Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 24, Issue 2, 2024
PRINT ISSN 2284-7995, E-ISSN 2285-3952

TESTING THE POTENTIAL OF INNOVATIVE TREATMENTS OF WHITE GRAPE MUST WITH VEGETAL PROTEINS – SENSORY IMPACT ON WINE

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Abstract

In white wine production, for some grape varieties, it is beneficial to technologically reduce the concentration of the polyphenols to make the final wine less bitter and astringent. Normally, the removal of excessive polyphenols is addressed by fining the wine with polyvinylpolypyrrolidone (PVPP), a synthetic polymer, or animal proteins, which bind the tannins. However, the nowadays trend is to replace the products of animal or synthetic origin with vegetal or inorganic ones. In this paper innovative technologies based on treatments with pea protein, alone and in combinations with other vegetal or inorganic products, were tested. Also, as another innovation, the treatments were performed directly on the must, to remove some of the polyphenols before they can be oxidized. Six variants of Tamâioasa romaneasca, treated with pea proteins and combinations of agents, were compared with a non-treated variant and with the classical treatment with PVPP. The resulting wines were evaluated by professional tasters based on a complex sensory evaluation sheet and sensory profiles were determined for all variants. Multivariate statistics analysis was also applied to determine the most promising alternative treatments acceptable for the wine consumers.

Key words: *innovative wine fining, pea protein, wine consumers, wine sensory profile*

INTRODUCTION

White wines, especially those produced with maceration, as it is the case of aromatic wines, have often too many bitter substances extracted from the skins and seeds, most of them polyphenols. To remove some of the polyphenols and reduce the bitterness a very good oenological product is the polyvinylpolypyrrolidone (PVPP) [6, 18, 5]. Due to its synthetic origin [18], PVPP is a controversial oenological product for which alternatives are actively sought. As it acts by absorption of polyphenols in a similar way as proteins [8], animal and vegetal proteins are the first choice for its replacement, with the vegetal proteins [11] being the most acceptable alternative. The newest researches have focussed on several vegetal alternatives, of which some are already accepted for use including by the OIV [13], namely the potato [3] and pea protein. However, besides the proteins, chitosan, a polysaccharide used for protein haze prevention [14] and other purposes [21], also proved to be effective on removing some types of polyphenols [4, 19]. Chitosan is also approved for the use in oenology as long as it is not obtained from crustaceans, but from fungi, such as *Aspergillus niger* [7] or *Agaricus bisporus* [12], in this way being of a vegetal origin.

Combinations of these new materials with other already consecrated materials such as bentonite or activated carbon are also actively tested in several laboratories.

In the present paper pea protein was tested as an alternative for removing bitterness from an aromatic wine of Tamaioasa romaneasca. Several combinations of pea protein and other approved oenological materials were applied and compared with the classical non treated or PVPP treated variants. The main focus was to determine how these treatments affect the sensory perception of the resulted wines, as each of these substances can remove different compounds, thus potentially changing the overall sensory profile. Applying alternative technologies is crucial to modulate the wine quality and attract more consumers in a market in which production and consumption face various challenges [17].

MATERIALS AND METHODS

The oenological materials used in this study were purchased from authorized providers of oenological materials. Thus, the pea protein Proveget 100 is from Agrovin (Spain), PVVP SMARTVIN, chitosan Kitosmart andactive carbon Acticarbone 2SW are from Enologica Vason (Italy), and calcium bentonite Microcol CL G and yeast hulls OENOLEES are from Laffort (France).

To produce the variants, the following combinations of materials were used (Table 1):

Table 1. Variants and oenological materials used for treatments

Variant code	Pea protein (PP)	Chitosan (K)	Yeast hulls (Y)	Carbon (C)	Bentonite (B)	PVPP
V0	-	-	-	-	-	-
PV	-	-	-	-	-	√
PP	√	-	-	-	-	-
PPYB	√	-	√	-	√	-
PPCB	√	-	-	√	√	-
PPCY	√		√	√	-	-
PPKY	√	√	√	-	-	-
PPKC	√	√	-	√	-	-

Source: Own experimental design.

