

## APPLICATION OF SOY PROTEIN ISOLATE IN THE FINING OF RED WINE

### APLICAÇÃO DE ISOLADO PROTEICO DE SOJA NA CLARIFICAÇÃO DE VINHO TINTO

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#### SUMMARY

Soy protein isolate was evaluated as a potential fining agent as an alternative to the predominant protein commercial fining agents (ovalbumin, porcine gelatin, and pea protein isolate). Two red wines (cv. 'Merlot' and cv. 'Lambrusco Maestri') were finned, bottled, and analyzed for phenolic content, color, turbidity, and sensory profile. Independent of the protein used, fining promoted a reduction in a majority of the phenolic compound, a slight (but significant) reduction of chromatic properties, and a decrease in the turbidity of the wines. A decrease in astringency, persistence, bitterness, and wine body, as well as an increase in brightness, clarity, and acidity, was also observed in all treatments. The application of soy protein isolates yielded similar results to those obtained with other commercial fining agents, both in the physicochemical and the sensory measures, which favors its usage as an alternative to the traditional fining agents of animal-protein origin.

#### RESUMO

Avaliou-se o isolado proteico de soja como potencial agente clarificante, comparativamente aos principais clarificantes proteicos comerciais (ovoalbumina, gelatina suína e isolado proteico de ervilha). Dois vinhos tintos (cv. 'Merlot' e cv. 'Lambrusco Maestri'), foram clarificados, engarrafados e analisados quanto à composição fenólica, cor, turbidez e ao perfil sensorial. Independente da proteína utilizada, a clarificação promoveu redução na maioria dos compostos fenólicos avaliados, ligeira (mas significativa) diminuição das características cromáticas, e decréscimo na turbidez dos vinhos. Também houve redução da adstringência, da persistência, do amargor e do corpo/estrutura do vinho, sendo realçadas características como brilho, limpidez e a acidez, em todos os tratamentos. A aplicação do isolado proteico de soja proporcionou resultados similares àqueles obtidos com os demais clarificantes comerciais, tanto sob o aspecto físico-químico como sensorial, sendo uma alternativa técnica aos tradicionais clarificantes proteicos de origem animal.

**Key words:** vegetable protein, color, turbidity, sensory, alternative additive.

**Palavras-chave:** proteína vegetal, cor, turbidez, sensorial, aditivo alternativo.

#### INTRODUCTION

Many technical questions arise while making decisions on enological practices, such as the choice of grape variety, harvest time, maceration and fermentation types, and the lack or addition of winemaking inputs. Moreover, while they may be cultural, ideological, or purely commercial, there is an increase in the incorporation of new concepts in enology (Cargnello, 2009).

The fining of wines is a widely employed enological practice, even though a wide range of variations exists in the process (Tschiersch *et al.*, 2010). The primary goal of fining is to promote the clarification of wine and to improve its stability. In red wines, fining agents are added to remove the phenolic substances, thereby avoiding colloidal precipitation and making the wine more clear and stable (Yokotsuka and Singleton, 1995; Marchal *et al.*,

2002). However, the modification of the colloidal matrix of wine to promote fining and stability can change the sensory characteristics, predominantly due to a reduction in the concentration of tannins (Maury *et al.*, 2003).

Most of the widespread commercial protein fining agents for wine, such as egg albumin (ovalbumin) and gelatin, are of animal origin. However, with the discovery of the transmissible nature of Bovine Spongiform Encephalopathy (BSE) and concerns about the potential risk of allergies and food intolerance from products of animal origin, the search for a new protein source was initiated (Marchal *et al.*, 2002; Maury *et al.*, 2003; Jiménez-Martínez *et al.*, 2017). As a result, several fining agents were studied and proposed as an alternative to traditional agents, for example, proteins of cereals, legumes, potatoes and grape seeds (Simonato *et al.*, 2009; Noriega-Dominguez *et al.*, 2010; Gambuti *et al.*, 2012; Simonato *et al.*, 2013; Vincenzi *et al.*, 2013; Jiménez-Martínez *et al.*, 2017; Togores, 2018; Vernhet, 2019).

Granato *et al.* (2018) evaluated in recent work that vegetable proteins are as effective as proteins of animal origin in the fining of red wines. Moreover, previous studies have reported that compared to gelatin, plant protein isolates are more efficient in the removal of phenolic compounds, as they can remove oxidized tannins, and promote a reduction of bitterness and astringency of the red wine (Aziz *et al.*, 2017a). Therefore, seeks out alternate plant-based fining agents, derived from rich sources of protein, and are easily obtainable, preferably with low cost, be healthy and safe for consumers, and promote fining without negatively affecting wine quality.

The soy protein isolates are widely known and characterized and contain, in their final composition, 7S ( $\beta$  conglycinin) and 11S (glycinin) protein fractions (Hermansson, 1978; Kinsella *et al.*, 1985). Because these fractions possess good solubility and a positive charge at the pH typically found in red wines (3.2 to 3.8), they are thought to have the capacity to act as a fining agent.

The use of plant-derived proteins as wine fining agents can be a great alternative, ensuring food safety, not having the risk of BSE and other possible allergic reactions that the consumer may suffer from proteins of animal origin (Bindon and Smith, 2013). In addition, the use of vegetable proteins does not require the labeling of this additive, thus, avoiding the limitation of the marketing of wine to specific consumer segments, as well as avoiding an additional analytical cost (Rolland *et al.*, 2006). Based on the previous reports, the present work aimed to analyze

the efficiency of pea and soy isolates as possible fining agents.

