2014	2015
Yearly mid temperature in zone	9.7	10.4	10.9	10.5	11.7	11.7
Y. m. t. in Hungary	10.2	10.8	11.3	11.1	11.9	11.7
Number of sunny days (per year)	1893	2289	2297	2031	1938	2153
Number of rainy days	185	109	113	150	146	130
Yearly precipitation (mm)	1166	483.9	518.6	774	725.7	569.5

In the experimental year (2013), the total amount of precipitation in Szerencs was 614 mm. 103 mm came down in May of which about 30 mm stormy fraction was of scarce utilization. The mid temperature in the vegetative period was approximately 1 °C lower, compared to the average.

WINE MAKING TECHNOLOGY

The last trial of ripening parameters was checked on September, 26th, 2013, and the next day the harvest was accomplished (22,7 MM° (252,3 g/l) Hungarian sugar degree of the must; pH 3,24). 'Furmint' and 'Hárslevelű' were processed together, rachis (stem) was not removed. 'Muscat lunel' was harvested earlier, it was not included in the experiment.

A 5 g/hl dosage of potassium metabisulfite was added and mixed into the pomace before a 12-hour maceration (traditional method). Pressing was performed on low pressure. Finally pressed must (5 to 10 %) was not retrieved. Fermentation was delayed with the addition

of further 25 g/hl potassium metabisulfite in order to allow 12 hours of sedimentation. The temperature was lowered to 10 °C. The clear fraction of the must was decanted and pumped to a fermentation tank. Then, yeast nutrient was added to facilitate the proceeding of the fermentation (Vitamon® Combi, Erbslöh, Geisenheim, Germany; 50 g/hl). Analytical check performed with ICP OES showed no mineral constituents in the product. (Method detailed later. Result not presented.) Inoculation was done with cold resistant *Saccharomyces cerevisiae* subsp. *bayanus* (Interker-Wein Kft, Eger, Hungary, 30 g/hl, double amount as suggested by the manufacturer). Fermentation at 10 °C was complete in two weeks. Six weeks after fermentation, the lot was racked from over yeast layer to a 50 litre glass jug, topped with added CO₂ and closed with airlock. For the first sulfite addition 5 g potassium metabisulfite was added to the 50 l lot, then the new wine was fragmented into 5 l glass jugs. Racking was carried out excluding contact with air. Jugs were closed with a layer of CO₂. Fining with bentonite was performed 2 weeks after first racking.

BENTONITE PRODUCTS IN THE TRIAL AND EVALUATION OF PROTEIN STABILITY

For the evaluation of the effects of the bentonite products, 8 times 5 l were taken. One of these was the control, not treated with any fining medium, the other 7 batches were fined with the average recommended

amount of different bentonite products according to their corresponding instruction manuals (Table 2), only the dosage of Deriton was modified, according to previous experiences. Portions were dosed with an analytical scale.

Table 2: Dosage of bentonite products in the trial

Product name	Suggested dosage (g/hl)	Applied dosage (g/hl)	Characterisation	Distributor
Everclar Ω	30-50	25	Montmorillonit, fish-glue	EVER (It)
Nucleobent	10-40	25	Activated Na-bentonite	EVER (It)
BW200	60-90	75	Na-bentonite	Sud Chemie (D)
Gélbenton	10-30	20	Na-K-bentonite	Dal Cin (It)
Tükrös	60-80	70	Bentonite, illite	Geoproduct Kft., Mád (Hun)
Deriton	100-150	50	Alcalically activated bentonite	Bentonit Kft., Felsőzsolca (Hun)
Bentonit	60-130	80	Na-bentonite	Unikén Kft., Kecel (Hun*)

**only distributor can be given*

After two weeks, according to the treatments, 8 times 4 bottles of wines were taken without any filtration. At each step, wines were topped with CO₂. During the fermentation, considerable tartrate formation was observed. After fining, no further crystallization was detected. Cellar temperature was consistent at 13 to 14 °C, no illumination was used. Before bottling neither cold stabilization, nor malolactic fermentation was performed. Samples were kept in the cellar up to the time points of measurements. Transportation to the laboratory took

about 2 hours. In the laboratory, wines were kept in a refrigerator at 4 °C.

