Food Science and Applied Biotechnology, 2022, **5**(2), 140-150

Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2022.v5.i2



OPEN ACCESS

Research Article

Protein change in Chardonnay wine from Bulgaria after treatment with different types of bentonites

Ivan Bakardzhiyski[1]

[1] *Department of Wine and Beer Technology, Faculty Technological, University of Food Technologies, Plovdiv, Bulgaria*

Abstract

Protein profile change of Bulgarian Chardonnay wine under the influence of treatment with bentonite was studied. Protein concentration was determined using the Bradford assay, while fractionation was performed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The amount of protein found in the original wine was 147.1 $mg.L^{-1}$, expressed as bovine serum albumin divided into 9 electrophoretic fractions. Low molecular weight fractions with molecular weights of 20, 22 and 24 kDa, were predominant whose share was equal to 65 % of the total electrophoretic fraction profile of wine. The examined wine was treated with four different types of bentonite-two sodium, one activated calcium and one sodium-calcium bentonites. They were added in increasing doses, covering the range of 0.2-3 $g.L^{-1}$. At lower treatment doses (0.2-0.8 $g.L^{-1}$), differences in protein concentration reduction were observed. Above the dose of 0.8 $g.L^{-1}$, an equalization of their effect compared to the reduction of total protein content was reported. Affinity, however small, of the studied bentonites to the different molecular fractions of proteins was observed.

Keywords: white wine, bentonite, proteins, Bradford assay, SDS-PAGE, colloidal stability

Abbreviations: B1 – activated calcium bentonite; B2 – sodium-calcium bentonite; B3 – sodium bentonite; B4 – sodium bentonite; BSA – bovine serum albumin; SDS-PAGE – sodium dodecyl sulphate-polyacrylamide gel electrophoresis; CBB – coomassie brilliant blue

✉*Corresponding author: Ivan Bakardzhiyski, Department of Wine and Beer Technology, Faculty Technological, University of Food Technologies, Maritza blvd. 26, Plovdiv, Bulgaria, tel.: +359 32 603 642; mobile: +359 888 098861; E-mail: ivanbak@uft-plovdiv.bg*

Article history:
Received 3 April 2022
Reviewed 27 September 2022
Accepted 10 October 2022
Available on-line 13 October 2922

https://doi.org/10.30721/fsab2022.v5.i2.189
© 2022 The Authors. UFT Academic publishing house, Plovdiv

Introduction

It has long been well-known that proteins, in particular those derived from grapes, play a leading role in the formation of colloidal haze in white wines (Bayly and Berg 1967; Dupin et al. 2000; Chagas et al 2017; Reynolds 2022; Silva-Barbieri et al. 2022; Jackson 2022). Immediately after alcoholic fermentation, some of them are still in the state of solution. Under the influence of a number of factors (alcohol, pH, phenolic compounds, ambient storage temperature, heavy metals, aeration, other colloids etc.), they can lose their solubility and become the cause for the so-called protein haze in white and rosé wines (Chobanova 1988; Boulton et al. 1996; Siebert et al. 1996; Pocock and Waters 2006; Pocock et al. 2007; Esteruelas et al. 2009; Melnik et al. 2015; Chagas et al 2016; Celotti et al. 2021; Jones-Moore et al. 2022; Morata 2022).

In some previous scientific studies, attempts were made to find a relationship between protein concentration and wine stability with respect to colloidal haze. For example, according to Troost (Chobanova 1988), if a wine contains proteins below 20 $mg.L^{-1}$, it is stable. According to other researchers (Fukui and Yokotsuka 2003), the threshold for wine stability is at a concentration of 35 $mg.L^{-1}$. There are examples indicating a much wider range of 27-72 $mg.L^{-1}$ (Hung 2010) and those where the concentration is 9-11 $mg.L^{-1}$ and the wine is still not colloidally stable (Hsu et al. 1987b).

In respect of the fraction profile, in the middle of the last century it was believed that bentonite did not act selectively compared to protein fractions of different molecular weight (Koch and Sajak 1959). More recent studies (Hsu et al. 1987a; Sauvage et al. 2010; Pargoletti et al. 2021; Osorio-Macías et al. 2022) refute these claims and illustrate a certain affinity of bentonite to specific fractions at a given dose.

This paper aims to study the change in proteins in white wine when treated with different types of bentonites added in increasing doses.

Materials and Methods

The present research was conducted on Bulgarian Chardonnay wine from the Danube Plain Region. It was colloidally unstable, taken at the stage after alcoholic fermentation and before any processing had been carried out on it. It was treated with 4 different bentonite available on Bulgarian market. They were delivered from official distributors on Bulgarian market and were designated as: B1-activated calcium bentonite, B2-sodium-calcium bentonite, B3-sodium bentonite and B4-sodium bentonite.