Despite several works comparing fining agents, so far, there has been no published data that evaluated the effect of fining agents on the sensorial and physicochemical characteristics in two red wines. In addition, there lack enough data comparing various traditional and alternative agents, allowing a broader study on their effect on red wine. Therefore, a comparative study was performed; the trials were conducted in a manner to simulate industrial procedures, using commercial preparations of egg albumin, porcine gelatin, pea protein isolate, and soy protein isolate (non-commercial) on red wines of cv. 'Lambrusco Maestri' and cv. 'Merlot'. As the essential requirement of a fining agent is to diminish astringency and preserve the chromatic characteristics of wine, the objective of this study was to evaluate the turbidity, color, phenolic composition, and the sensory changes in the treated wine.

## MATERIAL AND METHODS

### Wine

For the experiment, two red wines were vinified with grapes from the 2012 vintage, in the Serra Gaucha (Brazil). The winemaking process for both cultivars, started with a homogenous volume of 200 L of must, prepared following destemming, crushing, enzyme treatment (commercial pectinase 20 mg/L), addition of sulphite (50 mg/L), yeast (250 mg/L) and supplementation with nutrient (250 mg/L), maceration (seven days), racking, and additional sulphite (adjusted with 30 mg/L free SO<sub>2</sub>) addition after spontaneous malolactic fermentation.

The wine of 'Lambrusco Maestri' (L. Maestri) was chosen because of its deep color, high acidity (Bowman, 2018), and polyphenolic content, and as the wine often needs to be treated with fining agents such as albumin and gelatin to diminish the content of astringent tannins. The 'Merlot' wine represents the most significant portion of wine *Vitis vinifera* processed in Brazil (Rizzon and Miele, 2003; Machado *et al.*, 2017).

The physicochemical characteristics of the wines used in the experiment are shown in Table I. The alcoholic strength (mL/L) of wines was determined using an enochemical distilling unit (Gibertini, Milan, Italy). Determination of density, total and volatile acidity, total, and free SO<sub>2</sub> were performed according to OIV methods (OIV, 2009). The determination of sugar content (g/L) was performed based on the methodology of Lane-Eynon (Instrução Normativa n°

24, 2005). The pH determination in the wines was performed with an MPA210 benchtop pH meter (Tecnopon, Brazil) according to Brazilian legislation (Instrução Normativa nº 24, 2005). The analyses were performed one month after the malolactic fermentation.

**TABLE I**

Analytical parameters of the red wines before fining

*Parâmetros analíticos dos vinhos tintos antes da clarificação*

Parameter <sup>a</sup>	L. Maestri	Merlot
Density (g/L)	995	994
Alcohol content (mL/L)	123	118
Sugar content (g/L)	1.9	1.6
Volatile acidity (g/L acetic acid)	0.4	0.5
Total acidity (g/L tartaric acid)	5.5	4.9
pH	3.42	3.39
Total SO <sub>2</sub> (mg/L)	97.2	115.2
Free SO <sub>2</sub> (mg/L)	36.8	31.4
Turbidity (NTU) <sup>b</sup>	73.0	29.0
420 nm	4.67	3.78
520 nm	7.51	7.02
620 nm	1.67	1.16
Color intensity	13.85	11.96
Total Tannins (mg/L)	3.91	2.37
Total phenolic index (280 nm)	85.0	58.4
Total anthocyanin (mg/L)	2834.9	2423.4
Monomeric flavan-3-ols (mg/L)	13.23	11.22
Oligomeric flavan-3-ols (mg/L)	74.05	71.09
Polymeric flavan-3-ols (mg/L)	310.19	281.89
Total flavan-3-ols (mg/L)	397.47	374.20

<sup>a</sup>Mean values obtained from three replicates. <sup>b</sup>NTU: Nephelometric Turbidity Units.

### Fining agents

For fining, the following proteins were tested: egg albumin (AEB Group, San Polo, Italy), porcine gelatin (Esseco, San Martino, Italy), pea protein isolate (Enologica Vason, Verona, Italy), and soy protein isolate (SPI), which was extracted according to the method described by Wally-Vallim *et al.* (2014) and provided by Bremil - Industry Food Products, Rio Grande do Sul, Brazil. A dose of 0.20 g/L was utilized for all proteins because it represents the maximum recommended dose for commercial pea protein and is close to the maximum value normally used for gelatin and albumin (Marchal *et al.*, 2002; Tschiersch *et al.*, 2010).

### Fining experiments

The protein applications were performed after malolactic fermentation, by adding a total of 50 mg L<sup>-1</sup> total sulfur dioxide (30 mg/L final free SO<sub>2</sub>) into a volume of 4.6 L of wine. The protein solution fining agent (10% w/v in distilled water) was introduced using a centrifugal pump with a capacity of 280 L/h to promote its homogeneous incorporation into the wine. The same procedure was performed for the control wine that received no fining agent but received the same volume of water used for the dilution of all fining agent. After the incorporation of the agent, 4.6 L of the wine was packaged in glass containers and kept at a controlled temperature of 17 °C for seven days. The stabilized wine was separated from the precipitate by siphoning and was poured in 0.75 L bottles for analysis. All analyses were performed two months after bottling.

