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Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics

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ABSTRACT

The use of potassium caseinate for wine fining can cause allergic reactions and must be included on wine bottle labels. The focus of this research was to determine if non-allergenic pea protein or polyvinylpyrrolidone (PVPP) are possible alternatives for wine fining. Trials were performed to compare the effects of these potential fining agents on phenolic compounds, browning potential, turbidity and sensory attributes. The results indicate that flavonoid and non-flavonoid phenols decreased ($p < 0.05$) in the wines treated with potassium caseinate, pea protein, and PVPP. All fining agents decreased ($p < 0.05$) wine colour. Potassium caseinate was the most effective fining agent for reducing browning potential. Pea protein and potassium caseinate resulted in increased clarity of the final wines. No sensorial differences ($p < 0.05$) were detected among the wines prepared with the different fining agents. This study provides information that could be used to select an appropriate alternative to potassium caseinate.

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1. Introduction

Fining agents are commonly used in wine production to clarify, to control browning and to improve stability and/or organoleptic characteristics (Lefebvre, Sieczkowiak, & Vidal, 2005; Marchal et al., 2002).

After identifying contaminated cow products as the origin of Creutzfeldt-Jacob disease (Bovine Spongiform Encephalopathy), the risks associated with the use of animal proteins for wine fining were evaluated, and some were immediately banned from foods and beverages (e.g., blood albumin). This has led to several markets only selecting/selling wines fined with vegetable proteins (Lefebvre, Restani, & Scotti, 2003). Since 2005, European legislation has authorised the use of vegetable proteins as fining agents and also authorised the use of pea protein, according to recent legislation (Reg. CE606/2009 Annex IA). An additional issue is the allergenic characteristics of animal proteins used in food/beverage manufacturing, which could present a public health risk; in response, the European Parliament adopted Directive 2007/68/EC amending 2000/13/EC (Weber, Steinhart, & Paschke, 2007; 2009).

Therefore, the use of milk proteins, particularly casein, in wine processing will need a labelled declaration. This was initially mandated to take effect the 31st of May 2009, but has been suspended until the 31st of June 2012 due to the limited available scientific data (Teissedre, 2011). This protein is characterised by sequences of hydrophobic and hydrophilic amino acids, resulting in an amphiphilic character with micelle-forming properties (Weber et al., 2007). Casein is insoluble in the normally acidic pH of wine but soluble at alkaline pH. Potassium caseinate is derived from casein by dissolving it in aqueous potassium hydroxide and then spray drying the solution. It is used in wine production rather than casein due to its higher solubility (Weber et al., 2009). Fining agents are used to remove oxidisable and oxidised phenolic compounds of white and red wines by adsorption (Caillet, 1994). These proteins are employed in large quantities in wine production. For example, in France, 2060 million L per year of wine are fined with casein, which corresponds to 41% of French wines (Weber et al., 2007). In addition, up to 20% of German wines are fined with casein (Deutscher Weinverband, 2010).

A potential alternative to casein is the synthetic water-insoluble polymer polyvinylpyrrolidone (PVPP), which can effectively adsorb phenols from wine (Laborde et al., 2006). PVPP could be used alone or in combination with much smaller quantities of casein and still effectively reduce wine oxidation through removal

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Table 1
Total phenolic index, non-flavonoids, flavonoids, total phenols, turbidity, browning potential, chromatic characteristics and colour of both fined and unfined white wine at a laboratory scale (mean $\pm$ SD).

