

IMPACT ON UV-VISIBLE SPECTROSCOPY PARAMETERS OF TAMAIOASA ROMANEASCA WINES FROM MUSTS CLARIFIED WITH PEA PROTEIN BASED FINING AGENTS

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Abstract

Pea protein is a plant-based fining agent recently approved for the clarification of musts and wines. Vegetal proteins are intended to replace the classical fining agents based on proteins of animal origin or the synthetic polymer polyvinylpolypyrrolidone (PVPP), which both proved very efficient for partial removal of polyphenols, but are less accepted by vegetarian or eco-friendly consumers. As an alternative, pea protein can be used for clarification either alone or in complex products containing other non-animal materials. This paper focuses on the evaluation of several pea protein based fining agents used to clarify the must of Tămăioasa românească, an aromatic grape variety which is vinified with a short maceration, leading to wines with a higher content of polyphenols. Variants with no fining as well as PVPP fining were also produced. For all fining variants, the clarification was performed both with oxygen protection and in the presence of oxygen. UV-visible spectroscopy was used to determine parameters related to the content of phenols in the resulted wines (total phenol index as OD 280 nm, flavonoids as OD 365 nm, CIELab parameters and colour differences), after must clarification and completion of the fermentation.

Key words: white wine; pea protein fining agents; CIELab, total phenol index

INTRODUCTION

One new trend in food and beverages is a movement towards less processed products based on natural ingredients. Moreover, vegetarians and vegans demand for their wines that no animal product be used in winemaking (Goodman, 2023). Therefore, even fining agents, which technically are adjuvants and do not remain in the final wines (Kemp et al., 2022), have come into question, when they are of animal or synthetic origin. This is the case with PVPP (polyvinylpolypyrrolidone), a much appreciated synthetic polymer of pyrrolidone (Haaf et al., 1985), which is useful for removing fast some of the small polyphenol molecules, such as leucoanthocyanidins and catechins (Donner et al., 1993; Laborde et al., 2006), which are prone to oxidation and cause "browning" or "pinkening" (Gil et al., 2017; Cojocaru and Antocea, 2019; Ugliano et al., 2021). PVPP is also known for reducing bitterness and astringency, through the same mechanism of polyphenol removal, even though it does not act on large polyphenol

molecules, such as tannins. For these large polyphenol molecules, proteins are more effective fining agents (Cosme et al., 2008), especially the ones of animal origin, which were traditionally used and proved in time. Nowadays, there is interest in replacing PVPP and animal proteins with natural/vegan alternatives (Cosme et al., 2012; Versari et al., 2022), more acceptable from the viewpoint of many consumers. For this reason, several studies were recently done attempting to replace PVPP with vegetal proteins such as those from rice, potato, soy or pea (Gambutti et al., 2012; Kang et al., 2018; Marangon et al., 2019; Cojocaru and Antocea, 2022), as well as anorganic materials, such as activated carbon or bentonite. Some of these materials, used in single treatments, can lead to a reduction of other desirable compounds in beverages (Seriš et al., 2024).

There are variations in the polyphenol removing efficiency of these materials (Río Segade et al., 2020), as well as effects on aroma (Lambri et al., 2010; Vincenzi, et al., 2015) and colour (González-Neves et al.,

2014), therefore tests are necessary to determine the optimum agents or combination of agents for each type and style of wine.

Regarding the allergenic potential, proteins of either animal or vegetal origin may be a concern, but in wine fining it was proven that usually the final wines do not contain allergenic proteins (Peñas et al., 2015). Moreover, plant proteins have a much lower allergenic potential and for this reason pea and potato protein fining agents need not be mentioned on the labels in accordance to the legislation of EU (Peñas et al., 2015; EGTOP, 2015).

In this study the d/or inorganic materials (activated carbon, bentonite).