There are many tests for evaluating the protein stability (TOLAND et al., 1996). We used the heat test. Filtered wine samples were stored at 80 °C for six hours and 4 °C for three days. After the heat shock the samples were allowed to stand at room temperature overnight. On the next day the samples were eye-tested against a light source in comparison with an untreated control sample of the filtered wine (TOLAND et al., 1996).

TIME POINTS OF MEASUREMENTS

As illustrated in Table 3 the alcoholic content, total titratable acidity and pH were measured three times for the sake of the critical control of the trial. The targeted parameters with respect to mineral composition were measured (ICP OES inductively coupled plasma optical emission spectrometry) three times. This

basically would have pointed to checking secondary tartaric formation and precipitation, which was not performed. Aromatic composition was analysed only at the end date of the trial. Furmint wines usually need some years of ageing.

Table 3: Order of measurements in the trial

Basic parameters	Method	Date of measurement (dd,mm,yyyy)			
		14.03.2014	10.05.2014	10.06.2016	10.01.2017
- Alcohol content	Malligand ebulliometer	x	x	x	-
- Total titratable acidity	OIV-MA-AS313-01:R2009	x	x	x	-
- pH	Mettler Toledo FiveEasy	x	x	x	-
- Sugar content of wine	Rebelein-probe (REBELEIN, 1973)			x	-
- Extracts	Evaporation for 2 days at 60 °C (minus sugar)			x	-
Focused parameters					
- Mineral composition	ICP OES	x	x	-	x
- Aromatic composition	Bruker Scion 456-gas chromatograph	-	-	x	-

SAMPLE PREPARATION AND THE ANALYSIS OF ELEMENTS

The bentonite treated wine samples and a sample of untreated control wine were diluted ten-fold with 5 (m/V) % nitric acid (VWR ARISTAR® for trace analysis) before analysis. As deionized water type-1 grade (Milli-Q® water purification system, Merck-Millipore, Molsheim, France) water was used. All samples were analyzed in triplicates. The analysis was made by a ThermoFischer

Scientific (Cambridge, UK) iCAP 6300 ICP-OES instrument equipped with CETAC ASX-520 autosampler and a Meinhard-type concentric nebulizer attached to a cyclonic spray chamber. All measurements were made in axial-mode.

The quantification of desired elements was made by external calibration. As a stock solution, a multi-element

solution was made from monoelement standards (1000 mg/l in 2 % nitric acid, Scharlau, Scharlab, Sentmenat, Spain) and to construct calibration curves appropriate dilutions were made from that. Samples were measured in triplicates.

ANALYSIS OF VOLATILE COMPOUNDS

The profiling of the volatiles of the wines was carried out with a Bruker Scion 456-gas chromatograph equipped with Bruker SHS-40 Headspace Sampler. The gas chromatograph was coupled to a Bruker SQ mass spectrometer and was fitted with a Br-5 capillary column (30 m 0.25 mm i. d. 1.0 μ m film thickness). The carrier gas was helium, flow rate was 1 ml/min in constant flow mode. 5000 μ l samples were incubated in headspace vials at 60 °C for 20 minutes in the automatic sampler with no agitation. 1000 μ l headspace sample was injected into the column. The transfer line was maintained at 230 °C, the injector temperature was 250 °C (20:1 split ratio). The oven was held at an initial temperature of 40 °C for 2 minutes, then increased to 280 °C at 10 °C/min, and held at this temperature for 3 minutes. The mass spectrometer was operated in electron impact ionisation mode (70eV) source temperature: 180 °C; scanning rate: 1 scan per second, and the mass spectra were recorded in full scan mode. The identification of the volatile compounds was based on mass spectrometric data obtained from the National Institute of Standards and Technology (NIST) (Version 2005) mass spectral library.

STATISTICAL ANALYSIS OF MINERAL AND AROMATIC COMPOSITION

Meta analysis is a powerful statistical tool that can combine the mean differences of multiple samples compared to a specific control sample. The main logic is that samples were measured with certain errors and we would like to provide only one pooled estimate of the mean difference closest to the real one with respect to within- and between-sample errors. Y_i denotes the actual mean differences between samples and control, θ indicates the pooled mean difference, while θ_i is the sample effect on the pooled mean difference, τ^2 is the between-sample variance and V_i represents the within-sample variance. We

assume that both within- and between-sample variances follow normal distribution. The so-called random effect model can be written in the following form:

$$Y_i = \theta + \theta_i + e_i, \text{ where } \theta_i \sim N(0, \tau^2); e_i \sim N(0, V_i) \quad (1)$$

$i = 1-9$ (3 periods, with 3 replications/period) period effect, checked with a mixed effect model and included as a covariate, was found not significant. Duncan's test was applied to measure the pairwise differences between samples.