The studied wine was divided in volumes of 100 mL. To these, while constantly stirring (for 5 minutes), bentonite was added in increasing doses of 0.2 to 3 $g.L^{-1}$ in the form of a 5 % suspension. After a settling of 48 hours, the wine from each examined variant was decanted separating sediment from liquid, filtered through K5 filter sheet and subjected to analysis according to the following methods:

- protein concentration - via the Bradford assay, with the modification of Stosheck (Owusu-Apenten 2002). The method is spectrophotometric and was carried out using a Shimadzu UV-1800 UV-VIS spectrophotometer. The protein reagent was prepared daily according to the method described by Bradford (1976). The results of the quantitative analyses are expressed in $mg.L^{-1}$ of bovine serum albumin (BSA).

- protein recovery - via trichloroacetic acid (TCA) precipitation (Tattersall et al. 1997). Centrifugation was performed using an Eppendorf miniSpin microcentrifuge.

- protein fraction composition - via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to the Laemmli (1970) procedure, at acrylamide concentration in the separating gel of 15 %. Fractionation was performed via a Clever Scientific OmniPAGE Mini Vertical Protein Electrophoresis System, powered by a Clever Scientific PowerPRO 300 Power Supply. The separation was performed at a constant current providing 30 mA for each gel. The protein gels were developed by coomassie staining (with solution of coomassie brilliant blue R-250, CBB-R250) and then destaining whit solution containing 10 % of ethanol and 7 % of acetic acid. Protein molecular weight was determined using protein markers with known molecular weight SigmaMarker™ 6,500-200,000 Da and Totallab-trial version a specialized software for image analysis of electrophoretic gels. The presence of individual fractions in the total protein fraction profile was determined according to Totallab image analysis software data.

- wine colloidal stability test-on two parallel samples. The first one was heated at 70°C and the second was cooled at -7°C for 16 h. The turbidity was observed after temperate to room temperature for 2 hours as described previously by Chobanova (2007).

Statistical data processing was done using EXCEL software.

Results and Discussion

The protein concentration in the untreated wine was 147.1 mg.L^{-1} of BSA. The change in the protein concentration in the wine treated with the tested bentonite variants, depending on the applied dose, is shown in Fig. 1. It can be stated that at lower doses (of up to 0.8 g.L^{-1}), different bentonites affect the concentration of the total protein differently.

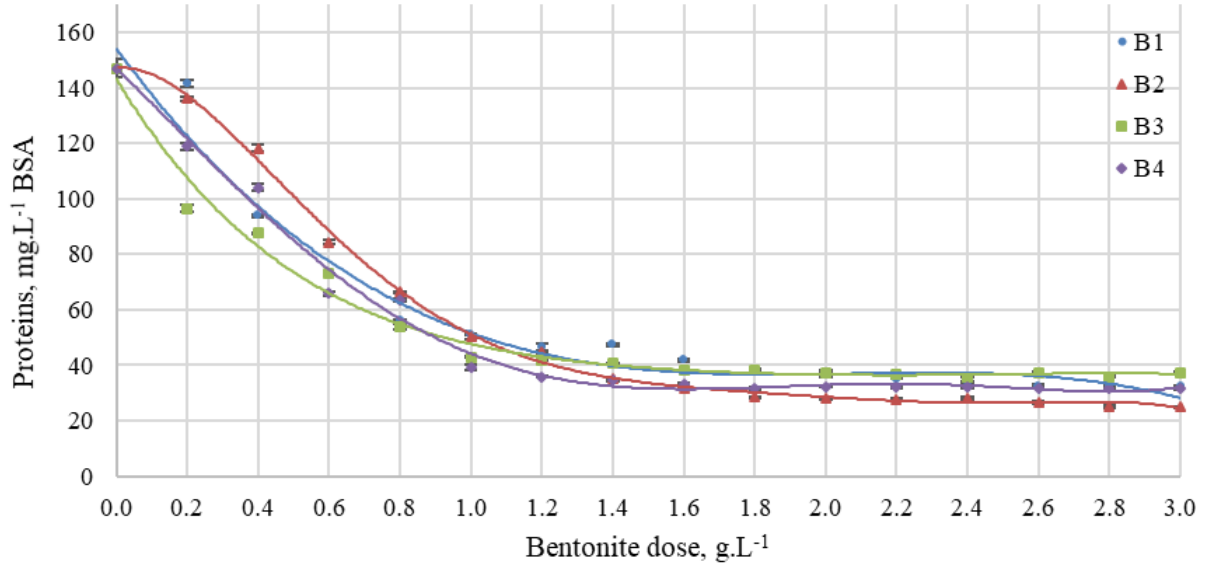


Figure 1. Change in the protein concentration of the experimental wines treated with different bentonites depending on the added dose

The strongest decrease was reported for B3 bentonite (34-63 %), and the weakest was in the series of samples treated with B2 bentonite (7-55 %). The remaining bentonites (B1 and B4) demonstrated an almost identical behavior of reduction with intermediate values comparable to the previously mentioned bentonites with respect to the considered index. These results can be explained by the different deproteinization capacity of the bentonites tested. According to the data from our previous study (Bakardzhiyski et al. 2016), B3 bentonite demonstrated the highest deproteinization capacity, and the one marked as B2-the lowest. B1 and B4 bentonites had relatively close values of this index.

