Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 1, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

USE OF VEGETABLE PROTEINS AS ALTERNATIVES TO PVVP AND CASEINATE FOR REMOVING POLYPHENOLS FROM WHITE GRAPE MUSTS

George Adrian COJOCARU, Arina Oana ANTOCE

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems,
59, Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: arina.antoce@horticultura-bucuresti.ro

Abstract

Polyvinilpolypirrolidone (PVPP) and potassium caseinate (KCas) are the standard treatments used for removing before fermentation a part of the polyphenolic compounds from white grape musts, in order to prevent their oxidation in the resulted wines. As PVPP is a synthetic polymer and KCas is an animal protein with allergenic potential, in line with the movements toward more natural and vegan products, alternative fining agents are being proposed in the form of vegetal proteins. In this study the fining potential of pea and potato proteins was evaluated compared to PVPP and KCas, by determining the change in colour (CIELab parameters) and the amount of total polyphenols removed from the wines of Welsch Riesling. Each fining agent was applied to the must before fermentation in doses of 10, 20 and 30 g/hl and their effect in wine was analysed. The treatments applied tend to reduce wine colour yellowness (parameter b), shift more toward greener (parameter a), and decrease colour saturation (C). For each treatment the parameters determined spectrophotometrically were in direct correlation with the dose used, even though the total colour differences (ΔE values) of the musts were not perceivable by the naked eye, in the young wines. However, clarification of must with any fining agent significantly removed a part of the total polyphenols in a dose-dependent manner, the efficiency of the fining agents being in the following order: PVPP>Pea>Potato=KCas>Control. To also evaluate the economic impact of using these new alternatives, sensory analysis was also carried out and the costs of treatments were determined.

Key words*: white wine, pre-fermentative, fining agents, CIELab, total polyphenols.*

INTRODUCTION

Modern white winemaking relies more and more on treatments applied during pre-fermentative stages to ensure a better clarification of the grape juice and to remove certain undesirable solids or compounds before the alcoholic fermentation begins. Commercial pectolytic enzymes are used to lower the viscosity of juice by breaking down the pectin macromolecules, increasing in the same time the yield of juice (Claus and Mojsov, 2018). Lowering the viscosity of grape juice improves the rate of sedimentation of many undesirable solid particles such as dust, fungicides and microorganisms. Many winemakers support the natural sedimentation or the flotation procedure by adding fining agents, which accelerate these processes and can also bring some more benefits such as lowering the concentration of polyphenols, improving the colour of the final product and extending the shelf life of the product (OIV, 2021a).

Nowadays, alternatives are sought, because these materials tend to be rejected by the general public due to their synthetic or animal or origin.

The PVPP is very effective for removing compounds which confer undesirable wine pigmentation or bitterness, but it is a synthetic homopolymer (Ribéreau-Gayon *et al.*, 1999; Laborde *et al.*, 2006; Cosme *et al.*, 2012). Potassium caseinate is a very effective treatment for oxidized juices and wines, reducing browning and maderisation due to its capability of casein-quinone conjugates formation, but it is a phosphoprotein derived from milk (Hurrel *et al.*, 1982; Kroll *et al.*, 2003; Ribéreau-Gayon *et al.*, 1999; Cosme *et al.*, 2012). Due to the risk of allergenic reactions and the requirement of the mention for the use of animal proteins on the label,

many winemakers tend to avoid casein and use PVPP as an alternative treatment.

Indeed, some residues of allergenic fining agents can be present in some wines, but most of the wines prepared for bottling were free of allergenic proteins (Peñas *et al.*, 2015). Casein is not usually an issue, but fining agents containing egg white proteins can leave more frequently residues than those based on milk proteins (Peñas *et al.*, 2015). Following the OIV guidelines for the use of fining agents with allergenic potential (OIV, 2014), helps very much in limiting the presence of allergenic residues in the final wine.