### Polyphenols quantification

The total amount of tannins in the wines was determined based on the method described by Ribéreau-Gayon and Stonestreet (1966), using the principle of acid hydrolysis. The Total Polyphenol Index (TPI) was obtained by reading the optical density at 280 nm (Ribéreau-Gayon, 1970). The anthocyanin content was determined by an adaptation of the bisulfite method, according to the procedure previously described by Somers and Evans (1977). The flavan-3-ol fractions were obtained by preparatory C18 Sep-Pak separation into three major fractions (monomeric, oligomeric and polymeric), following the method proposed by Sun *et al.* (1998), fractionation of neutral phenolic compounds was carried out using a C18 Sep-Pak cartridge (Waters Associates, Philadelphia, USA) according to Oszmianski *et al.* (1988), Mayén *et al.* (1995), and Gil-Muñoz *et al.* (1999). The neutral polyphenols retained in the cartridge were eluted using 16 % acetonitrile at a pH of 2.0 to collect flavan-3-ol derivatives.

### Color and turbidity analysis

The color indices were determined by a direct absorbance reading (420 nm, 520 nm, 620 nm) using a spectrophotometer, model T92+ (PG Instruments, Lutterworth, United Kingdom) having a quartz cuvette of 1 mm optical path. The color intensity was represented as the sum of the readings obtained at 420 nm, 520 nm, and 620 nm. All values were expressed for an optical path of 10 mm (Zamora, 2003). The turbidity of the wine was directly measured in a nephelometric turbidimeter (model AP2000, Brand PoliControl, Diadema, Brazil) that was previously calibrated with standard solutions of formazine.

Turbidity was expressed in NTU (Nephelometric Turbidity Units).

### Sensory analysis

A quantitative descriptive sensory analysis was performed on the premises of IFRS-Campus Bento Gonçalves, four months after bottling. The panel (with extensive wine tasting experience) consisted of 15 active winemakers from the Serra Gaucha region, all with at least five years of experience. The judges were provided with training, to adapt, and to record on an unstructured scale (9 cm) and with the sensory terms (17 attributes) used in the evaluation. These procedures were based on models described by Jackson (2002), Kemp *et al.* (2009), and Dutcosky (2011). An initial group of 10 panellists was selected, and the evaluation of the wines took place in two sessions (two different days), where 10 samples (five treatments in duplicate) were evaluated per session. The use of two sessions (one kind of wine per day) allowed a better evaluation of the parameters, avoiding the saturation of tasters at the time of evaluation. Samples were served in ISO approved wineglasses labeled with 3-digit random codes in random order and varied for each judge. The order of presentation of samples within each session was also randomized.

### Experimental design and statistical analysis

A factorial experiment was arranged in a split-plot, and consisted of the combination of two different wines (plots) from the 2012 vintage ('Merlot' and 'Lambrusco Maestri') and five treatments: a control (no added fining agent) and four proteins designated

as fining agent, two of animal origin (egg albumin and porcine gelatin) and two proteins isolated from vegetables (soy and pea). The experiments were carried out in triplicates. The physicochemical data were statistically evaluated by analysis of variance ( $p < 0.01$  and  $p < 0.05$ ), using the F-test (Fisher), and subjected to comparison by Tukey test ( $p < 0.05$ ).

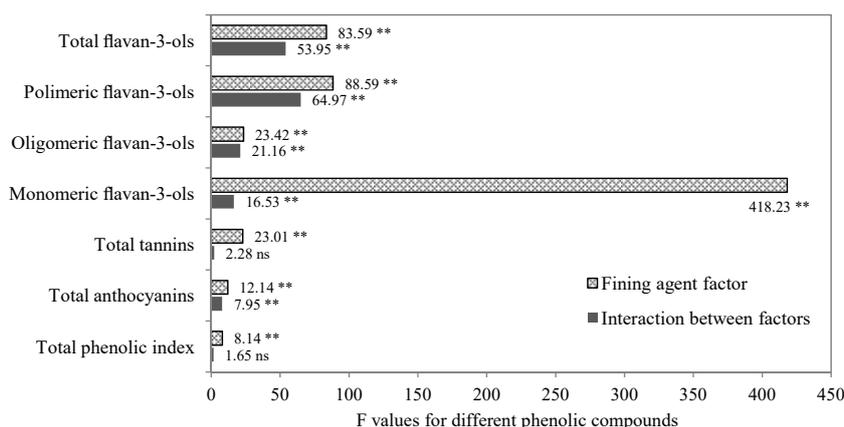
For the quantitative descriptive sensory analysis, the experiment was arranged in split-plots, but in randomized blocks (evaluators) with two replications. All results were subjected to analysis of variance ( $p < 0.01$  and  $p < 0.05$ ), using the F-test (Fisher), followed by Tukey test ( $p < 0.05$ ).

The significance level for the principal component analysis (PCA) was set at  $p < 0.05$ , and the statistical software Paleontological Statistics (PAST, version 3.2, Oslo, Norway) was used for analysis (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

### Phenolic composition of wines

In general, all the fining agents studied caused a reduction in the phenolic content of the wines. However, except for the total tannin content and the total polyphenol index (TPI) (Figure 1), for which, no effect was observed, the results depend on the interaction between the wine type and the fining agent. Some authors have suggested that the high capacity of some protein agents for eliminating tannins could be due to their hydrophobic interactions (Cosme *et al.*, 2009; Granato *et al.*, 2014).