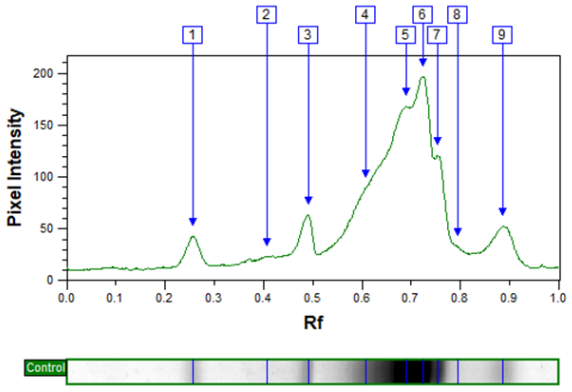
The discussed observations are valid up to a dose of 0.8 g.L^{-1}, above which the deproteinization capacity acquires an almost identical nature regardless of the origin, composition and manner of activation of the studied bentonites.

Despite the relatively similar nature of the protein concentration reduction curves, a stabilizing effect was obtained at different doses for different preparations. The lowest dose providing stability was reported for B1 bentonite-1.8 g.L^{-1}, the highest for B2-2.8 g.L^{-1}. The stabilizing doses for B3 and B4 bentonites were 2.2 and 2.6 g.L^{-1} respectively.

Protein concentration of the wines stabilized with the various bentonites was as follows: 37.8; 25.2; 36.5 and 31.9 mg.L^{-1} BSA for B1, B2, B3 and B4 bentonites. These results do not confirm a Troost's thesis (Chobanova 1988), according to which wines can be considered stable at protein concentrations below 20 mg.L^{-1}. In the studied experimental series of wines, stability was reported at a slightly higher value of this index. In fact, the world scientific literature is devoid of unanimity on this issue as stated in the introduction to this paper. The commented discrepancies can be explained by the specifics of the composition of different wines and,

more precisely, the flocculation abilities of different substances in the composition of wine in terms of proteins (alcohol, phenolic content, acidity, presence of heavy metals, other high molecular compounds, sulfur dioxide, etc.). Also, the fact that the methods used to determine protein content in different studies over the years may also lead to different conclusions should be taken into consideration.

The initial protein fraction profile of the wine used included 9 fractions (Fig. 2). Two of them were of medium molecular weight (with molecular weights of 43 and 61 kDa) and the other seven were of low molecular weight (13-32 kDa).



A.

Fracti-on, №	1	2	3	4	5	6	7	8	9
Mol. weight, kDa	61	43	32	24	22	20	19	17	13
Share, %	5	4	5	23	22	20	8	3	9

B.

Figure 2. Protein fractional profile of untreated (control) Chardonnay wine
A-electrophoregram; **B**-molecular weight and percentage of each fraction in the total fractional profile

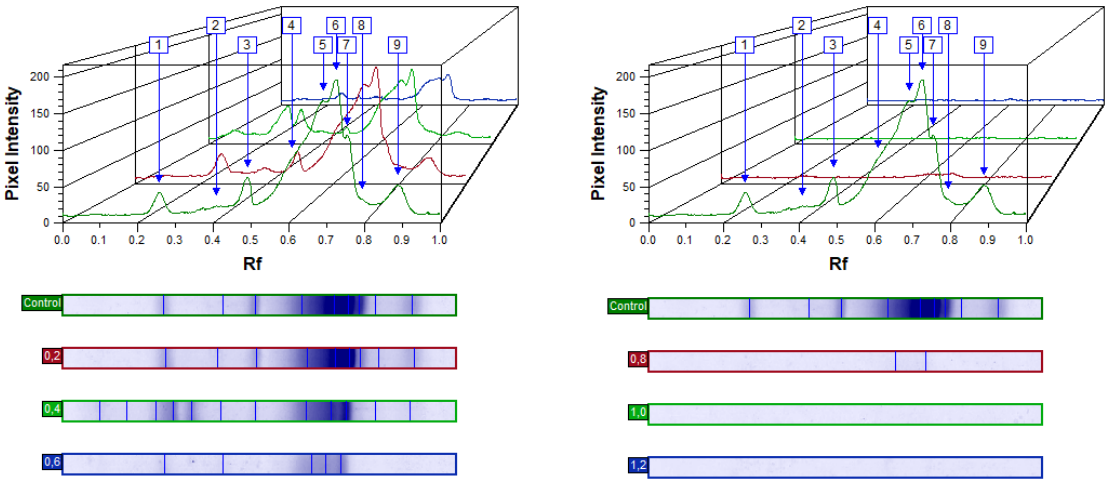
No high molecular weight compounds were reported. Of all the fractions those with molecular weights of 24, 22 and 20 kDa had the largest share of 23, 22 and 20 % respectively. Those with molecular weights of 17 and 43 kDa were the ones with the smallest share, and their percentage was 3 and 4 % respectively. In confirmation of a number of our previous studies (Bakardzhiyski and Chobanova 2013; Bakardzhiyski et al. 2015), as well as those found in the world scientific literature related to this topic (Hsu et al. 1987c; Dorrestein et al. 1995; Fusia et al. 2010; Hung 2010; Marangon et al. 2013; Van Sluyter et al. 2013), low molecular weight fractions predominated, accounting for 91 % of the fraction profile. The remaining 9 % was represented by medium molecular weight compounds.