On the other hand, synthetic polymers such as PVPP or PVP raised some safety concerns, as they can allegedly contain certain impurities, residual monomers or degradation products with potential effects on human health (Schubert and Glomb, 2010; EFSA ANS Panel, 2020). However, the latest conclusion of the European Food Safety Agency regarding the use of typical doses of PVP and PVPP as food additives, revealed they are not of safety concern (EFSA ANS Panel, 2020). Nevertheless, natural alternatives to synthetic polymers or to potentially allergenic proteins are still desired, especially for the application to organic wine production. Renouncing to these fining procedures is not an option, as the management of phenolic compounds during white wine production is essential to achieve the sensorial characteristics of quality modern wines. Vegetable proteins are receiving lately a lot of attention, as they have similar functions as PVPP and caseinate (Marangon *et al*., 2019). Pea and potato proteins, for example, are allowed as fining agents for conventional or organic wine production (EU Regulation 2021/1165). Even though certain vegetal proteins, including from pea and potato, may cause allergic reactions as well, these are less frequent than in the case milk casein (Taylor *et al.*, 2021; Castells *et al.*, 1986; Seppälä *et al.*, 1999; Martorell *et al.*, 2006). In accordance to European legislation, the use of fining agents derived from pea and potato may not be mentioned on the wine labels, as they are generally non-allergenic protein isolates (Peñas *et al.*, 2015; EGTOP, 2015). As such, these vegetal proteins may be good alternatives to PVPP and caseinate in white wine production, but more evaluation is needed to determine their other possible effects on sensory wine quality.

MATERIALS AND METHODS

The grapes of Welsch Riesling variety were harvested in 22 September 2020 from Săhăteni Pietroasa wine region. The processing of grapes followed the classical white winemaking protocol: destemming, crushing and then the must was separated using a vertical hydraulic press. A dose of 50 mg/l SO_2, using a solution of 10% w/v potassium metabisulphite, and a dose of 3 g/hl pectolytic enzyme (Zimafruit, Enologica Vason) were added in to the resulted free-run juice (100 l) to speed up clarification. To evaluate the effect of several fining agents and doses, the homogenized must was transferred in 39 containers of 2-liter capacity, thus producing 13 experimental variants with 3 replicates for each. The experimental samples are described in the Table 1.

Table 1. Experimental variants produced to test the effect of fining agents and doses added

Experimental variants	Fining agent	Dose, g/hl
Control	-	-
KCas_10	Potassium caseinate (Clarito Spray Dry, Enologica Vason)	10
KCas_20		20
KCas_30		30
PVPP_10	Polyvinylpolypyrrolidone (PVPP, Enologica Vason)	10
PVPP_20		20
PVPP_30		30
Pea_10	Pea protein (Proveget 100, Agrovin)	10
Pea_20		20
Pea_30		30
Potato_10	Potato protein (Proveget Fine, Agrovin)	10
Potato_20		20
Potato_30		30

Colloidal dispersions of 5% w/v concentration were prepared in distilled water for each fining agent. The following amounts of dispersions were added to must: 4 ml of dispersion for the dose of 10 g/hl, 8 ml for the dose of 20 g/hl and 12 ml for the dose of 30 g/hl.

The samples thus treated, were well homogenized, kept at 10°C for 48 hours and then racked off the lees. Subsequently, 1.4 liters of each limpid must variant and repetition was transferred into 1.5 liters containers. The physio-chemical parameters of the limpid free run juice are presented in the Table 2.

Table 2. Physio chemical parameters of the limpid free run juice

Brix, %	20.2
pH	3.38
Total titratable acidity, g/l expressed as tartaric acid	5.81
Sugars, g/l	194
Potential alcohol, % vol.	11.8

The musts were inoculated with 25 g/hl active dry yeast Fermol Arome Plus, AEB and 24 hours later, a dose of organic nutrient of 40 g/hl (Nutristart ORG, Laffort) was added to each sample. The alcoholic fermentation continued for 7 days at a constant ambiental temperature of ≈20ºC. The resulted wines were racked off, treated with 70 mg/l SO_2, using 10% w/v $K_2S_2O_5$ solution and 0.6 g/l granulated sodic bentonite, using a 5% w/v gel to remove thermolabile proteins. Then, they were left for cold stabilization at -4ºC for 14 days. After that, the wines were racked again and transferred in 0.75 l bottles, then stored for 6 months in the cellar, to assess if their sensory qualities preserve well.