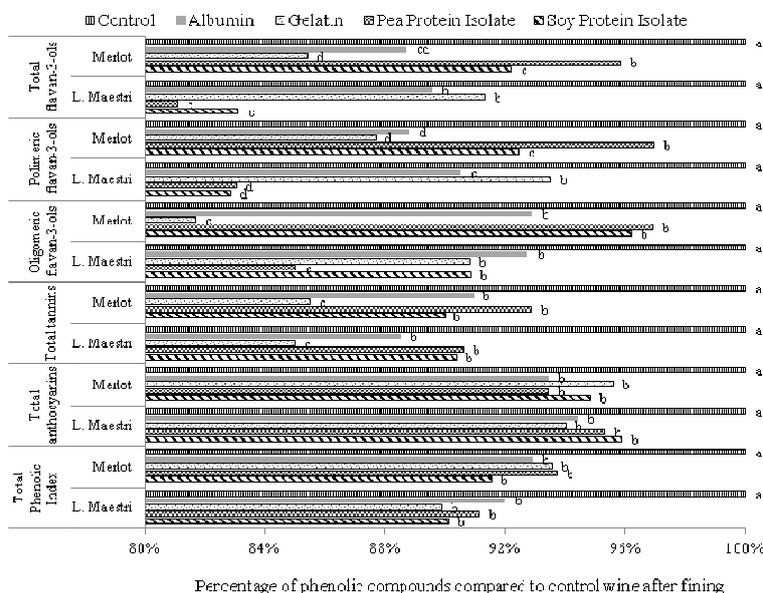


**Figure 1.** F values for fining agent factor and the interaction between wine and fining agent factors obtained in the analysis of phenolic compounds. ns - not significant; \*significantly different ( $p < 0.05$ ); \*\* very significantly different ( $p < 0.01$ ).

Valores de F para o fator cola e a interação entre os fatores vinho e clarificante obtidos na análise de compostos fenólicos. ns – não significativo; \* diferença significativa ( $p < 0.05$ ); \*\* diferença muito significativa ( $p < 0.01$ ).

All proteins promoted a reduction in the levels of the phenolic compounds (Figure 2). These results were expected since fining proteins such as ovalbumin and

collagen can complex with phenolic compounds (Robichaud and Noble, 1990; Cheynier *et al.*, 2000; Brossaud *et al.*, 2001).



**Figure 2.** Phenolic compounds content depending on the application of protein fractions (egg albumin, porcine gelatin, pea protein isolate and soy protein isolate) in ‘Lambrusco Maestri’ and ‘Merlot’ wines. For each fining protein values followed by different letters are significantly different ( $p < 0.05$ , Tukey’s test).

*Conteúdo de compostos fenólicos conforme aplicação de frações de proteína (albumina de ovo, gelatina de suíno, isolado de proteína de ervilha e isolado de proteína de soja) em vinhos ‘Lambrusco Maestri’ e ‘Merlot’. Para cada clarificante proteico, valores seguidos por letras diferentes são significativamente diferentes ( $p < 0.05$ , teste de Tukey).*

In this study, the most notable interference occurred with the levels of monomeric flavan-3-ols (Table II). The results obtained are in agreement with those of previous experiments using model wine (Granato *et al.*, 2010), ‘Catalanesca’ white wine (Granato *et al.*,

2014), ‘Cabernet Sauvignon’ press wine (Aziz *et al.*, 2017a), ‘Aglianico’ red wine (Granato *et al.*, 2018), and ‘Monastrell’ red wines (Jiménez-Martínez *et al.*, 2019).

**TABLE II**

Monomeric flavan-3-ols content (mg/L) and percentage of decrease  
*Teor em flavan-3-ol monoméricos (mg/L) e percentagem de decréscimo*

Fining agent	L. Maestri		Merlot		Mean	
	Value	Reduction (%)	Value	Reduction (%)	Value	Reduction (%)
Control	13.23 ± 0.45 aA		11.22 ± 0.05 bA		12.23 ± 0.79 A	
Albumin	5.68 ± 0.35 aD	57%	5.59 ± 0.29 aC	50%	5.63 ± 0.19 D	54%
Gelatin	6.6 ± 0.09 aC	50%	6.75 ± 0.28 aB	40%	6.67 ± 0.14 BC	45%
PPI	7.6 ± 0.59 aB	43%	6.74 ± 0.19 bB	40%	7.17 ± 0.43 B	41%
SPI	6.05 ± 0.83 aCD	54%	6.74 ± 0.24 aB	40%	6.39 ± 0.47 C	47%
Mean	7.83 ± 1.29 a		7.41 ± 0.92 a		7.62 ± 0.79	

Values with different capital letters in the same column (fining agent) and different small letters in the same row (wines) are statistically different Tukey’s test ( $p < 0.05$ ). Mean values ± standard deviation. PPI: pea protein isolate. SPI: soy protein isolate.

There was an approximated 50 % reduction of these compounds, and they contribute to the browning more intensely than other phenolics (Lee and Jaworski, 1988), and there is strong evidence of epicatechin being the most relevant browning agent among redox-active polyphenols (Sioumis *et al.*, 2006). As for the oligomeric flavan-3-ols, the pea protein was found to reduce their levels in the two evaluated wines, while the IPS did not differ from the animal proteins in the two wines. Granato *et al.* (2018) observed the opposite behavior in the clarification of a young red wine, where the IPS reduced more oligomeric flavan-3-ols than IPE. This finding is important because the molecular size of the polyphenolic compounds present in wine affects its bitterness and astringency (Peleg *et al.*, 1999).

In all cases, the addition of fining agents caused a decrease in total tannins. The reduction promoted by IPS was similar to animal protein (10 % in both wines), while the most significant decrease was obtained by PPI (15 %). In a study by Jiménez-Martínez *et al.* (2017) with clarification of red wine, IPE promoted greater reduction (64 %) of tannins when compared to two porcine origin gelatins, one mannoprotein-rich yeast autolyzed product, and pomace cell wall material.