The results obtained from the electrophoretic analysis of the studied wine samples treated with different bentonites in increasing doses are given in figures 3-6.

The B1 bentonite added to the wine used had the greatest effect on the fraction with a molecular weight of 19 kDa. Even with the lowest added dose (0.2 $g.L^{-1}$), a decrease of 50 % was observed (Fig. 3), and with a subsequent increase of the dose it disappeared from the electrophoretic image. In terms of degree of reduction, the protein fraction with a molecular weight of 13 kDa ranked second. At the first dose, its reduction was about ¼ of its initial concentration in the control sample. The next step of increasing the dose (0.4 $g.L^{-1}$) caused the removal of more than 50 % of it, compared to the original wine. When 0.6 $g.L^{-1}$ of B1 bentonite was added, it was no longer observed in the resulting electrophoregram.

Significantly weaker in the beginning was the change in fractions with molecular weights of 22, 20 and 32 kDa. A more significant reduction (40-68 %) was achieved with doses of above 0.4-0.6 $g.L^{-1}$. The lower treatment doses hardly affected the fractions with molecular weights of 17, 24, 43 and 61 kDa. Increasing the dose of bentonite to 0.6 $g.L^{-1}$ was sufficient to completely remove the fractions with molecular weights of 17 and 32 kDa. To achieve this effect with respect to proteins with molecular weights of 20 and 24 kDa, a dose of 1.0 $g.L^{-1}$ was required, probably due to their higher concentration in the original wine.

An interesting situation was observed in the series of wines treated with this bentonite type-in the sample treated with a dose of 0.4 $g.L^{-1}$ the appearance of new fractions, which were not present



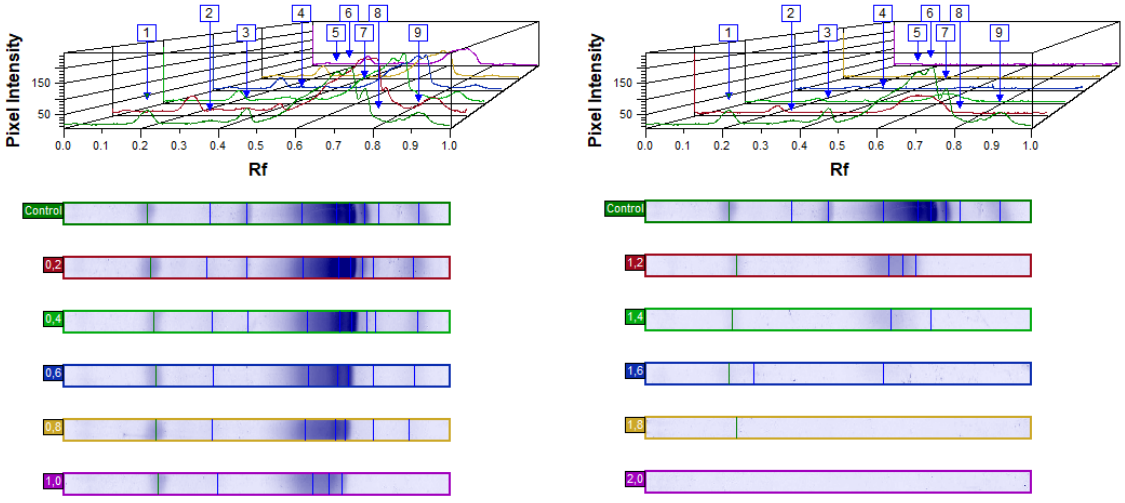
A.

Molecular weight, kDa	Bentonite dose, g.L^{-1}						
	0	0.2	0.4	0.6	0.8	1	1.2
165	-	-	100	-	-	-	-
99	-	-	100	-	-	-	-
65	-	-	100	-	-	-	-
61	100	0	85	-29	-100	-100	-100
53	-	-	100	-	-	-	-
43	100	0	-50	-21	-100	-100	-100
32	100	-16	-56	-100	-100	-100	-100
24	100	0	-51	-62	-93	-100	-100
22	100	-13	0	-68	-100	-100	-100
20	100	-14	-40	-66	-93	-100	-100
19	100	-50	-100	-100	-100	-100	-100
17	100	0	0	-100	-100	-100	-100
13	100	-25	-53	-100	-100	-100	-100

B.

Figure 3. Experimental data of wines treated with increasing doses of bentonite B1.
A-electrophoregrams; **B**-change in the percentage of protein fractions in experimental wines compared to the control

in the original wine (53, 65, 99 and 165 kDa), was observed, as well as an increase in the concentration (by 85 %) of some of those that had previously tended to decrease (61 kDa). A probable reason for these observations may be the electrostatic breakdown processes caused by bentonite with respect to high molecular weight proteins and/or complexes with protein participation, which could not have entered the separating gel and were later not visualized in the electropherogram. After detachment of smaller protein fragments, as a result of the increased dose of bentonite, some of them may have remained in solution and precipitated in the process of protein extraction and purification prior to the electrophoretic analysis. Interestingly, these proteins were removed from the wine with the next step of increasing the dose of bentonite (0.4 g.L^{-1}), probably due to their lack of stability as a separate unit.