After the storage period, all the samples were sensorially evaluated and spectrophotometrically analyzed using CIELab method (OIV, 2021b) and the measurement of OD_{280} of a 10% diluted sample. The sensorial analysis was performed by a panel of 5 experienced wine-tasters, using a methodology developed in our laboratory and patented at the Romanian Office of Inventions and Trademarks (Antoce and Năməloşanu, 2007). The CIELab method and OD_{280} were carried out running the software WinAspect version 2.2.7. coupled with a double beam UV-VIS spectrophotometer Specord 250 from Analytik Jena AG. All the spectrophotometric determinations were performed in quartz or glass cuvettes and conventionally referred to the optical path of 10 mm.

RESULTS AND DISCUSSIONS

After 6 months of storage in bottles, all the fining agents applied to the must before alcoholic fermentation led to an increase in the lightness parameter (L) and decrease of colour saturation (C). These changes can be observed in Figure 1. The effect of lightness increase and colour saturation decrease is enhanced by the quantity of the fining agent added, in a dose-dependent manner, confirmed by the linear regression equations included in Figure 1, with coefficients of determination varying from 0.86-0.99. In accordance to the equation slopes, the maximum effect is produced by potato protein or potassium caseinate. The samples treated with PVPP and pea protein showed milder effect on these changes.

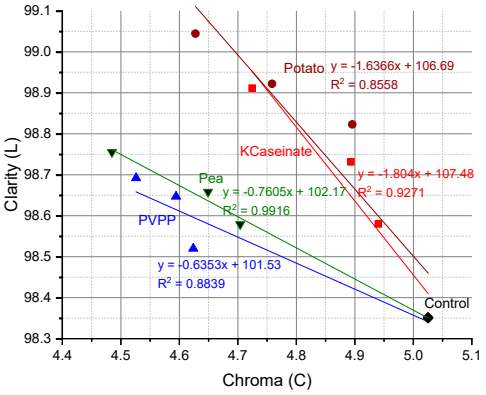

Figure 1. Variations of clarity (L) and chroma (C) parameters in experimental samples depending on fining agent type and dose

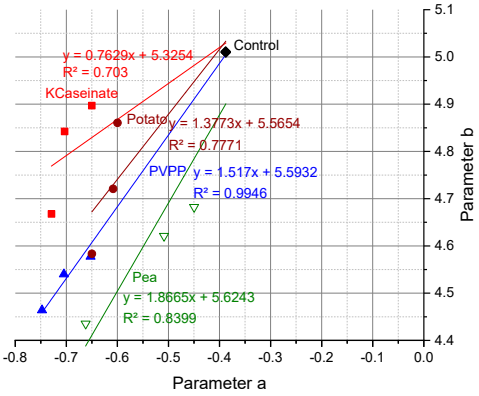

Figure 2. Variations of *a* and *b* parameters in experimental samples depending on fining agent type and dose

On the other hand, we can observe in Figure 2 the effect of fining agents on parameters *a* and *b*. The applied fining agents showed a reduction of yellowness (parameter *b*) and a shift more toward greener (parameter *a*). These effects were more evident in the samples treated with higher doses. The pea protein and PVPP were able to reduce more the parameter *b* (yellowness) than potato protein or potassium

caseinate at similar doses of treatment. Thus, the most effective treatments to obtain lower values for parameter *b* remain those with pea protein and PVPP.
However, the PVPP seems to change the most the colour towards greener, by decreasing more the value of the parameter *a*.

In Table 4 are presented the average values of the Total Polyphenolic Index (TPI) along with standard deviations.
The TPI showed a decreasing trend with an increase of the fining agent dose. The most powerful fining agent in reducing the TPI was PVPP and followed by pea protein.

Table 3. The effect of dose and of clarifying agent on TPI, CIELab parameters and colour difference