A total dose of 20 g/hL oenological materials was used for each variant and repetition. Three repetitions were produced for each experimental variant in stainless steel tanks of 50 l volume.

The treatments were applied directly in the must obtained from Tamaioasa romaneasca grapes, harvested on October 16, 2023. The main parameters of the must, determined in accordance to the OIV methods [15], before treatments, were: density = 1.111 g/mL, fermentable sugars 246 g/L, total acidity 4.28 g/L as tartaric acid, pH= 3.66, acetic acid 0.04 g/L, L-malic acid 0.7 g/L and polyphenols 526 mg/L. For the clarification and polyphenol removal the materials were allowed to stay in contact with the must for 24 hours, after which the must was racked and inoculated with the same *Saccharomyces cerevisiae* yeast (Arome plus Fermol AEB yeast). After 2 weeks of fermentation at 14ºC the resulted wines were racked again, treated with a dose of 50 mg/L sulfur dioxide for antioxidant protection and bottled in glass recipients of 0.75 L. A month after bottling the wines were submitted to sensory analysis using a method patented in our laboratory [1]. Based on this method, which was fully explained in previous papers [2], the main taste, visual and aroma descriptors were evaluated on intensity scales from 0-10 by a team of professional tasters. Where possible, detailed aroma descriptors were also provided by the wine tasters. Scores on an evaluation sheet of 100 points were also allocated to each wine (variants and repetitions).

The results collected from the wine tasters were analyzed with appropriate statistical methods (ANOVA, post-hoc Tukey test and PCA) using the software packages Origin 2018 (OriginLab, USA).

RESULTS AND DISCUSSIONS

Influence of oenological treatment on main sensory parameters

To determine the sensory influence of the various fining treatments, the results collected on evaluation sheets from each taster for each experimental variant and repetition were gathered in a database and statistically analysed.

For the main taste parameters (acidity, sweetness, astringency, bitterness and extract perceptions) and for the perception of colour intensity the results are included in Table 2.

As it can be seen, the perception of acidity is inversely correlated with the perception of sweetness.

The wine variants which did not receive any treatment were perceived as sweeter and less acid than others, which shows that in the absence of a good clarification by any type of fining, the fermentation was not completed up to dryness.

Table 2. Main taste and visual sensory parameters of experimental wines (values on intensity scales up to 10)

Wine sample groups	**Taste related and visual parameters**					
	Acidity	**Sweetness**	**Astringency**	**Bitterness**	**Extract**	**Colour intensity**
V0	2.4±0.5^{a}	4.0±1.9^{a}	2.2±0.7^{a}	4.5±0.8^{a}	6.0±0.0^{a}	5.7±1.3^{a}
PV	3.5±0.3^{b}	2.1±0.3^{b}	1.8±0.3^{ac}	4.3±0.6^{a}	5.8±0.3^{a}	3.8±0.6^{b}
PP	3.2±0.3^{bc}	2.4±0.2^{b}	1.7±0.3^{ac}	2.2±0.6^{b}	5.3±0.3^{b}	4.5±0.4^{bc}
PPYB	2.8±0.3^{ac}	2.5±0.3^{b}	1.8±0.4^{ac}	2.3±0.5^{b}	6.6±0.1^{c}	4.9±0.3^{abc}
PPCB	3.6±0.5^{b}	2.2±0.3^{b}	2.5±0.4^{b}	3.1±0.4^{b}	6.0±0.1^{a}	4.3±0.5^{b}
PPCY	3.7±0.5^{b}	2.2±0.3^{b}	1.7±0.5^{ac}	2.5±1.2^{b}	5.1±0.1^{b}	5.0±0.0^{ab}
PPKY	3.0±0.0^{ac}	3.0±0.0^{a}	1.3±0.5^{c}	2.8±0.7^{b}	4.7±0.5^{b}	5.7±0.8^{ac}
PPKC	3.5±0.0^{b}	3.5±0.0^{a}	1.0±0.0^{c}	1.3±0.4^{c}	4.3±0.5^{b}	4.8±0.7^{abc}

[*] Average values ± standard errors (n=3). The letters show the statistical difference among results for p<0.05. For the same compound, common letters for 2 or more variants show no significant difference among them. Post-hoc analysis was performed using the Tukey test.
Source: Own results.