The tests were performed on the must of the aromatic white variety Tamăioasa românească, which is alternatives tested were based on pea protein, combined also with two other materials. The two extra materials could be of non-animal organic materials (yeast hulls, chitosan) an known for having higher loads of polyphenols transferred from the seeds and skins, due to the maceration process carried out for aroma extraction (Stoica and Gheorghita, 2008).

MATERIALS AND METHODS

The grapes of Tamăioasă românească variety were harvested on September 11th, 2023 from Pietroasa wine region. At harvest the grapes had the parameters specific for the production of a quality wine: sugars 22.61 °Bx; glucose+fructose 213.1 g/l; density 1.0941 g/ml; pH=3.97; total acidity 5.52 g/l; volatile acidity 0 g/l; malic acid 2.33 g/l; lactic acid 0 g/l; tartaric acid 2.82 g/l; extract 33.7 g/l; assimilable 233 mg/l, ethanol 0.15%; polyphenols 862 mg/l, potassium 2617 mg/l.

The grapes were processed in the same day, starting with the destemming and crushing. The free-run must, which resulted from the grapes pressed in a pneumatic horizontal wine press after a short enzyme maceration of 6 h, was treated for the partial removal of the polyphenols with various fining agents. Volumes of 40 l of must were used for each variant and repetition. For the reductive process, the treatment, the racking from the deposit and the subsequent winemaking was

performed in stainless steel tanks of 50 l, while glass demijohns were used for the oxidative process.

For the reductive process 3 repetitions were prepared for each variant. For the sake of comparison with a less controlled process, in which the presence of more oxygen is unavoidable, for each variant a single repetition in demijohns was prepared, too. The treatments were similar for the must in both type of recipients, as described in Table 1.

Table 1. Variants of must treatments and the fining agents used alone or in combinations

Variant name	PVPP	Pea protein (P)	Chitosan (K)	Yeast hulls (Y)	Carbon (C)	Bentonite (B)
V0	-	-	-	-	-	-
PV	☑	-	-	-	-	-
PP	-	☑	-	-	-	-
PYB	-	☑	-	☑	-	☑
PCB	-	☑	-	-	☑	☑
PCY	-	☑	-	☑	☑	-
PKC	-	☑	☑	-	☑	-
PKY	-	☑	☑	☑	-	-

Each treatment consisted of a dose of 20 g/hl of a fining agent or a combination of them. The fining agents used in this study are commercially available. PVPP SMARTVIN, chitosan Kitosmart and active carbon Acticarbone 2SW are from Enologica Vason (Italy), pea protein Proveget 100 is purchased from Agrovin (Spain), yeast hulls OENOLEES and calcium bentonite Microcol CL G are from Laffort (France).

After keeping the samples 24 h in the presence of the fining agents, each recipient was racked, separating the lees from the limpid must. Each variant and repetition were then inoculated with 25 g/hl *Saccharomyces cerevisiae* yeast and let for 10-14 days to complete fermentation to dryness. Fermentation activators were added in the beginning of the fermentation and again after 3 days of fermentation. In the stainless steel tanks the temperature was controlled and kept at around 15°C during the entire period of fermentation. In the demijohns, even kept in a cool room, the temperature fluctuated between 15 and 20°C. After the fermentation ceased, the wines were racked and sulfited with 50 mg/l sulfur dioxide. After one week the wines were racked again and analyzed.

Total phenolic index (TPI) was determined by measuring the optical density (absorbance) at

280 nm, the wavelength which is absorbed by the phenolic rings, especially from flavonoids such as catechins and condensed and hydrolysable tannins (Harbertson and Spayd, 2006). Flavonols, such as quercetin and kaempferol, absorb and can be estimated by measuring the optical density at 365 nm (Harbertson and Spayd, 2006).

The colour CIELab parameters were determined in accordance to the OIV method (OIV, 2021).