	TPI	**Clarity (L)**	**Parameter a**	**Parameter b**	**Chroma (C)**	**hab°**	****ΔE Total colour difference**
Dose effect	F=(2, 262.335) p<0.05 $\widehat{\omega}_p^2 = 0.93$	F=(2, 34.823) p<0.05 $\widehat{\omega}_p^2 = 0.64$	F=(2, 21.792) p<0.05 $\widehat{\omega}_p^2 = 0.52$	F=(2, 43.336) p<0.05 $\widehat{\omega}_p^2 = 0.69$	F=(2, 37.279) p<0.05 $\widehat{\omega}_p^2 = 0.66$	F=(2, 33.347) p<0.05 $\widehat{\omega}_p^2 = 0.63$	F=(2, 45.741) p<0.05 $\widehat{\omega}_p^2 = 0.72$
Control (without)	6.32±0.02^{d}	98.35±0.09^{d}	-0.388±0.03^{c}	5.010±0.12^{c}	5.025±0.12^{c}	94.43±0.46^{c}	-
Low dose (10 g/hl)	6.13±0.07^{c}	98.63±0.13^{c}	-0.588±0.09^{b}	4.754±0.14^{b}	4.791±0.14^{b}	97.05±1.04^{b}	0.476±0.10^{c}
Medium dose (20 g/hl)	6.00±0.08^{b}	98.74±0.13^{b}	-0.631±0.09^{b}	4.681±0.12^{b}	4.724±0.12^{b}	97.68±1.06^{b}	0.603±0.09^{b}
High dose (30 g/hl)	5.91±0.08^{a}	98.85±0.15^{a}	-0.697±0.05^{a}	4.537±0.11^{a}	4.591±0.11^{a}	98.74±0.69^{a}	**0.780±0.09^{a}**
Fining agent effect	F=(3, 96.596) p<0.05 $\widehat{\omega}_p^2 = 0.88$	F=(3, 38.880) p<0.05 $\widehat{\omega}_p^2 = 0.75$	F=(3, 30.854) p<0.05 $\widehat{\omega}_p^2 = 0.70$	F=(3, 42.764) p<0.05 $\widehat{\omega}_p^2 = 0.77$	F=(3, 43.691) p<0.05 $\widehat{\omega}_p^2 = 0.77$	F=(3, 27.629) p<0.05 $\widehat{\omega}_p^2 = 0.68$	F=(3, 6.408) p<0.05 $\widehat{\omega}_p^2 = 0.32$
Control (without)	6.32±0.02^{d}	98.35±0.08^{d}	-0.388±0.03^{d}	5.010±0.12^{c}	5.025±0.12^{c}	94.43±0.46^{d}	-
KCas	6.05±0.10^{c}	98.74±0.16^{b}	-0.694±0.04^{a}	4.802±0.13^{b}	4.852±0.12^{b}	98.24±0.59^{ab}	0.562±0.17^{b}
PVPP	**5.90±0.11^{a}**	98.62±0.08^{c}	-0.702±0.05^{a}	4.527±0.05^{a}	4.581±0.04^{a}	98.82±0.65^{a}	0.644±0.11^{ab}
Pea	6.01±0.09^{b}	98.67±0.09^{bc}	-0.540±0.10^{c}	4.579±0.11^{a}	4.613±0.10^{a}	96.75±1.44^{c}	0.571±0.17^{b}
Potato	6.09±0.10^{c}	98.93±0.11^{a}	-0.619±0.04^{b}	4.720±0.12^{b}	4.760±0.12^{b}	97.48±0.60^{bc}	**0.702±0.14^{a}**

*Average values ±SD and **Two-way ANOVA – Tukey HSD (p<0.05).

Table 4. Total polyphenolic index and total colour differences towards control samples

Variants	**TPI**	**ΔL**	**Δa**	**Δb**	****ΔE Total colour difference** F=(2, 9.927), p<0.05 $\widehat{\omega}_p^2 = 0.73$
Control	6.32 ± 0.02	-	-	-	-
KCas_10	6.17 ± 0.02	0.23 ± 0.09	-0.262 ± 0.03	-0.113 ± 0.13	0.389 ± 0.03^{c}
KCas_20	6.04 ± 0.03	0.38 ± 0.18	-0.316 ± 0.03	-0.168 ± 0.17	0.554 ± 0.12^{bcde}
KCas_30	5.95 ± 0.06	0.56 ± 0.12	-0.341 ± 0.06	-0.342 ± 0.09	**0.746 ± 0.11^{abc}**
PVPP_10	6.05 ± 0.03	0.17 ± 0.05	-0.265 ± 0.03	-0.433 ± 0.12	0.541 ± 0.09^{bcde}
PVPP_20	5.89 ± 0.01	0.3 ± 0.08	-0.317 ± 0.03	-0.470 ± 0.11	0.648 ± 0.07^{abcd}
PVPP_30	**5.79 ± 0.04**	0.34 ± 0.06	-0.360 ± 0.04	-0.546 ± 0.13	**0.744 ± 0.09^{abc}**
Pea_10	6.11 ± 0.03	0.23 ± 0.09	-0.062 ± 0.02	-0.328 ± 0.13	0.418 ± 0.10^{de}
Pea_20	6.00 ± 0.02	0.31 ± 0.08	-0.121 ± 0.05	-0.389 ± 0.14	0.526 ± 0.08^{cde}
Pea_30	**5.92 ± 0.01**	0.41 ± 0.11	-0.274 ± 0.10	-0.575 ± 0.14	**0.770 ± 0.10^{ab}**
Potato_10	6.22 ± 0.02	0.47 ± 0.15	-0.212 ± 0.04	-0.151 ± 0.10	0.558 ± 0.08^{bcde}
Potato_20	6.09 ± 0.02	0.57 ± 0.03	-0.220 ± 0.03	-0.291 ± 0.12	0.687 ± 0.03^{abc}
Potato_30	5.98 ± 0.02	0.69 ± 0.02	-0.262 ± 0.01	-0.428 ± 0.14	**0.863 ± 0.07^{a}**