Table 3. Aroma sensory parameters of experimental wines (values on intensity scales up to 10*)

Wine sample groups	**Aroma related parameters**					
	Aroma	**Flower**	**Fruits**	**Vegetal**	**Spicy/Burnt**	**Complex**
V0	2.3±0.5^{a}	1.0±0.9^{a}	1.7±1.4^{a}	2.8±1.0^{a}	4.7±0.5^{a}	1.7±0.8^{a}
PV	3.3±1.5^{ab}	1.2±0.4^{a}	2.8±1.2^{ab}	3.8±1.0^{a}	3.8±0.4^{a}	1.6±0.5^{a}
PP	5.2±0.3^{bc}	2.7±0.5^{b}	5.5±0.5^{bc}	4.3±0.5^{b}	5.0±0.0^{b}	2.8±0.8^{b}
PPYB	5.0±0.4^{b}	0.7±0.5^{ac}	3.3±2.0^{ab}	3.0±0.0^{a}	3.7±0.5^{b}	0.3±0.5^{c}
PPCB	4.3±0.5^{b}	0.0±0.0^{c}	4.3±1.4^{b}	2.7±0.5^{a}	2.3±0.5^{c}	0.3±0.5^{c}
PPCY	5.3±0.3^{ab}	2.0±0.0^{b}	3.7±0.5^{abc}	2.7±0.5^{ab}	2.7±0.5^{c}	2.3±0.5^{ab}
PPKY	4.3±0.3^{ab}	0.2±0.4^{ac}	5.3±0.5^{bc}	3.3±0.5^{ab}	3.3±0.5^{b}	1.5±0.8^{a}
PPKC	3.8±3.0^{ab}	0.2±0.4^{ac}	5.7±0.5^{c}	3.3±0.5^{ab}	2.7±0.5^{c}	1.2±0.4^{a}

[*] Average values ± standard errors (n=3). The letters show the statistical difference among results for p<0.05.For the same compound, common letters for 2 or more variants show no significant difference among them. Post-hoc analysis was performed using the Tukey test.
Source: Own result.

The samples containing also chitosan (PPK) were perceived as sweeter too, similarly to the control wines, but their acidity was maintained as in the rest of the PP treated samples. This sensation of sweetness is perhaps induced by the chitosan itself, which has the ability to mask some tastes, blocking taste receptors, especially those detecting bitterness [22]. For the astringency, as well, the samples containing PP and chitosan (PPKY, PPKC) were the least astringent, while the control samples were the most astringent. Bitterness, however, was lowest in the PPKC samples (containing also activated carbon in the fining combination), while he PPKY (containing also yeast hulls in the fining combination) and the rest of the PP-containing samples had medium bitterness. The highest bitterness was present, as expected, in the control samples, but also in the samples treated with PVPP. As explained, chitosan has the ability to mask the bitter taste, by blocking the nucleophilic groups of the bitter substances, hindering their reaction with the bitter taste receptors [22]. In PKKY samples this mechanism may be affected by the presence of yeast hulls, which also have nucleophilic groups which can bind the chitosan. This assumption is based on the documented fact that one of the antifungal mechanisms of chitosan is binding with the yeast cell wall [10].

It is also noticeable the lowest extract perception in the samples also containing chitosan in the fining combination with PP.