A.

Molecular weight, kDa	Bentonite dose, g.L^{-1}										
	0	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2.0
61	100	0	-2	-2	-2	-15	-47	-64	-58	-62	-100
58	-	-	-	-	-	-	-	-	100	-	-
43	100	-1	-2	-35	-40	-45	-100	-100	-100	-100	-100
32	100	-57	-64	-100	-100	-100	-100	-100	-100	-100	-100
24	100	-6	-7	-8	-6	-27	-48	-60	-81	-100	-100
22	100	-10	-13	-31	-35	-60	-71	-100	-100	-100	-100
20	100	-13	-16	-35	-58	-73	-78	-100	-100	-100	-100
19	100	-47	-59	-100	-100	-100	-100	-100	-100	-100	-100
17	100	-2	-7	-46	-61	-100	-100	-100	-100	-100	-100
13	100	-3	-22	-48	-74	-100	-100	-100	-100	-100	-100

B.

Figure 4. Experimental data of wines treated with increasing doses of bentonite B2. **A**-electrophoregrams; **B**-change in the percentage of protein fractions in experimental wines compared to the control

The electropherograms of the series of wines treated with increasing doses of B2 bentonite are shown in Fig. 4.

Fractions with molecular weights of 19 and 32 kDa were the most amenable to the effects of B2 bentonite. Their concentration decreased by about and over 50 % even at the lowest applied dose (0.2 g.L^{-1}). With the increase of the dose to 0.4 g.L^{-1}, the decrease grew by about 10 % and at a dose of 0.6 g.L^{-1} they no longer appeared in the electropherogram. Compared to the observed effect on the one with a MW of 19 kDa, when treated with the previous bentonite (B1), its complete elimination was achieved via a one-step lower dose.

Of all the available fractions, the most difficult and slow was the reduction of those with molecular weights of 61 and 24 kDa. For this purpose, it was necessary to apply B2 bentonite in doses of 2.0 g.L^{-1} and 1.8 g.L^{-1} respectively. Interestingly, both proteins were relatively stable when treated with the previous B1 bentonite, but this time via using B2 bentonite their complete removal was achieved with doses almost twice as high. The first significant decrease in their concentration was observed after

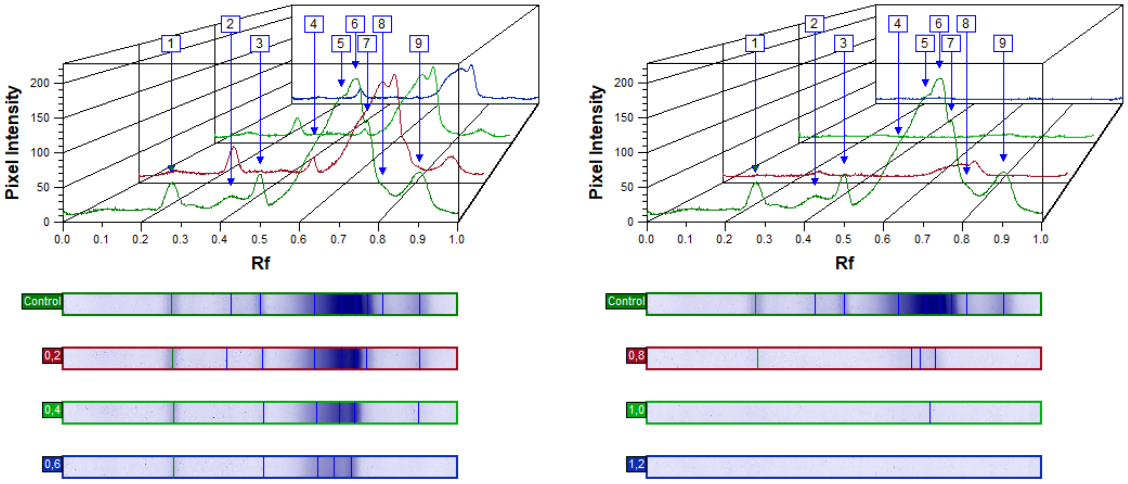
the introduction of B2 bentonite in doses of 1.2 and 1.0 g.L^{-1} respectively.

This is probably due to the different deproteinization capacity of the two bentonites. Their stability in comparison to the other fractions may be a result of their composition. Some scientific publications comment on the possible glycosylation of these fractions (Dambrouck et al. 2003; Fusia et al. 2010). It is possible that the glycosidic part affects their charge and their spatial structure, which could complicate the mutual flocculation with the oppositely charged bentonite or sorption by the latter.

The concentration of the remaining molecular fractions decreased more significantly with increasing the dose to and above 0.6 g.L^{-1}. Similar to the previous bentonite, the appearance of a new fraction was observed, which was missing in the initial protein fraction profile. This is the one with a MW of 58 kDa, appearing in the sample treated with a dose of 1.6 g.L^{-1}. Like the course already observed above, this fraction disappeared from the gel with the next step of increasing the dose to 1.8 g.L^{-1}.