Concerning the total anthocyanin content for both wines, the reduction was similar in all treatments, presenting only significant difference compared to the control wine. Similar results were reported by Jiménez-Martínez *et al.* (2019), where an evaluation in 'Monastrell' and 'Merlot' wines did not show a statistical difference for the total anthocyanins when treated with gelatin, albumin, or plant protein. In another study, gelatin presented a greater reduction of anthocyanins compared to the pea protein isolate (Aziz *et al.*, 2017a).

Regarding the soy protein isolate, the trend of responses was similar to that of the other tested protein fractions, which is an important contribution, given the ease of obtaining this product. The wine to be fined influences the protein and its effect on the reduction of phenolic compounds. The action of SPI in fining is similar to the animal proteins, such as for the monomeric and oligomeric flavan-3-ols ('Lambrusco Maestri' wine), and is similar to pea protein isolate, as in the case of the polymeric flavan-3-ols ('L. Maestri wine'). However, for the 'Merlot' wine, this pattern was not observed, reinforcing that

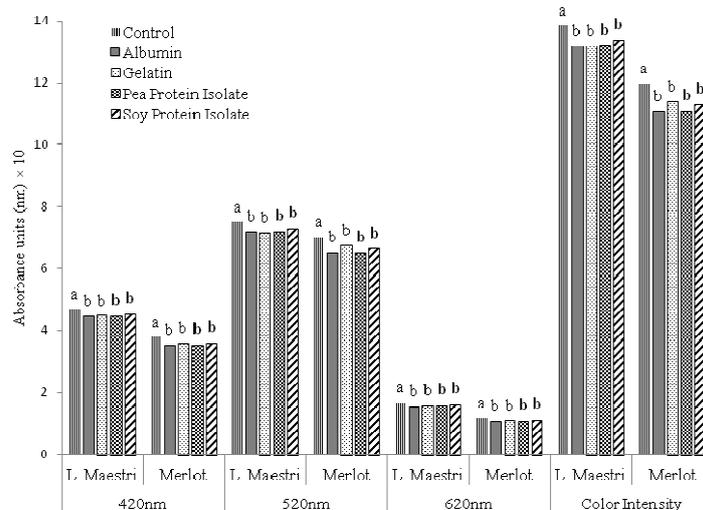
the optimal fining protein might vary depending on the wine.

### Wine color

Changes in the red wine color related to the fining treatments were analyzed by UV/V spectrophotometry at 420, 520, and 620 nm. For all the color parameters evaluated, no significant differences were observed between the four fining agents tested, even though they differed from the control wine. In a previous study, the fining of 'Raboso' red wine caused the reduction in red color (520 nm), and similar results were obtained using gelatin, albumin or pea protein as fining agents (Gazzola *et al.*, 2017). In another study by Jiménez-Martínez *et al.* (2019) the color intensity obtained after fining of 'Monastrell' and 'Merlot' red wines with albumin, gelatin or plant protein did not show significant differences but were smaller than the control wine.

All treatments using the fining agent proteins presented a reduction in chromatic characteristics (Figure 3). There was a decrease in absorbance at 420, 520, and 620 nm, representing yellow, red, and blue colors, respectively. A similar result was reported by Iturmendi *et al.* (2010), Cosme *et al.* (2012), Granato *et al.* (2018), and Kang *et al.* (2018), who studied the fining by vegetable protein from different sources. The SPI exhibited similar behavior to other commercial protein fining agents.

The reduction of color intensity is an expected result of fining treatments (Cosme *et al.*, 2007; Gambuti *et al.*, 2012). The anthocyanin content as well as the molecules that interact with anthocyanins, such as the tannins (Boulton, 2001), were reduced by the addition of fining proteins; the reduction of color intensity can be justified by this phenomenon, as reported by Harrison (2018). In this context, SPI was similar to other protein agents. Aziz *et al.* (2017b) did not find significant differences for the color intensity while fining with polyvinylpyrrolidone, gelatin, or pea protein isolate on 'Cabernet Sauvignon' wine. González-Neves *et al.* (2014) found that anthocyanin content was slightly reduced by the use of albumin and egg gelatin, and was not affected by the use of vegetable proteins (gluten proteins) in most of the red wines of 'Tannat' evaluated in the study. A similar behavior concerning the decrease in color intensity compared to the control wines was observed in the present study.



**Figure 3.** Chromatic characteristics of ‘Lambrusco Maestri’ and ‘Merlot’ wines treated with egg albumin, porcine gelatin, pea protein isolate and soy protein isolate. For each fining protein values followed by different letters are significantly different ( $p < 0.05$ , Tukey’s test).

*Características cromáticas dos vinhos ‘Lambrusco Maestri’ e ‘Merlot’ tratados com albumina de ovo, gelatina suína, isolado proteico de ervilha e isolado proteico de soja. Para cada clarificante proteico, valores seguidos por letras diferentes são significativamente diferentes ( $p < 0.05$ , teste de Tukey).*

### Turbidity

After seven days, all treated wines had turbidity ranging between 1.4 and 18.2 NTU; similar values to those reported by Granato *et al.* (2018). The turbidity was significantly reduced by the use of fining

proteins, and there was no significant difference between the responses generated by SPI and PPI (Table III). Furthermore, the animal proteins promoted a greater reduction than the proteins of vegetable origin.