*Average values ±STDEV.S and **One-way ANOVA – Tukey HSD (p<0.05).

The changes induced by fining agents on the CIELab parameters, TPI and total colour difference were statistically analysed using additive model Two-way ANOVA coupled with post-hoc test Tukey HSD (p<0.05), in order to evaluate, on one hand, the main effects of fining agents irrespective of the dose and, on the other hand, the main effect of a low, medium or high dose applied, irrespective of the type of fining agent (Table 3). Both of factors were statistically significant, which means that they affect the parameters of wine at p<0.05, inducing reductions of total polyphenol index (TPI) and differences in total colour (ΔE). The effect sizes were large for both factors with bigger values for the dose effect (TPI $\widehat{\omega}_p^2 = 0.93$; ΔE $\widehat{\omega}_p^2 = 0.72$) and slightly lower values for the fining agent effect (TPI $\widehat{\omega}_p^2 = 0.88$; ΔE $\widehat{\omega}_p^2 = 0.32$), meaning that the correction of TPI or ΔE can be easier achieved by adjusting the dose of any of the tested fining agents (Table 3). In the case of the CIELab parameters (L, a,

b, C, hab°), the dose and fining agents were also statistically significant ($p<0.05$), but the effect magnitude was opposite comparing with the case of TPI and ΔE. The effect magnitude was large for both factors, with bigger values for the fining agent type (L $\hat{\omega}_p^2 = 0.75$; parameter a $\hat{\omega}_p^2 = 0.70$; parameter b $\hat{\omega}_p^2 = 0.77$; C $\hat{\omega}_p^2 = 0.77$; hab° $\hat{\omega}_p^2 = 0.68$), followed by the effect of the applied dose (L $\hat{\omega}_p^2 = 0.64$; parameter a $\hat{\omega}_p^2 = 0.52$; parameter b $\hat{\omega}_p^2 = 0.69$; C $\hat{\omega}_p^2 = 0.66$; hab° $\hat{\omega}_p^2 = 0.63$) meaning that to achieve a certain correction for any specific CIELab parameter, selecting the appropriate fining agent is more efficient than changing the dose (Table 3). However, it is clear from the Table 3 that the use of any fining agent, even at low dosage, may induce significantly favourable changes of any of the parameters, when compared with the parameters of control sample. Obviously, the higher the dose, the bigger the effect on colour and polyphenols, but the stripping effect of these fining agents on aroma and mouthfeel may also increase, lowering complexity of the wine. Nevertheless, each grape variety, vintage and terroir will lead to a different level of phenolic compounds in the must, therefore the dose of the fining agent should be adjusted in accordance with the desires wine style. For a lighter body and a fruity white wine of Welsch Riesling, the lowest dose of any of the tested fining agent was determined to be enough. For selecting the appropriate fining agent to be used during the pre-fermentation stage, the following features can be exploited, taking into account their effectiveness (Table 3):

- **to lower TPI:** PVPP > Pea > KCas= Potato > Control.
- **to increase clarity/lightness (L):** Potato > KCas ≥ Pea ≥ PVPP > Control.
- **to shift the colour towards green (parameter a), thus reducing the red component associated with oxidation:** PVPP = KCas > Potato > Pea > Control.
- **to reduce yellowness (parameter b), also associated with some phenolic oxidation:** PVPP = Pea > Potato = KCas > Control.
- **to reduce colour saturation (C):** PVPP = Pea > Potato = KCas > Control.
- **to induce a beneficial total colour difference (ΔE):** Potato ≥ PVPP ≥ Pea = KCas.