For absorbance and color measurements a UV-visible spectrophotometer Specord 250 (Analytik Jena, Germany) was used. Quartz cuvettes were used for the UV determinations and glass cuvettes for the visible spectrum determinations. For absorbance determinations a dilution of 10 was applied, therefore the final result was multiplied by the dilution factor. Whenever a cuvette with a smaller path length was used, the final result was also multiplied to adjust the final result for a cuvette of a 1 cm path length. For CIELab data acquisition and analysis the software WinAspect version 2.2.7 (Analytik Jena, Germany) was used.

CIELab parameters and their significance are described in detail in a previous paper (Antoce et al., 2022). Total colour difference (ΔE) was determined in accordance to the formula: $\Delta E = ((L_c - L_s)^2 + (a_c - a_s)^2 + (b_c - b_s)^2)^{1/2}$, where c=control and s=sample.

Statistical analysis was performed with the software package Origin 2018 (OriginLab, USA), applying, where appropriate, the statistical methods of Principal Component Analysis (PCA), one-way ANOVA, two-way ANOVA and post-hoc Tukey test.

RESULTS AND DISCUSSIONS

To estimate the impact of treatments on the overall polyphenol load, first the total phenolic index (TPI) was determined. For differences between samples within the same conditions (TPI_{red} and TPI_{ox}, respectively), one-way ANOVA was used for the calculations and Tukey test was applied for comparison of means. The differences induced by treatments and conditions, respectively, were calculated using two-way ANOVA and mean comparison by Tukey test at $p < 0.05$. This analysis has confirmed that there is a significant difference

between the total phenolic index of wines obtained in reductive and oxidative conditions, respectively. The differences between treatments, irrespective of the winemaking conditions, are shown in Table 2.

Table 2. Total phenolic index of wines made from must samples fined in reductive and oxidative conditions

Variant	TPI _{red}	TPI _{ox}	TPI differences induced by treatments, irrespective of winemaking conditions
	One-way ANOVA		Two-way ANOVA
V0	10.94±0.0003 ^a	12.95±0.016 ^a	a
PV	9.85±0.003 ^b	12.26±0.011 ^b	b
PP	10.31±0.006 ^c	13.50±0.014 ^a	c
PYB	10.65±0.004 ^d	12.73±0.018 ^a	c
PCB	10.35±0.002 ^c	12.24±0.013 ^b	bc
PYC	10.36±0.005 ^c	12.85±0.020 ^a	ac
PKY	10.57±0.002 ^d	11.36±0.009 ^b	bc
PKC	10.69±0.002 ^d	11.80±0.083 ^b	bc

Average values ± standard deviations and Tukey test for mean comparison ($p < 0.05$); different letters represent significant differences between variants.

As it can be observed, all the applied treatments, irrespective of the winemaking conditions, have the ability to partly reduce the polyphenols contained in the Tămăioasa românească must, leading to wines with lower TPI compared to the control V0, which was not treated with any fining agent.

However, differences are also observable among the variants with specific fining agents or combinations. The treatments with PVPP, irrespective of the winemaking conditions, proved to be the most effective, significantly reducing the TPI, compared to all the other variants applied. In the used total dose of 20 g/hl, pea protein and its combinations with other fining agents reduced the TPI compared to control, but not as much as the PVPP in the same concentration.

As expected, the most obvious difference in the final wine TPI was induced by the winemaking conditions, the presence of oxygen leading to higher TPIs in all similarly-treated variants (Figure 1). It also proves that the oxidative conditions were not enough to lead to the precipitation of the oxidized polyphenols, which remained in the final wine, affecting the overall quality.