**TABLE III**

Turbidity (NTU) and percentage of turbidity decrease in the wines after treatments

*Turbidez (NTU) e percentagem de diminuição da turbidez nos vinhos após os tratamentos*

Finishing agent	L. Maestri		Merlot		Mean	
	Value	Reduction (%)	Value	Reduction (%)	Value	Reduction (%)
Control	69 ± 1.2		26.5 ± 1.0		47.7 ± 9.5 A	
Albumin	14.1 ± 1.4	79%	1.4 ± 0.5	95%	7.8 ± 2.9 C	84%
Gelatin	14.9 ± 1.4	78%	2.3 ± 1.0	91%	8.6 ± 2.9 C	82%
PPI	17.5 ± 0.9	75%	5.1 ± 0.3	81%	11.3 ± 2.8 B	76%
SPI	18.2 ± 0.5	74%	5.7 ± 0.7	79%	11.9 ± 2.8 B	75%
Mean	26.7 ± 5.7 a		8.2 ± 2.5 b		17.5 ± 3.5	

Values with different capital letters in the same column (finishing agent) and different small letters in the same row (wines) are statistically different Tukey’s test ( $p < 0.05$ ). Mean values ± standard deviation. PPI: pea protein isolate. SPI: soy protein isolate.

This effect on reduction was observed in both wines, indicating that for turbidity, there was no interaction between the factors and the wine proteins. The reduction promoted by animal proteins (albumin and gelatin) was 84 % and 82 %, respectively, while the

vegetable protein promoted a 76 % and 75 % reduction for the PPI and SPI, respectively. These values are similar to those found by Granato (2010).

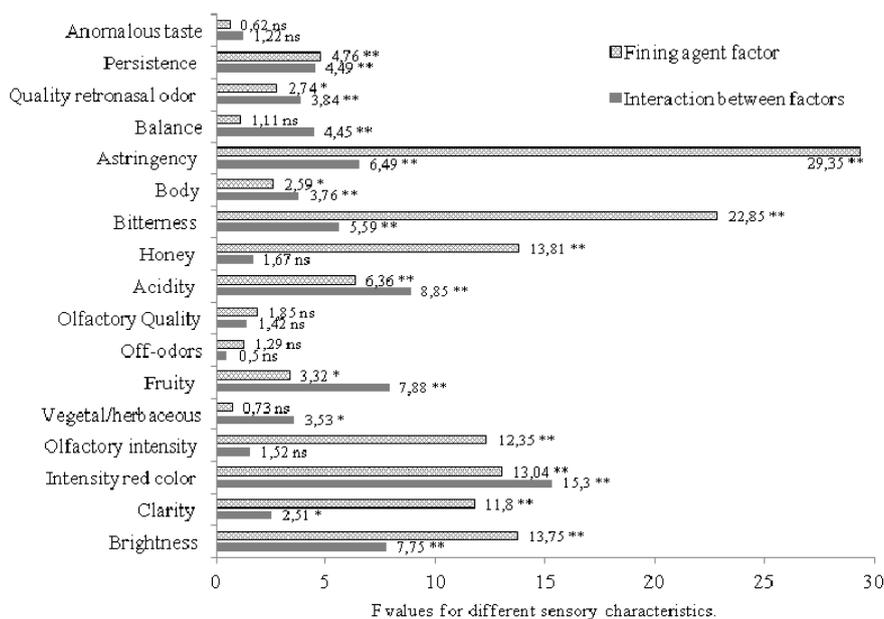
For the ‘L. Maestri’ wine, the results presented minor differences between the plant and animal proteins,

with a reduction of 74 % (SPI) up to 79 % (albumin), while for ‘Merlot’ wine the reduction results were between 79 % (SPI) up to 95 % (albumin). In the clarification of ‘Raboso’ red wine, Gazzola *et al.* (2017) observed that the most effective fining agent was egg albumin, while the pea proteins could not clarify the wine tested. Granato *et al.* (2018) observed lower turbidity values for fining with pea and soy protein isolate compared to gelatin application, after seven days of the fining in red wine.

### Sensory analysis

The treatments (fining agent) and the interaction between factors (wine type and the fining agent) showed significant differences for most of the properties evaluated (Figure 4).

F-values for the interaction between factors (wine and the fining agent) were significant ( $p < 0.01$ ) for 10 of the 17 attributes, indicating the influence of the characteristics of the wine on the sensory effects of fining using different proteins. Of the 17 sensory characteristics evaluated, 12 showed significant F-values for the fining agent factor, indicating that the astringency and bitterness attributes, as well as the visual properties and olfactory intensity, were the most affected by protein fining agents. A handful of previous studies have reported similar results on the interaction between the fining agent and the wine type (Sims *et al.*, 1995; Sarni-Manchado *et al.*, 1999; Gómez-Plaza *et al.*, 2000; Maury *et al.*, 2001; Bonerz *et al.*, 2004; Karamanidou *et al.*, 2011; Vincenzi *et al.*, 2013; Granato *et al.*, 2018; Kang *et al.*, 2018).



**Figure 4.** F values for fining agent factor and the interaction between wine and fining agent factors obtained in sensory evaluation by descriptive quantitative analysis. ns - not significant; \* significantly different ( $p < 0.05$ ); \*\* very significantly different ( $p < 0.01$ ).

Valores de F para o fator cola e a interação entre os fatores vinho e clarificante obtidos na avaliação sensorial por análise quantitativa descritiva. ns – não significativo; \* diferença significativa ( $p < 0.05$ ); \*\* diferença muito significativa ( $p < 0.01$ ).