The total colour difference (ΔE) indicated in Table 3 and 4, has values less than 1, so, for all samples a human observer will not see with the naked eye the colour differences between the wines.

These total colour differences (ΔE) are statistically significant (Table 3), but can be observed only by means of the spectrophotometer. Even not perceivable now, these colour differences have technological importance, as they show a reduction of oxidisable polyphenols from the must, thus reducing their presence in the wines, where in time, after a period of storage, could lead to visible effects. The evolution of the experimental samples over time could make a difference through slow oxidation reactions and in the presence of different oxidation substrates.

These small changes of the CIELab parameters and lowering TPI could be even more important for certain white wines which are produced from varieties more susceptible to oxidative phenomenon such as browning and pinking, as it is demonstrated that the fining agents could reduce to a certain degree the concentration of small-molecule phenolic compounds and even anthocyanins (Salacha *et al.*, 2008; Cojocaru and Antoce, 2019; Cosme *et al.*, 2019;). Browning rate is significantly correlated with flavanol concentration (r^2=0.84) and with total phenolic (r^2=0.79) (Salacha *et al*., 2008).

The results presented in Table 4 show that the total colour changes (ΔE) were influenced the most by the highest dose (30 g/hl) of any fining agent, practically no significant differences being obtained. For potato protein and PVPP, however, the medium dose of 20 g/hl produced also a similar effect as the doses of 30 g/hl. This is an important finding, as reducing the dose has also a beneficial economic impact.

According to the data indicated in Figure 3 we can observe that Potato protein increase the most the price of resulted wine, being followed by the PVPP. However, the Pea protein alternative is more or less close to K Caseinate, with only a lower price increase as compared to the other fining agents.

From a sensory point of view, with the exception of bitterness, the perception of taste parameters (acidity, sweetness, astringency, extract) and visual (colour intensity) were statistically insignificant (Figure 4). The tasters

pointed out that the minimum doses (10 g/hl) better preserved the complexity of the Welsch Riesling wines, while the higher doses, especially in the case of Potato protein, led to a reduction in complexity of the resulted wines. Following the sensory analysis, the overall quality of the wines was established to be in the following descending order of the applied treatments: PVPP > Pea > KCas > Potato > Control.

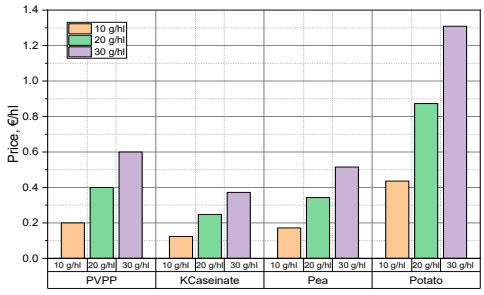

Figure 3. Price increase per hl of wine due to the application in musts of the fining agents in certain doses

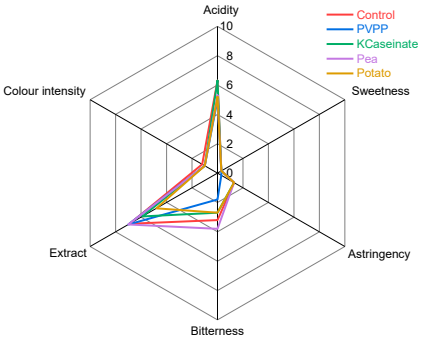

Figure 4. The overall effect of the fining agents on taste and the perceived colour intensity (average values)

Considering the price increase induced by these treatments (Figure 3) and the sensory panel agreement that the Potato protein reduces the wine complexity, the other vegetal protein, the Pea protein, appears to be a good alternative for PVPP and KCas.

By applying the principal components analysis (PCA) to the results of the sensory analysis, some correlations of fining agents to the sensory attributes can be highlighted (Figure 5). As there were no significant changes in the main taste characteristics except bitterness, only this one was included in the PCA chart, along with the perceived aroma attributes.