Heterogeneity was quantified using the τ^2 statistics across samples. The amount of residual heterogeneity was estimated in the random effect model using the restricted Maximum Likelihood estimator, model selection was based on the Log Likelihood and Akaike Information. The Null hypothesis is that $\theta_i = 0$ is tested with Z-test while $\tau^2 = 0$ is tested using Cochran's Q-test. Sample bias was assessed through statistical tests (EGGER et al., 1997). Confidence intervals were obtained under the assumption of normality and the confidence interval for the heterogeneity was obtained with the method described by VIECHTBAUER, 2007. The whole study was conducted following the guidelines of (VIECHTBAUER, 2010) and (HUZSVAI and BALOGH, 2015) for linear models in R. In our study, we investigated the pairwise differences between samples using Duncan's new multiple range test (MRT) (DUNCAN, 1955). Each comparison was performed at a significance level of 5 %. Meta analysis was performed by R 3.3.2 software and its metafor package while Duncan test was performed using Agricolae package (R CORE TEAM, 2016).

We also applied the so-called variable clustering around latent variables (VARHCA) using Tanagra 1.4.50 software (RAKOTOMALALA, 2005) which implements VIGNEAU and QANNARI'S work (2003). Clustering aroma compounds in our case is more useful because there are 23 compounds and only 8 samples. We also applied this method for determining the group of the most relevant aroma compounds and for representing aroma groups with one factor. VARHCA arranges attributes into homogenous clusters using hierarchical clustering by computing similarities with the correlation coefficient and associates each group with a latent component. These latent components can be used to characterise samples more easily.

RESULTS AND DISCUSSION

BASIC WINE PARAMETERS

Considerable decrease was detected in the alcoholic content of wines during two years, between 2 to 9 % (4.2 % in average), with a maximum for Everclar Omega (12,8 to 11,6 v/v%; 90.6 %) and minimum for Tükrös (12,4 to 12,2 v/v%; 98.3 %) bentonite products. The pH of wines increased with 6.7 % on average (about 0.2 in value) with the lowest change for Everclar (3,1 to 3,17 pH; 102,26 %) and highest values and measure of chan-

ge for Deriton (3,02 to 3,275 pH; 108.4 %) and unfinned control (2,99 to 3,275 pH; 109.5 %), which correspond to data on total titratable acid content: 85.2 % on average (-14.7 % change), showing the lowest decrease in the case of Everclar (6,938 to 6,075 g/l; 87,568 %) and Nucleobent (6,9 to 5,925 g/l; 85,87 %), biggest decrease in the case of Deriton (6,75 to 5,175g/l; 76,67 %) and unfinned control (6,562 to 5,175 g/l; 78,86 %) (Table 4).