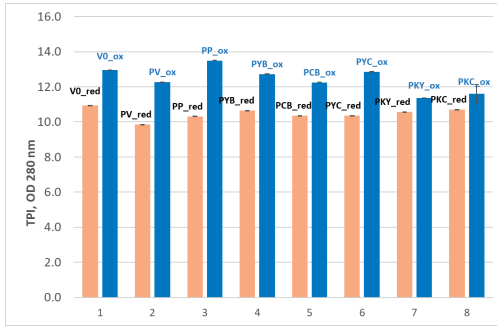


Figure 1. Total phenolic index (TPI) for wines resulted from must treated with various fining agents and combinations, in reductive (orange bars) and oxidative (blue bars) conditions

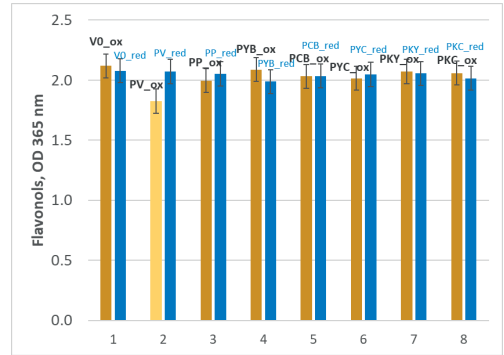


Figure 2. Flavonols content estimated as absorbance at 365 nm for the wine samples resulted from must treated with various fining agents

For the estimation of the flavonols in the final wines, it was similarly observed that the PVPP is the most effective in reducing the concentration of these compounds, compared with the same dose of pea protein and its combinations with other fining agents (Figure 2).

For an overall idea of how the treatments and winemaking techniques influence the wine colour, all the CIELab parameters were determined and are presented in Table 3.

Table 3. Colour CIELab parameters of wine must samples obtained from musts fined in reductive and oxidative conditions and differences calculated based on CIELab parameters *L*, *a* and *b*

Variant	<i>L</i> _{red}	<i>L</i> _{ox}	<i>a</i> _{red}	<i>a</i> _{ox}	<i>b</i> _{red}	<i>b</i> _{ox}	<i>c</i> _{red}	<i>c</i> _{ox}	<i>h</i> _{red}	<i>h</i> _{ox}	ΔE_{red}	ΔE_{ox}
V0	96.6±0.0	93.1	0.2±0.0	-0.3	8.9±0.0	15.3	8.9±0.0	15.3	1.5±0.0	-1.6		
PV	95.8±1.1	87.1	0.1±0.4	4.2	8.6±0.5	21.8	8.6±0.5	22.2	0.0±2.2	1.4	1.80±0.02	9.95
PP	95.7±1.2	87.5	0.3±0.1	5.0	8.3±0.2	22.6	8.3±0.2	23.1	1.5±0.0	1.4	1.81±0.63	10.63
PYB	96.4±1.3	90.3	0.1±0.1	3.2	8.7±0.1	20.7	8.7±0.1	20.9	0.5±1.8	1.4	1.81±0.52	7.04
PCB	95.8±1.4	93.7	0.1±0.1	2.0	8.4±0.7	13.6	8.4±0.7	13.7	0.5±1.8	1.4	1.27±0.05	2.88
PYC	97.4±0.0	85.8	0.2±0.0	4.7	7.7±0.0	21.4	7.7±0.0	21.9	1.5±0.0	1.4	0.73±0.02	10.80
PKY	94.5±0.0	93.0	0.6±0.0	0.9	9.3±0.0	17.2	9.3±0.0	17.2	1.5±0.0	1.5	0.73±0.04	2.29
PKC	96.6±0.0	89.6	0.2±0.0	3.6	8.5±0.0	21.5	8.5±0.0	21.8	1.5±0.0	1.4	1.17±0.02	8.11

Average values ± standard deviations

Luminosity *L* was not significantly affected by the treatment under reductive conditions, while some differences appeared in oxidative conditions, but mainly due to the delay in sedimentation of the particles in suspension. Based on the CIELab parameters, the main difference between the oxidative and reductive winemaking is determined by the luminosity *L*, as it is shown by a Principal Component Analysis in which all reductive samples are grouped towards a lower impact of luminosity, because the samples are better clarified and *L* values are higher (Figure 3).

The extracted eigenvectors (Table 4) show that all the CIELab parameters are included in the component PC1, which accounts for 85.59% of the variance, but the Luminosity has an opposite impact (negative values) as compared

to the parameters which relate to the actual colour (positive values).