The effect of the introduction of B3 bentonite in increasing doses to the protein fraction profile of the used Chardonnay wine is shown in Fig. 5.

The results show that B3 bentonite is most effective against protein fractions with MW of 17, 19 and 43 kDa.



A.

Molecular weight, kDa	Bentonite dose, g.L^{-1}						
	0	0.2	0.4	0.6	0.8	1	1.2
61	100	0	-32	-47	-69	-100	-100
43	100	-52	-100	-100	-100	-100	-100
32	100	-44	-67	-86	-100	-100	-100
24	100	-8	-31	-61	-79	-100	-100
22	100	-12	-27	-57	-89	-95	-100
20	100	-34	-46	-65	-86	-100	-100
19	100	-47	-100	-100	-100	-100	-100
17	100	-100	-100	-100	-100	-100	-100
13	100	-51	-78	-100	-100	-100	-100

B.

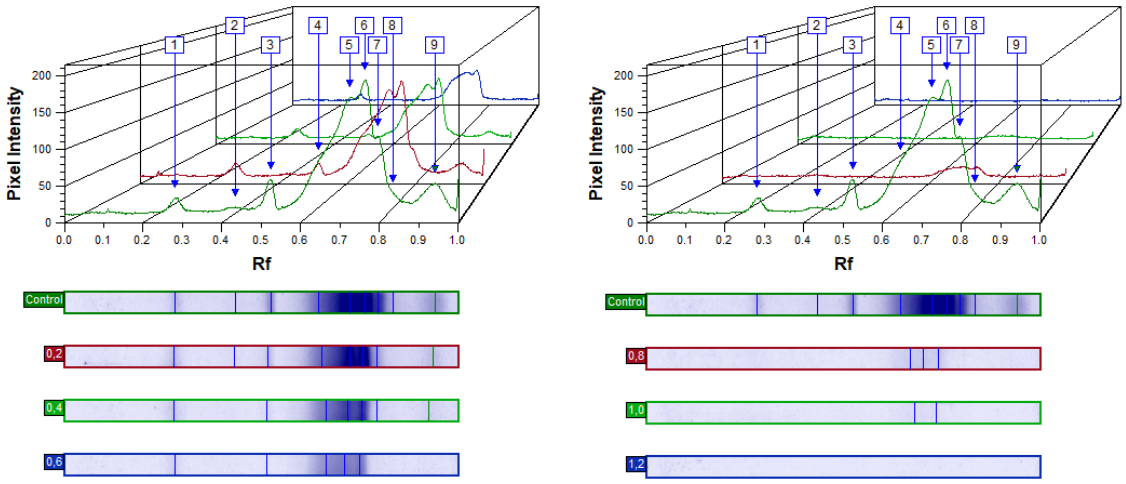
Figure 5. Experimental data of wines treated with increasing doses of bentonite B3. **A**-electrophoregrams; **B**-change in the percentage of protein fractions in experimental wines compared to the control

The first two have already been mentioned in the commentary on the previous bentonites. Probably their faster decrease is due to their lower concentration in wine.

The protein fraction with a MW of 22 proved to be the most difficult to remove. A dose of 1.2 g.L^{-1} was required for this purpose. The same effect was achieved with a one-step lower dose for B1 bentonite and a one-step higher dose for the one labelled as B2. With regard to the other fractions, the efficiency of B3 was comparable to that of B1 and there was a gradual decrease in its presence in the electrophoretic fraction profile.

All protein fractions were completely removed at a dose of 1.2 g.L^{-1}.

The electropherograms showing the change of the fraction profiles of the series of samples with added B4 bentonite are shown in Fig. 6.



A.

Molecular weight, kDa	Bentonite dose, g.L^{-1}						
	0	0.2	0.4	0.6	0.8	1	1.2
61	100	-4	-16	-38	-100	-100	-100
43	100	-36	-100	-100	-100	-100	-100
32	100	-50	-70	-79	-100	-100	-100
24	100	0	-39	-33	-73	-77	-100
22	100	-29	-39	-75	-91	-100	-100
20	100	-34	-46	-62	-87	-92	-100
19	100	-38	-70	-100	-100	-100	-100
17	100	-100	-100	-100	-100	-100	-100
13	100	-48	-73	-100	-100	-100	-100

B.

Figure 6. Experimental data of wines treated with increasing doses of bentonite B4.
A-electrophoregrams; **B**-change in the percentage of protein fractions in experimental wines compared to the control

The deproteinization capacity of B4 bentonite was most intense in the fractions with molecular weights of 17 and 43 kDa, followed by those with MW 13 and 19 kDa. The first one is completely eliminated at the lowest added dose, and the others via as follows: 0.4 and 0.6 $g.L^{-1}$. Like with B1 bentonite, it was the most difficult to remove fractions with MW 20 and 24 kDa. Moreover, the considered bentonite required a one-step higher dose-1.2 $g.L^{-1}$.

The amounts of the other fractions decreased relatively smoothly with the dose increase.