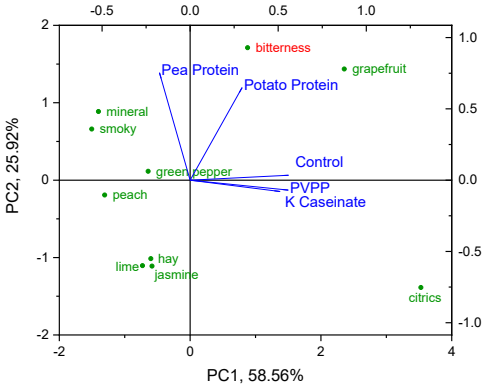

Figure 5. The overall effect of fining treatments on wines sensory characteristics

This technique reduces the data complexity and brings about some patterns and associations between the measured variables. It can be pointed out that the experimental variants treated with PVPP and those treated with KCas have certain sensory characteristics in common with the control samples, so they keep the complexity of the wines, similar to the control samples. These variants were associated with citrus, grapefruit and less with the mineral or smoky character. Experimental samples treated with Potato protein were more strongly associated with grapefruit and bitterness, while those treated with pea protein were more strongly associated with mineral, smoked and even green pepper. In all experimental samples, regardless of the treatment, there were highlighted dominant aromas of peach, hay and lime.

CONCLUSIONS

The pre-fermentative operations and treatments during white winemaking are important decisions for the quality of the final product. The reduction of TPI and inducing beneficial total colour differences can be achieved by using several fining agents in dose dependent manner, the higher the dose, the higher the produced effect. However, to fine tune the CIELab colour parameters not only the dose, but also the type of fining agent is important. Also, the sensory quality of the final wine, as well as the price, are significantly influenced by the fining agent used. The treatments with PVPP or KCas proved to be efficient and cost-effective, justifying their widespread use in

spite of their drawbacks as being synthetic or of animal origin, respectively. The present sensory analyses results, along with the spectrophotometric ones, highlighted that the Pea protein is a very good alternative to the treatments with PVPP or K Caseinate, while the Potato protein, even in low doses, was found to reduce too much the complexity of white wines and to be also the most expensive of all. The commercial products based on pea protein are allergen-free isolates, which give comparable results with PVPP or K Caseinate fining agents. Moreover, the pea protein isolates are allowed to be used in organic winemaking and the treatment cost is similar with the regular treatments with PVPP or K Caseinate.

REFERENCES

Antoce, A.O., Nămoloşanu, I.C. (2007). Method for sensory profile construction for defining and evaluating wine tipicality. *Romanian Patent, Official Bulletin for Industrial Property BOPI nr 6/2007, patent 123129 issued on 30.11.2010.*

Castells, M.C., Pascual, C., Martin Esteban, M., Ojeda, J.A. (1986). Allergy to white potato. *J. Allergy Clin. Immunol. 78*(6), pp 1110-1114

Claus, H., Mojsov, K. (2018). Enzymes for Wine Fermentation: Current and Perspective Applications. *Fermentation, 4* (52). doi: 10.3390/fermentation4030052

Cojocaru, G.A., Antoce, A. O. (2019). Effect of certain treatments to prevent or partially reverse the pinking phenomenon in susceptible white wines. *42nd World Congress of Vine and Wine, BIO Web Conf. Vol. 15*, doi: 10.1051/bioconf/20191502003

Cosme, F., Andrea-Silva, J., Filipe-Ribeiro, L., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Domingues, M.R.M., Nunes, F.M. (2019). The origin of pinking phenomena in white wines: An update. *41st World Congress of Vine and Wine, BIO Web Conf. Volume 12*, doi: 10.1051/bioconf/20191202013

Cosme, F., Capão, I., Filipe-Ribeiro, L., Bennett, R.N., Mendes-Faia, A. (2012). Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physiochemical and sensory characteristics. *LWT-Food Science and Technology 46* (2), pp. 382-387.