Concerning the rest of the samples treated with PP or combinations based on PP, the main sensory parameters are, in most cases, significantly different than those of the control samples, showing that the application of PP has good overall influence on the sensorial quality of the wine.

The samples treated with the classical PVPP are different than the control, as the clarification induced in must permitted a good fermentation, similar with the PP treatments (thus, acidity and sweetness are not significantly different than in the case of PP treatments). The most obvious change induced by PVPP is in the colour intensity, which is significantly and positively affected, showing that oxidizable polyphenols were removed in accordance to the documented mechanism of PVPP [5]. However, in our case, the expected effect of PVPP on astringency and bitterness was not significantly different as compared to control, being outperformed by almost all treatments with PP.

Aroma intensity (Table 3) as compared to the unclarified control samples proved to be higher, for all fining treatments, the samples with PVPP and the PPKC being slightly lower than the rest, the latter being maybe the effect of the activated carbon present in the fining combination.

The aroma components, split in several classes which contribute to the overall aroma sensation, presented many differences which are included in Table 3.

The flowery terpenic aroma, typical for this variety, was better preserved in the samples treated only with PP, and also in PPCY. Control and PVPP both had medium intensity flower aroma, while the samples treated with bentonite (PPCB, PPYB) or chitosan (PPKY, PPKC) in addition to PP had significantly lower flower aroma. However, the samples containing chitosan (PPKY, PPKC) and PP alone showed the highest intensity of fruity aroma, significantly increased as compared to control and PVPP samples. When used alone, PP increased the vegetal aroma as compared to combinations and even with control and PVPP samples. This vegetal not was not “beany”, typical of pea [20], but rather reminding of a freshly crushed green bell pepper. This can mean a better expression of the thiol compounds from the grape variety in the samples clarified with pea protein in full dose.

The typical spicy aroma of the grape variety – garden thyme, wild thyme and basil – is better preserved in the non-intervention samples (control V0), but also in the ones treated with PVPP and PP alone. All the other variants, through the other compounds present in combinations, attenuated in a significant way these aromas, better expressing the fruitiness of other compounds. The aroma complexity was significantly reduced only in the presence of bentonite (PPCB, PPYB), an effect which is not surprising when the bentonite is used [9].

Considering the diversity of the aroma present in Tamaioasa romaneasca, to have a clearer idea of the treatment effects, the results were also analysed by multivariate statistics, which allows for a reduction of the variables.

Aroma of wines evaluated by Principal Component Analysis

The large data sets obtained from analysing all the variants and repetitions were reduced by Principal Component Analysis, to better observe the main sensory influences induced by the oenological treatments.

For the main classes of aromatic descriptors, the Principal Component Analysis (Figure 1) showed that the first two components, PC1 and PC2, included most of the aroma variance, up to 81.01%.

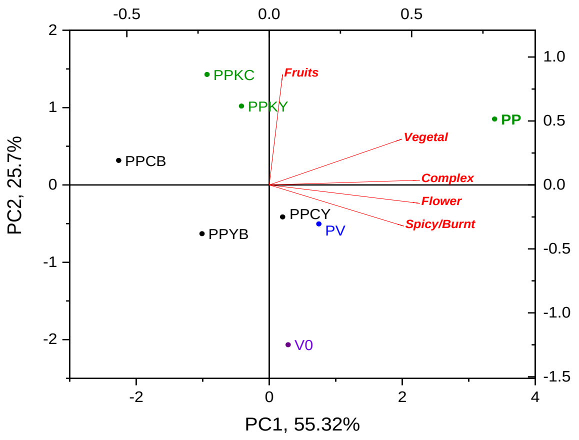

Fig. 1. Principal Component Analysis (PCA) of the main aroma descriptors for the experimental wines (V0-control-no treatment, PV-with PVPP, PP-with pea protein, K-with chitosan, C-with activated carbon, Y-with yeast cell walls, B-with bentonite).
Source: Own results.