Table 4: Basic wine parameters with respect to possible changes over 2 years

Product		Alcohol v/v %	pH	Acid* (g/l)	Sugar (g/l)	Extracts ** (g/l)
Everclar	2014.03.	12,8	3,1	6,938		
	2014.06.	12,6	2,96	6,713		
	2016.06.	11,6	3,17	6,075	0,7	20
	% change	90,625	102,26	87,568		
Nucleobent	2014.03.	12,5	3,03	6,900		
	2014.06.	12,4	2,98	6,787		
	2016.06.	12,2	3,18	5,925	0,7	19,5
	% change	97,6	104,95	85,87		
BW 200	2014.03.	12,6	3,02	6,675		
	2014.06.	12,6	3	6,600		
	2016.06.	11,8	3,235	5,850	0,5	19,74
	% change	93,651	107,12	87,64		
Gélbenton	2014.03.	12,6	2,98	6,637		
	2014.06.	12,6	2,96	6,787		
	2016.06.	12	3,19	6,000	0,5	19,92
	% change	95,238	107,05	90,402		
Tükrös	2014.03.	12,4	2,99	6,787		
	2014.06.	12,6	2,99	6,825		
	2016.06.	12,2	3,22	6,000	0,7	19,66
	% change	98,387	107,69	88,404		
Deriton	2014.03.	12,6	3,02	6,750		
	2014.06.	12,6	3	6,712		
	2016.06.	12,2	3,275	5,175	0,6	18,6
	% change	96,825	108,44	76,667		
Bentonit	2014.03.	12,6	3,01	6,825		
	2014.06.	12,6	2,98	6,825		
	2016.06.	12	3,215	5,925	0,6	19,58
	% change	95,238	106,81	86,813		
Control	2014.03.	12,6	2,99	6,562		
	2014.06.	12,8	2,97	7,012		
	2016.06.	12,4	3,275	5,175	0,3	18,38
	% change	98,413	109,53	78,863		
Average change (%)	95,747	106,73	85,278			
*in tartaric acid						
**without sugar						

Table 5: Significant differences in mineral composition of the pooled treatments

elements in ppm	Control		Bentonites							p-value for heterogeneity
	Mean	SD	Everclar Omega	Nucleobent	BW 200	Gélbenton	Tükrös	Deriton	Bentonit	
K	475.14	43.10	484.78 a	477.74 a	471.63 a	480.31 a	477.89 a	489.88 a	461.70 a	0.985
Ca	80.63	15.09	99.84 a	96.82 a	97.07 a	96.89 a	96.81 a	94.84 a	94.89 a	0.998
Mg	66.90	11.04	72.80 a	70.91 a	68.97 a	67.87 a	67.29 a	68.55 a	66.68 a	0.996
Na	4.06	1.70	7.25 c	8.18 c	16.50 a	8.17 c	11.69 b	12.40 b	8.80 c	<0.001
P	184.57	23.04	190.80 a	189.22 a	185.16 a	185.81 a	185.51 a	194.47 a	181.34 a	0.995
Fe	1.9948	0.18	2.1732 bc	2.0850 c	2.4459 a	2.0722 c	2.3339 abc	2.4032 ab	2.1444 bc	0.078
Mn	0.7160	0.11	0.7538 a	0.7377 a	0.7614 a	0.7410 a	0.7379 a	0.7304 a	0.7106 a	0.998
Sr	0.2931	0.03	0.3021 a	0.3070 a	0.3033 a	0.3032 a	0.3104 a	0.3305 a	0.3059 a	0.907
Cu	0.0208	0.01	0.0437 a	0.0227 b	0.0136 b	0.0196 b	0.0154 b	0.0190 b	0.0122 b	0.006
Ba	0.0405	0.01	0.0517 ab	0.0561 ab	0.0459 b	0.0427 b	0.0491 ab	0.0693 a	0.0430 b	0.435
Zn	0.4242	0.05	0.4875 a	0.4600 a	0.4592 a	0.4372 a	0.4620 a	0.4281 a	0.4172 a	0.768
Al	0.1012	0.01	0.3040 e	0.4810 d	0.5967 b	0.2248 f	0.5640 c	1.0117 a	0.6047 b	<0.001

The protein stability test showed haze-like precipitation in the control wine at both temperatures, and slight turbidity in the samples treated with Gélbenton and Tükrös at 80 °C.

THE MINERAL COMPOSITION OF WINES

In contrast to our expectations, no decrease in the mineral composition was detected after two years (2014 to 2016), nor secondary crystallization and sedimentation of tartrate was observed, despite our expectation. The general relatively low level of K content in wines (4 to 500 ppm) could be a consequence of the arid experimental year and the high topographic elevation.

Product Bentonit showed minimum levels of mineral constituents in most cases (K, Mg, P, Mn, Cu, Zn) with no extending maximum data on the other side. Fining with Deriton resulted in high concentration for most elements (K, Na, P, Fe, Sr, Ba and Al). In this case, the Al content reached the tenfold of the unfined control. With respect to the elements K, Ca, Mg, P, Mn, Sr and Zn, it is to be noted that there is no significant difference in comparing the different bentonite products.