In the four series of samples, no direct relationship was observed between the results of the quantitative (Bradford Assay) and qualitative analysis (SDS-PAGE). In particular, the quantification method reacted and showed the presence (albeit small) of proteins in the studied wines treated with high doses of bentonite (1.2-3 $g.L^{-1}$), but proteins were not detected in electropherograms of the qualitative analysis. Probably this is due to the fundamental differences between the quantitative and qualitative analyses, as well as the peculiarities in the composition of wines. It is possible that the remaining amount of protein was in the form of complexes with other substances that are indestructible under prior isolation and purification conditions for SDS-PAGE, but, on the other hand, are degradable under alkaline conditions during pre-treatment before quantification using the Bradford assay.

Another possible option is that small amounts of very high molecular weight proteins (which could not have entered the pores of the gel) or those of lower molecular weight (which were not retained by the gel) were present in the samples and thus not visualized in electropherograms, but still interacted with the CBB-G250 stain and reacted during the quantitative method used for their determination.

Conclusions

More noticeable differences in the total amount of protein are observed at lower doses (up to 0.8 $g.L^{-1}$) and they may be related to the composition and deproteinization capacity of the different bentonites. At higher treatment doses, their effectiveness is almost commensurate.

The thesis that no direct link between the value of protein concentration and the colloidal stability of white wines should be sought has been confirmed.

The molecular fractions of the lowest concentration are most easily removed by the added bentonite, and this is again due to their deproteinization capacity. Not very intense, but still existing, affinity of different types of bentonites to different molecular fractions of proteins has been found.

References

Bakardzhiyski I., Chobanova D. Study of the protein profile of wines from a variety Traminer obtained by different technological modes of alcoholic fermentation. *Lozarstvo and Vinarstvo*, 2013, (61)1: 25-35. [in Bulgarian]

Bakardzhiyski I., Chobanova D., Bambalov K. Research on some important from an enological point of view of bentonite properties offered on Bulgarian market. *Food Processing Industry Magazine*, 2016, 65(10): 29-32. [in Bulgarian]

Bayly F., Berg H. Grape and wine proteins of white wine varietals. *American Journal of Enology and Viticulture*, 1967, 18(1): 18-32.

Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Celotti E., Barahona M., Bellantuono E., Cardona J., Roman T., Nicolini G., Natolino A. High-power ultrasound on the protein stability of white wines: Preliminary study of amplitude and sonication time. *LWT - Food Science and Technology*, 2021, 147(7): 111602. https://doi.org/10.1016/j.lwt.2021.111602

Chagas R., Ferreira L., Laia C., Monteiro S., Ferreira R., The challenging SO_2-mediated chemical build-up of protein aggregates in wines. *Food Chemistry*, 2016, 192(1): 460-469. https://doi.org/10.1016/j.foodchem.2015.07.052

Chagas R., Lourenço A., Monteiro S., Ferreira R., Ferreira L. Is caffeic acid, as the major metabolite present in Moscatel wine protein haze hydrolysate, involved in protein haze formation? *Food Research International*, 2017, 98(8): 103-109. http://doi.org/10.1016/j.foodres.2016.09.007

Chobanova D. Enology (exercise guide). Academic Publishing House of UFT-Plovdiv, 2007, Print ISBN 978-954-24-0082-0. [in Bulgarian]

Chobanova D. Study of the influence of phenolic compounds on the stability of white wines. PhD thesis by VIHVP, Plovdiv of Bulgaria, 1988. [in Bulgarian]

Dambrouck T., Marchal R., Marchal-Delahaut L., Parmentier M., Maujean A., Jeandet P. Immunodetection of proteins from grapes and yeast in a white wine. *Journal of Agricultural and Food*

Chemistry, 2003, 51(9): 2727-2732. https://doi.org/10.1021/jf0208435

Dorrestein E., Ferreira R., Laureano O., Teixeira A. Electrophoretic and FPLC analysis of soluble poteins in four portuguese wines. *American Journal of Enology and Viticulture*, 1995, 46(1): 235-242.

Dupin I., McKinnon B., Ryan C., Boulay M., Markides A., Jones G., Williams P., Waters E. Saccharomyces cerevisiae mannoproteins that protect wine from protein haze: Their release during fermentation and lees contacts and a proposal for their mechanism of action. *Journal of Agricultural and Food Chemistry*, 2000, 48(8): 3098-3105. https://doi.org/10.1021/jf0002443

Fukui M., Yokotsuka K. Content and origin of protein in white and red wines: changes during fermentation and maturation. *American Journal of Enology and Viticulture*, 2003, 54(3): 178-188.

Fusia M., Mainente F., Rizzi C., Zoccatelli G., Simonato B. Wine hazing: A predictive assay based on protein and glycoprotein independent recovery and quantification. *Food Control*, 2010, 21(6): 830-834. https://doi.org/10.1016/j.foodcont.2009.11.009

Hsu J., Heatherbell D. Heat unstable proteins in wine. I. Characterization and removal by bentonite fining and heat treatment. *American Journal of Enology and Viticulture*, 1987a, 38(1): 11-16.

Hsu J., Heatherbell D. Heat-unstable proteins in grape juice and wine. II. Characterization and removal by ultrafiltration. *American Journal of Enology and Viticulture*, 1987b, 38(1): 17-22.