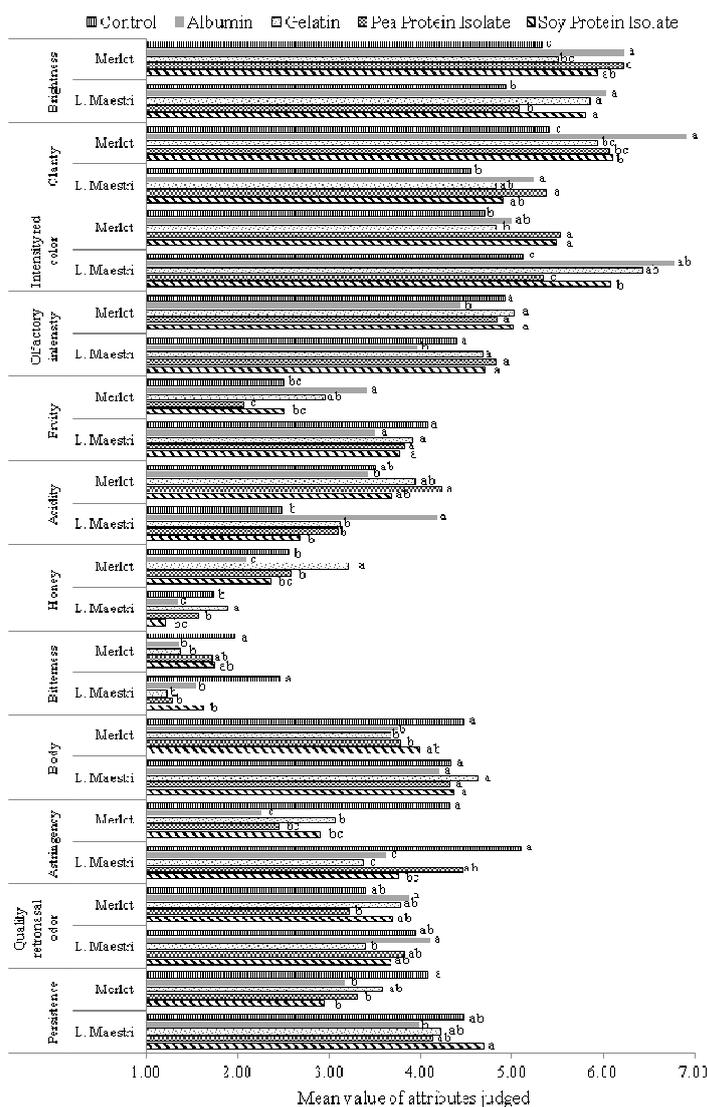
The fining with SPI did not provide negative sensory changes; in fact, its effects were close to other commercial proteins evaluated. In ‘L. Maestri’ wine, good results were observed with SPI, compared to PPI, regarding the brightness and intensity of the red color (Figure 5). For the sensorial profiles of fine wines (Figures 6a and 6b), only the characteristics that showed statistical differences ( $p < 0.05$ ) were presented.

The SPI decreased the bitterness and astringency, similarly to other commercial fining agents, showing no statistical differences ( $p > 0.5$ ) between the types of fining agents studied. These results corroborated with Kang *et al.* (2018), who observed that the application of gelatin, pea protein isolate, and soy protein isolate did not present statistical differences in the reduction of astringency. Gazzola *et al.* (2017) also observed similar results in the reduction of astringency using

albumin, soy protein isolate, and grape seed extract for red wine. However, both studies found no reduction in bitterness with these fining agents.

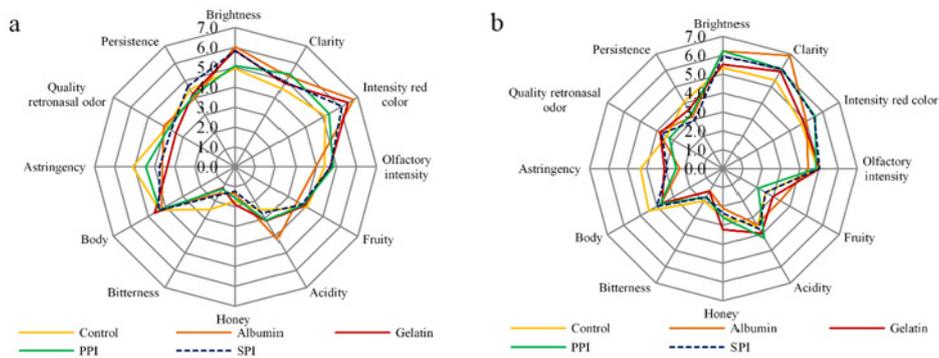
The reduction of bitterness and astringency is essential, as it is a requirement for a protein fining agent. The effect of fining proteins in reducing these characteristics is attributed to the interaction of the proteins with phenolic compounds, especially tannins,

which are the main components responsible for astringency (Luck *et al.*, 1994; Soares *et al.*, 2017). It is previously reported that low molecular weight phenolic compounds, such as monomeric and oligomeric flavan-3-ols, might influence the bitterness of wines (Hufnagel and Hofmann, 2008; Sáenz-Navajas *et al.*, 2010).



**Figure 5.** Values found for the studied attributes applied in sensorial evaluation of ‘Lambrusco Maestri’ and ‘Merlot’ wines treated with different fining agents (egg albumin, porcine gelatin, pea protein isolate and soy protein isolate). For each fining protein values followed by different letters are significantly different ( $p < 0.05$ , Tukey’s test).