It can be clearly seen that bentonite treatment significantly increases the Ca, Na, Fe and Al content of the wine (Table 5). This increase was between 10 to 50 % compared to the unfined wine. In the case of Na, Fe, Al content, significant pairwise differences between bentonite products can also be detected. Regarding Na and Fe, BW200 had the highest increase, but Tükrös and Deriton should also be highlighted. A respectively notable heterogeneity in the case of Na and Al should be mentioned. In other cases ($p > 0.05$), samples are homogeneous. Especially in the case of Al significant pairwise differences can be observed between almost every bentonite product. Data show that a two- to tenfold increase in Al content can be generally expected due to bentonite treatment.

AROMATIC COMPONENTS

Data on aromatic composition demonstrate that bentonite fining alters the presence of volatile compounds. First of all, it is visible that unfined control wine contained the most components, thus representing the highest complexity. Unfined wine showed 23 aromas, whilst bentonite fined items contained 8 to 9 components less. If we look for any linkage between bentonite products and aromatic components, clustering could give useful information concerning 23 components (attributes) and 8 treatments (samples).

In our dataset, VARHCA detected 4 clusters that can be seen in Table 6. All attributes seemed well-assigned to their groups based on R-squared and correlations except phenylethyl alcohol and 3-methyl-1-butanol. We also investigated the orthogonality between the clusters by calculating the correlation between the components. We obtained independent factors, therefore clusters are well represented by their components and the total explained variance is sufficiently large (79.4 %). The 11 most influential attributes from all clusters are marked with bold letters regarding R-squared value and correlation.

Clusters are represented by one latent component seen in Table 6. The component scores can be presented in a heat map for each sample (Fig. 1). This method enables the visualization and clear interpretation of the aroma depletion of specific bentonite products. The blue colour represents the depletion of clustered components. On the other side, the red colour shows the increasing presence of the aromas of the pointed cluster. The heat map shows that the control contained most clusters and components, but Everclar Omega rose over the control in aromas of "Cluster 1" even in the case of butanoic acid and hexanoic acid. In comparison, however, it is visible that Everclar Omega depleted components of "Cluster 3" and "Cluster 4" (ethyl acetate and propanoic acid), which remain abundant in unfined control wine. Nucleobent is to be highlighted, because in this case aroma components of "Cluster 1" rise over those of the unfined control.

Looking at "Cluster 4" we can see that Tükrös, Deriton and Bentonit eventuated the same aroma concentration like the unfined control.

In the case of "Cluster 2" Bentonite and Gélbenton could be compared to Everclar Omega and the unfined control. The presence of these components suggest the following descending order: Everclar Omega, control, Bentonit and Gélbenton (BW 200, Tükrös and Deriton equal zero).

In the case of triethyl borate ("Cluster 1"), negative values of "Correlaton to the first PCA" and "Latent Components from the clustering approach" indicate extendingly high concentration in the case of control. Another reason is that Nucleobent and Everclar Omega totally depleted this aroma component.

Table 6: Homogeneous clusters arranged by VARHCA

Cluster	Items	R-squared	Correlaton to the first PCA	Latent Components from the clustering approach			
				c1	c2	c3	c4
1	1-Propanol, 2-methyl-	0.885	0.941	0.338			
	Butanoic acid, 3-methyl-, ethyl ester	0.964	0.982	0.353			
	Hexanoic acid, ethyl ester	0.953	0.976	0.351			
	Butanoic acid, ethyl ester	0.751	0.866	0.312			
	Butanoic acid, 2-methyl-, ethyl ester	0.728	0.853	0.307			
	Triethyl borate	0.691	-0.831	-0.299			
	1-Butanol, 2-methyl-, (S)-	0.554	0.745	0.268			
	Propanoic acid, 2-methyl-, ethyl ester	0.715	0.845	0.304			
	<u>Phenylethyl Alcohol</u>	<u>0.433</u>	<u>0.658</u>	0.237			
	1-Butanol, 3-methyl-	<u>0.313</u>	<u>0.559</u>	0.201			
	1-Butanol, 3-methyl-, acetate	0.751	0.867	0.312			
2	1,3,5-pentanetriol	0.988	0.994		0.424		
	unknown	0.988	0.994		0.424		
	3,3-dimethyl-4-heptanol	0.988	0.994		0.424		
	unknown	0.988	0.994		0.424		
	2,4,5-trimethyl-1,3-dioxolane	0.987	0.993		0.424		
	unknown	0.555	0.994		0.424		
3	Acetic acid	0.795	0.892			0.508	
	Ethyl Acetate	0.894	0.946			0.539	
	Propanoic acid, ethyl ester	0.807	0.898			0.512	
4	Ethylene sulfide	0.582	0.763			0.435	
	Butanedioic acid, diethyl ester	0.981	0.991				0.707
	Octanoic acid, ethyl ester	0.981	0.991				0.707
% Tot. Var. Exp.		79.4		33.6	23.9	13.4	8.5