Hurrel, R.F., Finot, P.A., Cuq, J.L. (1982). Protein-polyphenol reactions. 1. Nutritional and metabolic consequences of the reaction between oxidized caffeic acid and the lysine residues of casein. *British Journal of Nutrition 47*, pp. 191-211

Kroll, J., Rawell, H.M., Rohn S. (2003). Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. *Food Science and Technology Research 9*, pp. 205-218

Laborde, B., Moine Ledoux, V., Richard, T., Saucier, C., Dubourdieu, D., Monti, J.-P. (2006). PVPP-Polyphenol complexes: a molecular approach. *Journal of Agricultural and Food Chemistry 54,* pp. 4383-4389

Marangon, M., Vincenzi, S., Curioni, A. (2019). Wine fining with plant proteins. *Molecules, Vol. 24*(11), pp. 2186, doi: 10.3390/molecules24112186

Martorell, A., Plaza, A.M., Boné, J., Nevot, S., García Ara, M.C., Echeverria, L., Alonso, E., Garde, J., Vila, B., Alvaro, M., Tauler, E., Hernando, V., Fernández, M. (2006). Cow's milk protein allergy. A multi-centre study: clinical and epidemiological aspects. *Allergol. et Immunopathol. 34*(2), pp 46-53

Peñas, E, di Lorenzo, C., Uberti, F, Restani, P. (2015). Allergenic Proteins in Enology: A Review on Technological Applications and Safety Aspects. *Molecules 20* (7), pp 13144-13164, doi: 10.3390/molecules200713144

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (1999). Handbook of Enology - The chemistry of wine and stabilization and treatments, Vol. 2, John Wiley & Sons, Ltd

Salacha, M.-I., Kallithraka, S., Tzourou, I. (2008). Browning of white wines: correlation with antioxidant characteristics, total polyphenolic composition and flavanol content. *International Journal of Food Science and Technology 43*, pp. 1073–1077, doi: 10.1111/j.1365-2621.2007.01567.x

Schubert, M., Glomb, M.A. (2010). Analysis and Chemistry of Migrants from Wine Fining Polymers. *J. Agric. Food Chem. 58*(14), 8300–8304, https://doi.org/10.1021/jf101127t

Seppälä, U., Alenius, H., Turjanmaa, K., Reunala, T., Palosuo, T., Kalkkinen, N. (1999). Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. *J. Allergy Clin. Immunol. 103* (1), Part 1, pp165-171, doi: 10.1016/S0091-6749(99)70541-5

Taylor, S.L., Marsh, J.T., Koppelman, S.J., Kabourek, J. L., Johnson, P.E., Baumert, J.L. (2021). A perspective on pea allergy and pea allergens. *Trends in Food Science & Technology 116*, pp 186-198, https://doi.org/10.1016/j.tifs.2021.07.017

***EFSA ANS Panel (2020). (EFSA ANS Panel on Food Additives and Nutrient Sources added to Food: Younes, M., Aquilina, G., Castle, L., Engel, K-H, Fowler, P., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, D.H., Wölfle, D, Wright, M., Boon, P., Crebelli, R., di Domenico, A., Filipič, M., Mortensen, A., Woutersen, R., van Loveren, H., a Giarola, A., Lodi, F., Rincon, A.M., Tard, A., Frutos Fernandez, M.J.). Re-evaluation of polyvinylpyrrolidone (E 1201) and polyvinylpolypyrrolidone (E 1202) as food additives and extension of use of polyvinylpyrrolidone (E 1201). *EFSA Journal 18* (8): 6215, doi: 10.2903/j.efsa.2020.6215

***EGTOP (2015). Expert Group for Technical Advice on Organic Production, Final Report on Wine.

***OIV (2021b). Determination of chromatic characteristics according to CIELab: Method OIV-MA-AS2-11. *Compendium of international methods of wine and must analysis, Ed. 2021, Vol. 1.*

Organisation Internationale de la Vigne et du Vin. https://www.oiv.int/

***OIV (2021a). Oenological Treatments and Practices. *International code of œnological practices, Part II.* Resolutions adopted in Geneva (Switzerland) 18th G.A. – 26 November 2020. Organisation Internationale de la Vigne et du Vin. https://www.oiv.int/

***OIV (2014). Code of good fining practices for wine to be applied in the use of proteinaceous wine fining agents with allergenic potential (casein and egg white). *Resolution OIV-OENO 520-2014, adopted in 2014 by the 12th OIV general assembly*. Organisation Internationale de la Vigne et du Vin. https://www.oiv.int/

***Commission Implementing Regulation (EU) 2021/1165 of 15 July 2021 authorising certain products and substances for use in organic production and establishing their lists. CELEX no. 32021R1165 on https://eur-lex.europa.eu/