Valores encontrados para os atributos estudados aplicados em avaliação sensorial de vinhos ‘Lambrusco Maestri’ e ‘Merlot’ tratados com diferentes colas (albumina de ovo, gelatina de suino, isolado de proteína de ervilha e isolado de proteína de soja). Para cada clarificante proteico, valores seguidos por letras diferentes são significativamente diferentes ( $p < 0.05$ , teste de Tukey).



**Figure 6.** Sensory profiles of red wine obtained by the mean of the scores given by the panellists. Impact of fining on the sensory characteristics of the ‘Lambrusco Maestri’ (a) and ‘Merlot’ (b) wines. PPI – pea protein isolate. SPI – soy protein isolate.

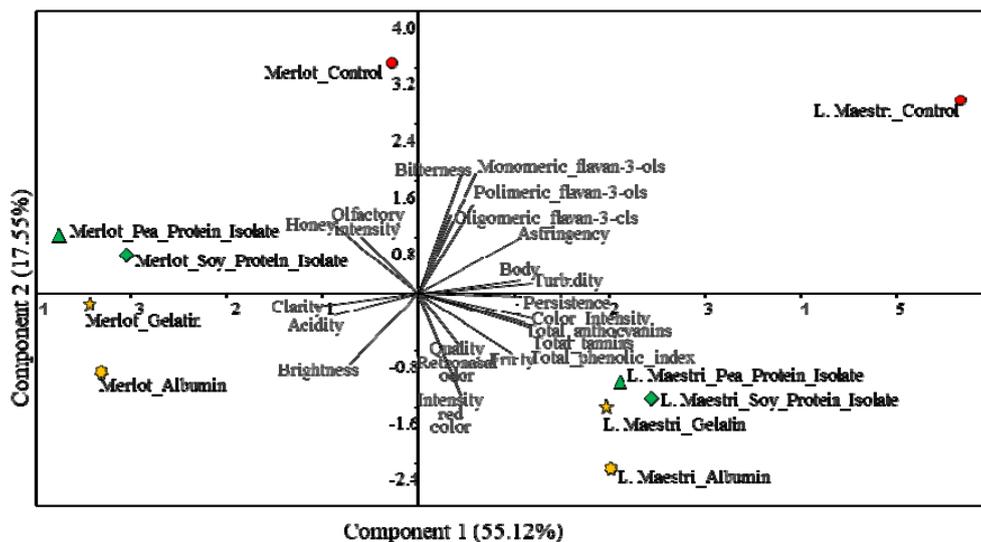
*Perfis sensoriais dos vinhos tintos obtidos pela média das pontuações dadas pelos painelistas. Impacto da clarificação sobre as características sensoriais dos vinhos ‘Lambrusco Maestri’ (a) e ‘Merlot’ (b). PPI – isolado proteico de ervilha. SPI – isolado proteico de soja.*

### Principal component analysis

To better evaluate the differences and similarities between the fining treatments, the data were standardized using Principal Component Analysis (PCA). Taken together, the first two principal components (PC1 and PC2) explain 72.67 % of the total variation. The choice of the parameters used was based on the analysis of variance, only the response variables that presented significant F for the fining agent factor were used in the analysis: turbidity, oligomeric flavan-3-ols, polymeric flavan-3-ols, monomeric flavan-3-ols, total phenolic index, total

tannins, total anthocyanins, color intensity, clarity, red color intensity, brightness, olfactory intensity, fruity, body, astringency, bitterness, honey, acidity, quality retronasal odor and persistence.

The two wines used in this study are mainly represented by PC1 (Figure 7), the ‘Lambrusco Maestri’ wine has positive values, while the ‘Merlot’ wine has negative values.



**Figure 7.** PCA representation of wines (control and fined ones) and the physico-chemical and sensory variables in the plane defined by the first two principal components.

*Representação PCA de vinhos (controle e clarificados) e as variáveis físico-químicas e sensoriais no plano definido pelos dois primeiros componentes principais.*

The control treatment had larger values in both wines and both components, appearing in the upper right coordinates for the wines subjected to fining. These have lower values (relative to their respective controls) in both PC1 and PC2, illustrating that protein fining, regardless of the protein, promotes a reduction in bitterness, astringency, turbidity, and body, and cause an increase in the clarity, brightness, intensity of the bright red color, and feel of acidity. The animal proteins, especially albumin, promoted, in a comprehensive manner, a considerable change from the original profiles of the wines. Moreover, compared to albumin and gelatin, the SPI had similar behavior, and PPI, promoting less significant changes compared to the control wines. In Figure 7, the results of PCA show that the fining agents have a similar effect in wine. However, the 'Merlot' wine is localized entirely on the left side, and the 'L. Maestri' wine is localized entirely on the right side of the factorial plan. This behavior is an indication that the performance of SPI is similar to other commercial proteins.

## CONCLUSIONS

The results of this study show that SPI is an enological alternative to the current commercial protein fining agents used in red wines. Its application responds similar to ovalbumin, porcine gelatin, and pea protein isolate, both in the physicochemical and sensory aspects. Like other protein agents, SPI reduced the phenolic composition of the two wines studied. The monomeric flavan-3-ols content was decreased, and a less astringency was observed in the two wines. There was a slight reduction in the anthocyanins and the color intensity of the wines with all proteins, including SPI, which was similar to the other results. SPI, like PPI, reduced the turbidity, but the animal proteins promoted a greater reduction. According to the PCA, the fining action of SPI is similar to that of the commercial proteins, especially PPI.

Further studies are necessary to investigate the binding reaction between SPI and the tannins present in wine at a molecular level, determining the most relevant factors that influence the formation of soluble and insoluble complexes.

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