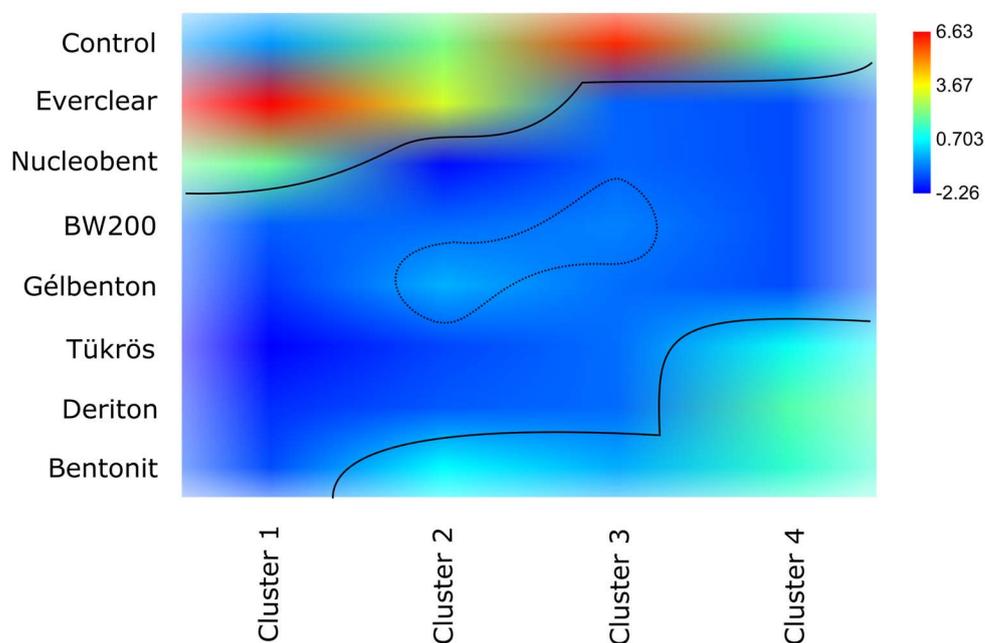


Fig. 1: Component scores presented in a heat map pointing depletion zones of specific bentonite products

CONCLUSIONS

Based on our data, in question of the most hazardous elements, bentonite treatment significantly increases the Fe content of the wine between 10 to 50 % compared to the unfined control with extending data for BW 200, Tükrös and Deriton. The use of bentonite products in general decreases the presence of Cu in wine, giving safety for longevity of the products, and lowering the possibility of Fe(II) oxidation. However, a more than 100 % rise of Cu content for Everclar Omega must be noted. The Mn content of wines did not show significant alteration, and no significant difference was found between the Mn content of wines treated with different bentonite products. Regarding Al, the detected two- to tenfold increase due to bentonite fining must be highlighted. It seems to be possible, that based on Latent Compo-

nents heat map could give a practical guideline for wine producers in their selection of bentonite products corresponding to the pointed final wine style. Based on the presented trial, BW 200 and Gélbenton could be highlighted for the strongest depletion of aromatic components.

However, the authors drive attention to the fact that the current study inspected only a narrow spectrum of aromas, because the trial was based on 'Furmint' wine with mostly fermentative aromas.

CONFLICT OF INTEREST STATEMENT

Authors declare they have no commercial interest with the presented products.

ACKNOWLEDGEMENTS

This article was supported by the János Bolyai research scholarship of the Hungarian Academy of Sciences and jointly supported by the ÚNKP-19-4-DE-5 New National Excellence Program of the Ministry for Innovation and Technology.

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Received December, 5th, 2018