The component PC1 accounted for 55.32% of the variance and includes flower, vegetal spicy/burnt and complex aroma, while PC2, with 25.70% is dominated by fruity aroma. This can be easily observed in Table 4 where the amount of variance retained by each principal component is expressed for each aroma descriptor as eigen values. The PC1 axis is therefore describing well the flowery-vegetal-spicy aspect of the aroma, while PC2 describes the fruity aroma.

Table 4. The extracted eigenvectors of the main aroma descriptors on the principal components

Aroma variable	Coefficients of PC1	Coefficients of PC2
Flower	0.52813	-0.14341
Fruits	0.04658	0.86367
Vegetal	0.46602	0.35895
Spicy/Burnt	0.47177	-0.32132
Complex	0.52836	0.03751

Source: Own results.

As observed, the alternative treatments induce detectable variations in the aroma of the final product. Control samples (V0), produced using the classical technology without any intervention, are the simplest wines, their sensory values being inversely associated with the fruity aroma and very little flowery-vegetal-spicy aroma. The samples treated with PVPP (PV) have better aromatic profile than the control wines, but their flowery-vegetal-spicy aroma is still low and the fruity aroma, even if more perceptible, the sensory values are still not directly associated with it. The alternative treatments with the vegetal protein from peas have, however, a detectable positive influence on the aroma profile. The most well balanced in terms of aroma are the wines treated only with pea protein (PP), whose sensory profile is positively associated with all aroma parameters, on both PC axes, being especially high in flowery-vegetal-spicy. The samples treated with both pea protein and chitosan (samples PP+K) display the highest fruity aroma and little flowery-vegetal-spicy (small inverse correlation of aroma profile is observed in Figure 2). The rest of the combined treatments, while maintaining a better fruitiness than the classical V0 and PV technologies, are stripping the wines of their flowery-vegetal-spicy components. This is especially the case of the samples which along with pea protein have also been treated with bentonite (samples PP+B).

In each aroma category, wherever possible to identify, the specific aroma was detailed.

The most cited descriptors were acacia flower for the flower aroma, grapefruit, lime, apples and apricot for fruity aroma, green pepper for vegetal, and thyme and basil for spicy.

Their perception intensity was also evaluated on numeric scales and the average values were used for another Principal Component Analysis which is shown in Figure 2.

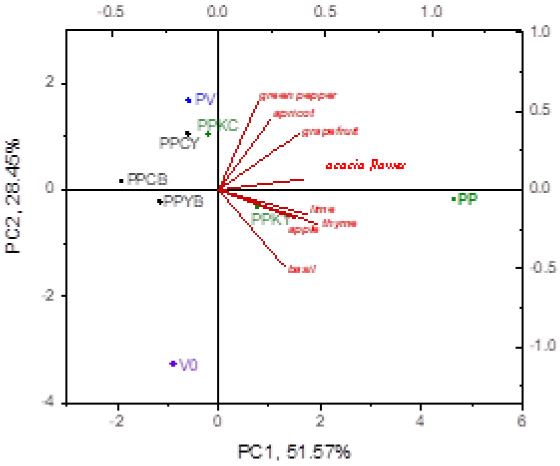

Fig. 2. Principal Component Analysis (PCA) of the specific aroma descriptors for the experimental wines (V0-control-no treatment, PV-with PVPP, PP-with pea protein, K-with chitosan, C-with activated carbon, Y-with yeast cell walls, B-with bentonite).
Source: Own results.

The first 2 components of the PCA explained only 80.02% of the total variance for the specific aroma identified by the tasters. Considering the complexity of these wine aromatic profiles, the result is very good. PC1 incorporates 51.57% of the total variance, including in it most of the fruity and all the spicy aroma. As presented in Table 5, grapefruit aroma is equally distributed in both components PC1 and PC2. All acacia flower, lime, apple, thyme and basil aromas are included in PC1, which can be considered to represent the axis of terpenic Tamaioasa varietal aroma. Green pepper and fresh apricot are mostly included in PC2which can be considered to represent the axis of thiolic Tamaioasa varietal aroma.