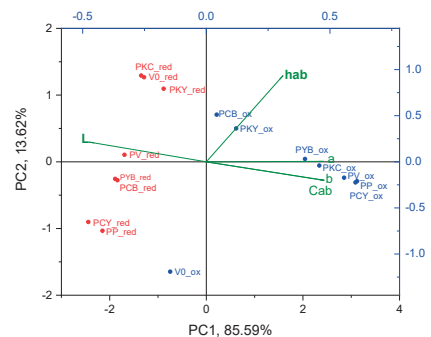


Figure 3. Principal Component Analysis plot based on CIELab parameters for wines resulted from must treated with various fining agents and combinations in reductive (red) and oxidative (blue) conditions.

Table 4. The extracted eigenvectors for the CIELab parameters

	Coefficients of PC1	Coefficients of PC2
L	-0.47379	0.21372
a	0.47418	0.00502
b	0.47604	-0.20116
C_{ab}	0.47722	-0.20007
h_{ab}	0.31036	0.93478

The other Principal Component, PC2, representing 13.62% of the total variance, is related mostly to the hue of the samples (h_{ab}). This means that the samples VO_{red} , PKC_{red} , PKY_{red} and PCB_{ox} and PKY_{ox} are associated with the deepest hues. This shows also the impact of the chitosan on the colour of the final wines, which retain generally more colour than the rest of the wines for which other fining treatments were used.

The effect on the colour is also more important when the oxidative winemaking is performed. This is easily observed in the colour space formed by the parameters a (variation of color from green to red) and b (variation between blue and yellow), where the samples produced in reductive conditions are clearly separated by the rest of the samples, having the lowest yellow and red components (Figure 4). The positive values of parameter a indicate that the colour is a shade of yellow and the positive values of parameter b indicate that the colour contains some red shades, but considering the very small values, these should be interpreted as giving the wine a brownish shadow, not red.

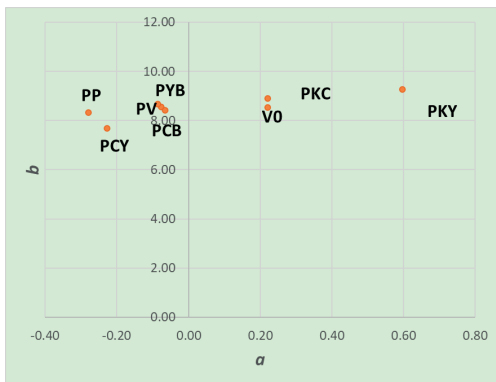


Figure 4. Placement in the colour space a (green to red) vs b (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in reductive (orange dots) and oxidative (blue dots) conditions

The effect of the treatments on the colour of the samples produced in a reductive way is low, the group of samples in Figure 4 being rather compact. However, the oxidative winemaking led to higher yellow and red components, with some differences among samples. The control oxidative sample was in this case the closest to the reductive samples group, showing that the fining treatments are not helping with regard to the quality of the final wines when winemaking is made in the presence of oxygen. Samples treated with pea protein in combination with chitosan and yeast (PKY) were also of a better colour in case of the oxidative conditions, as well as the samples treated with pea protein in combination with activated carbon and bentonite (PCB), which probably partially absorbed some of the oxidized components.

In order to differentiate the influence of the treatment type on the colour, in Figures 5 and 6 the samples vinified in reductive and oxidative conditions, respectively, are plotted in the space a vs. b .

For the samples prepared in reductive conditions (Figure 5) it can be observed that the most coloured, with a higher shade of red (giving overall a brownish shade) are the control wines, with no treatment and the wines obtained from musts treated with combinations of pea protein and chitosan (PKC and PKY). Taking into account the fact that the rest of combinations based on pea protein did not have positive values for the red shade, we can say that the chitosan is the fining agent responsible for adding this slight shift in the colour.