Hsu J., Heatherbell D. Isolation and characterization of soluble proteins in grapes, grape juice and wine. *American Journal of Enology and Viticulture*, 1987c, 38(1): 6-10.

Hung W. Protein stabilisation of New Zealand Sauvignon blanc. PhD thesis by Lincoln University of New Zealand, 2010. Available on-line at: https://researcharchive.lincoln.ac.nz/bitstream/handle/10182/2366/hung_phd.pdf?sequence=3

Jackson R., Wine science. Principles and applications, Fifth edition, Elsevier, 2020, ISBN: 978-0-12-816118-0. https://doi.org/10.1016/C2017-0-04224-6

Jones-Moore H., Jelley R., Marangon M., Fedrizzi B. The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking. *Food Hydrocolloids*, 2022, 123(2): 107150. https://doi.org/10.1016/j.foodhyd.2021.107150

Koch J., Sajak E. A review and some studies on grape protein. *American Journal of Enology and Viticulture*, 1959, 10(1): 114-123.

Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970, 227(8): 680–685. https://doi.org/10.1038/227680a0

Marangon M., Stockdale V., Munro P., Trethewey T., Schulkin A., Holt H., Smith P. Addition of carrageenan at diff erent stages of winemaking for white wine protein stabilization. *Journal of Agricultural and Food Chemistry*, 2013, 61, 26(6): 6516-6524. https://doi.org/10.1021/jf401712d

Melnik I., Chebukin P., Bochevar R., Mitev P., Stoyanov N. The comparative characteristic of "ENOGRUP" bentonites for stabilization of white table wine. *Proceedings Biotechnologies and Food Technologies, University of Ruse Angel Kanchev*, 2015, 54(10.2): 82-86. Available at: https://conf.uni-ruse.bg/bg/docs/cp15/10.2/10.2-14.pdf

Morata A. White Wine Technology. Elsevier, 2022, ISBN: 978-0-12-823497-6 https://doi.org/10.1016/C2020-0-00376-2

Osorio-Macías D., Bolinsson H., Linares-Pasten H., Ferrer-Gallego R., Choi J., Penarrieta J. M., Bergenståhl B. Characterization on the impact of different clarifiers on the white wine colloids using asymmetrical flow field-flow fractionation, *Food Chemistry*, 2022, 381(7): 132123. https://doi.org/10.1016/j.foodchem.2022.132123

Owusu-Apenten R. Food protein analysis: quantitative effects on processing. Marcel Dekker, New York, 2002, 463 pages. pp. 195-220, Print ISBN: 0-8247-0684-6. https://doi.org/10.1201/9780203910580

Pargoletti E., Sanarica L., Ceruti M., Elli F., Pisarra C., Cappelletti G. A comprehensive study on the effect of bentonite fining on wine charged model molecules. *Food Chemistry*, 2021, 338(2): 127840. https://doi.org/10.1016/j.foodchem.2020.127840

Pocock K., Waters E. Protein haze in bottled white wines: How well do stability tests and bentonite fining trials predict haze formation during storage and transport? *Australian Journal of Grape and Wine Research*, 2006, 12(3): 212-220. https://doi.org/10.1111/j.1755-0238.2006.tb00061.x

Pocock K., Alexander G., Hayasaka Y., Jones P., Waters E. Sulfate-a candidate for the missing essential factor that is required for the formation of protein haze in white wine. *Journal of Agricultural and Food Chemistry*, 2007, 55(5): 1799-1807. https://doi.org/10.1021/jf062658n

Reynolds A. Managing Wine Quality. Elsevier, 2022, ISBN: 978-0-08-102066-1 (online). https://doi.org/10.1016/B978-0-08-102065-4.09991-0

Sauvage F., Bach B., Moutounet M., Vernhet A. Proteins in white wines: Thermo-sensitivity and differential adsorbtion by bentonite. *Food Chemistry*, 2010, 118(1): 26-34. https://doi.org/10.1016/j.foodchem.2009.02.080

Siebert K., Carrasco A., Lynn, P. Formation of protein-polyphenol haze in beverages. *Journal of*

Agricultural and Food Chemistry, 1996, 44(8): 1997-2005. https://doi.org/10.1021/jf950716r

Silva-Barbieri D., Salazar F., López F., Brossard N., Escalona N., Pérez-Correa J. Advances in White Wine Protein Stabilization Technologies. *Molecules*, 2022, 27(4): 1251. https://doi.org/10.3390/molecules27041251

Tattersall D., Van Heeswijck R., Høj P. Identification and characterization of a fruit-specific, thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. *Plant Physiology*, 1997, 114(3): 759-769. https://doi.org/10.1104/pp.114.3.759

Van Sluyter S, Warnock N., Schmidt S., Anderson P., Van Kan J., Bacic A., Waters E. Aspartic acid protease from botrytis cinerea removes haze-forming proteins during white winemaking. *Journal of Agricultural and Food Chemistry*, 2013, 61(40): 9705-9711. https://doi.org/10.1021/jf402762k

TotalLab, trial version. Available at: www.totallab.com