Table 5. The extracted eigenvectors of the specific aroma descriptors on the principal components

	Coefficients of PC1	Coefficients of PC2
Grapefruit	0.38043	0.36293
Lime	0.4102	-0.1545
Apple	0.45936	-0.21602
Thyme	0.35307	-0.17989
Basil	0.312	-0.49191
Green pepper	0.18808	0.56964
Fresh apricot	0.24106	0.44041
Acacia flower	0.40064	0.07044

Source: Own results.

As can be observed in Figure 2, the group of samples only treated with pea protein (PP) display the most typical varietal aroma, with the notorious terpenic profile. Also, typical, but more complex are the samples prepared with chitosan in addition to the pea protein (PPKY, PPKC). The least typical are the groups of samples placed in the opposite quadrant to the one containing the vectors of the specific aromas, those containing bentonite especially, but also some with carbon (PPCY, PPCB, PPYB). The samples treated with PVPP (PV) are relatively close in aromatic profile to the samples treated with combinations of pea protein and other products. Only the control samples are far in their aromatic profile from all of the samples produced with fining treatments.

To evaluate if these differences in profile are positive or not, scores were also attributed for the wine quality.

Scores of wines on evaluation sheets of 100 points

The quality of wine was evaluated on the score sheet proposed by the OIV for the use in the wine contests [16]. These evaluation sheets contain scores for visual aspects, odour and taste quality and intensity, taste balance as overall quality, which summed go up to a maximum of 100 points.

The average scores for each group of treatment and the standard deviations are presented in Figure 3.

Following this evaluation, it became obvious that the control wines produced without any fining to remove polyphenols were the least appreciated, the score of 76 being not enough to obtain a medal in a wine contest. Samples treated with PVPP (PV) also received a low score, even though higher than the control wines, the difference being statistically significant.

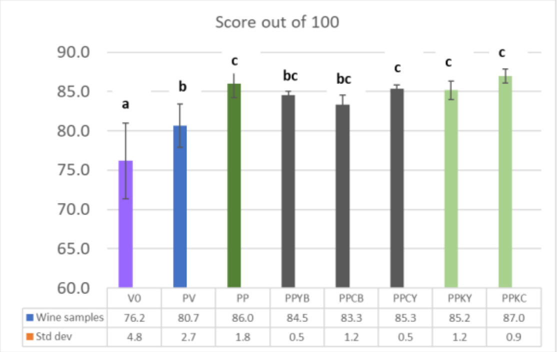

Fig. 3. Scores on the OIV wine contest evaluation sheets for the experimental wines (V0-control-no treatment, PV-with PVPP, PP-with pea protein, K-with chitosan, C-with activated carbon, Y-with yeast cell walls, B-with bentonite).

Source: Own results.

The best ranked wines were those produced with PP alone and the combination of PP and chitosan. With scores between 85-87, these samples could receive a gold medal in a wine contest.

CONCLUSIONS

According to these results it is clear that the alternative treatment of Tamaioasa romaneasca musts with the pea protein leads to wines with improved aromatic profile as compared to the wines produced by the usual technology with no interventions or with the use of the synthetic compound PVPP.

Pea protein used alone produces the most well-balanced aromatic wines, with all the aroma components specific to the variety well preserved. Chitosan, used together with pea protein, increases significantly the fruitiness. Although this combination also reduces the vegetal-spicy aroma, these wines are more appreciated by consumers and also by wine professionals due to the powerful and pleasant overall fruity aroma. Bentonite, however, even used together with pea protein, has a negative impact on the overall aroma, reducing both fruitiness and complexity as compared to the samples treated only with pea protein. The most important observation is that all the samples treated with pea protein, single or in combination with other agents, led to better quality wines than the control wines or those treated with PVPP, making this alternative treatment a viable solution that could be considered more acceptable by the environmentally-focused consumers.

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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