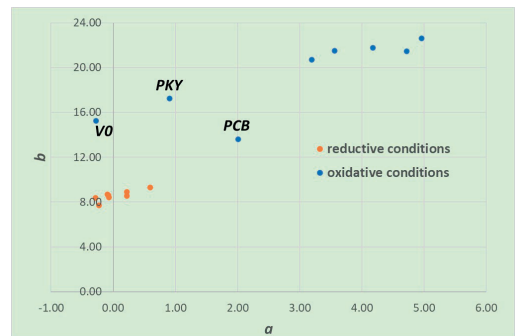


Figure 5. Placement in the colour space a (green to red) vs. b (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in reductive conditions

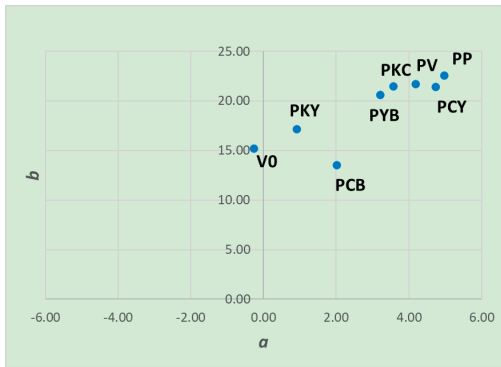


Figure 6. Placement in the colour space *a* (green to red) vs. *b* (blue to yellow), of the wine samples resulted from must treated with various fining agents and combinations in oxidative conditions

For the samples prepared in oxidative conditions (Figure 6) the red-brownish shadow is present in all samples (small positive values for parameter *a* in all of them), irrespective of the type of fining agent. Thus, it can be said that, under these conditions, the treatment cannot influence too much the colour.

As for the chromaticity, it increases by a factor of 2-3 for the samples obtained in demijohns (*c* parameter being between 13.7-23.1) as compared to the ones produced in stainless steel tanks (*c* parameter between 7.7 and 9.3).

The overall colour difference measured for each sample against the control showed small differences for samples prepared in reductive conditions, but still noticeable for various treatments. Thus, a visible difference in colour, compared to control, is present for the samples treated with PVPP (PV), pea protein (PP) and the combination pea protein, yeast and bentonite (PYB), all having the calculated ΔE around 1.8, the differences being hardly perceivable for the rest of the samples (ΔE between 0.73-1.27). For the oxidative winemaking the differences in colour were very visible, some of them with ΔE over the value of 5, being clearly of another colour, here signifying uncontrolled oxidation. The only samples produced in demijohns which maintained a non-oxidized colour were those with values of ΔE up to 3, which is the case for the fining combinations PCB and PKY. These are the same samples with the ones underlined in Figure 3, due to the difference of red shade incorporated in parameter *a*.

CONCLUSIONS

Fining treatments based on pea protein achieve a partial removal of phenolic compounds and can be a replacement for the use of synthetic PVPP. To ensure quality of the final wine, the treatments and winemaking process need to be controlled and under reductive conditions.

In the presence of oxygen, the oxidized polyphenols are not sufficiently removed by any of the fining agents. These oxidized polyphenols also significantly change the colour of the final wine. Thus, in oxidative conditions, irrespective of the fining treatment, the values for TPI are higher, along with the values for *a* and *b* parameters of CIELab.

In reductive conditions and for the doses of the fining agents of 20 g/hl, the higher reduction of TPI and flavonoids is still achieved by the treatment with PVVP, which is more effective than all the other treatments. Good TPI and flavonoids reductions are possible by using pea protein (PP) or combinations of pea protein with activated carbon (PCB and PYC). Therefore, in case the wine is addressed to consumers who avoid synthetic products or are environmentally minded citizens, pea protein and its combinations with activated carbon represent suitable alternatives. Further tests are, however, required to determine the sensory impact and the effect of all these treatments on the aromatic compounds.

ACKNOWLEDGEMENTS

This work was supported by a grant of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, project number 849, acronym Fine4Pietroasa, within IPC 2023.

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