Fining treatment	TPI	Non-flavonoids (mg/L gallic acid)	Flavonoids (mg/L gallic acid)	Total phenols (mg/L gallic acid)	Turbidity	Browning Potential ^{II}	L* (%)	a*	b*	Chromatic C*	Characteristics C*	h°	ΔE^*	Colour ^I
T	5.63 $\pm$ 0.08 ^a	89 $\pm$ 2 ^a	122 $\pm$ 1 ^a	210 $\pm$ 2 ^a	0.28 $\pm$ 0.04 ^b	0.011 $\pm$ 0.002 ^a	90.1 $\pm$ 0.1 ^a	-0.32 $\pm$ 0.02 ^a	2.44 $\pm$ 0.03 ^a	2.46 $\pm$ 0.03 ^a	97.48 $\pm$ 0.55 ^a	0.066 $\pm$ 0.003 ^a		
VP	5.01 $\pm$ 0.11 ^b	85 $\pm$ 1 ^{bc}	106 $\pm$ 4 ^{bc}	191 $\pm$ 2 ^b	0.08 $\pm$ 0.04 ^d	0.006 $\pm$ 0.001 ^{ab}	96.3 $\pm$ 0.1 ^e	-0.32 $\pm$ 0.02 ^a	1.73 $\pm$ 0.04 ^c	1.76 $\pm$ 0.05 ^c	100.50 $\pm$ 0.67 ^b	6.19 $\pm$ 0.11 ^a	0.058 $\pm$ 0.001 ^c	
CK	5.03 $\pm$ 0.12 ^b	84 $\pm$ 1 ^c	108 $\pm$ 5 ^{bc}	192 $\pm$ 4 ^b	0.13 $\pm$ 0.04 ^c	0.003 $\pm$ 0.002 ^b	95.3 $\pm$ 0.0 ^d	-0.49 $\pm$ 0.04 ^b	1.71 $\pm$ 0.04 ^c	1.78 $\pm$ 0.02 ^c	106.18 $\pm$ 2.07 ^c	5.17 $\pm$ 0.07 ^a	0.059 $\pm$ 0.009 ^{bc}	
PVPP	4.88 $\pm$ 0.09 ^b	83 $\pm$ 1 ^c	104 $\pm$ 3 ^c	187 $\pm$ 2 ^b	0.45 $\pm$ 0.04 ^a	0.007 $\pm$ 0.001 ^{ab}	90.6 $\pm$ 0.0 ^b	-0.34 $\pm$ 0.02 ^a	1.99 $\pm$ 0.05 ^b	2.02 $\pm$ 0.05 ^b	99.66 $\pm$ 0.65 ^b	0.63 $\pm$ 0.05 ^c	0.059 $\pm$ 0.002 ^{bc}	
VP/PVPP	5.56 $\pm$ 0.19 ^a	86 $\pm$ 1 ^b	118 $\pm$ 3 ^{ab}	208 $\pm$ 2 ^a	0.15 $\pm$ 0.00 ^c	0.008 $\pm$ 0.001 ^a	94.4 $\pm$ 0.0 ^c	-0.51 $\pm$ 0.01 ^b	1.70 $\pm$ 0.04 ^c	1.78 $\pm$ 0.05 ^c	106.52 $\pm$ 0.27 ^c	4.38 $\pm$ 0.05 ^b	0.054 $\pm$ 0.003 ^c	

Unfined wine (T), pea protein (VP), pea protein and PVPP (VP/PVPP), potassium caseinate (CK), TPI – total phenolic index, ^I – absorbance unit; ^{II} – difference between with and without nitrogen; L* (%) – lightness, a* – redness, b* – yellowness, ΔE^* – total colour difference. The values corresponding to ΔE^* were obtained taking as a reference the unfined wine (T). Means within a column followed by the same letter are not significantly different (LSD, 5%).

of polyphenols in reduced and oxidised (quinones and quinone methides) forms, which includes simple phenolic acids and flavonoids. PVPP binds (poly)phenolics by hydrogen bonding between the PVPP-carbonyl group with the phenolic-hydroxyl groups (Donel, Bécard, & Irwin, 1993; Verza, Pavei, & Ortega, 2008) of both simple phenolics and flavonoids. Casein directly inhibits the oxidation of (poly)phenols to quinones/quinone methides and may also remove these highly reactive oxidation products by non-enzymatic formation of casein–quinone conjugates (Hurrell, Finot, & Cuq, 1982; Kroll, Rawel, & Rohn, 2003; Rawel, Kroll, & Kulling, 2007; Ribéreau-Gayon, Glories, Maujaen, & Dubourdieu, 1999). Therefore, PVPP could be an allergen free alternative to casein; however, PVPP is considerably more expensive than casein (at least two-fold more) but is more effective at lower concentrations.

Commercial pea protein is a non-GMO protein, and it is used in vegan wine production. It is increasingly being considered as an alternative to casein because under current legislation, it would not have to be labelled as an allergen (Walker, Camarena, & Freeman, 2007). Cattaneo et al. (2003) detected no significant residues in red or white wine fined with pea protein. Similarly, in wines fined with pea protein at the maximum dose allowed (0.5 g/L) there were no protein residues or any allergenicity (Lefebvre et al., 2003; 2005). Additionally, it has been shown that wines treated with fining agents at commercial concentrations and made according to standard processes do not appear to present a risk to allergic individuals, and no detectable amounts of fining agent remain in the wines (Fischerleitner & Eder, 2007; Kirschner, Belloni, Kugler, Ring, & Brockoe, 2009; Lifrani et al., 2009; Weber et al., 2007). The wine industry is currently finding it difficult to replace casein, a fining agent with fully characterised effects on wine physico-chemical and sensory characteristics (Amati, Galassi, & Spinabelli, 1979; Braga, Cosme, Ricardo-da-Silva, & Laureano, 2007; Cosme, Ricardo-da-Silva, & Laureano, 2008; Jouve et al., 1989; Sims, East-ridge, & Bates, 1995). The effects of potential new allergen free alternatives on wine chemical and sensory properties, such as pea protein, have been insufficiently studied. The main purpose of this work was to evaluate the technological potential of some allergen free fining agents (pea protein and PVPP) as an alternative to potassium caseinate. Alternative fining agents were tested by measuring important parameters for the wine industry such as effects on wine colour, chromatic characteristics, phenolic compounds and sensory characteristics.

2. Material and methods

2.1. Characteristics of the wine

Young white wine of 2007 vintage, made from two white grape varieties (60% of Trajadura and 40% of Loureiro) from the Vinho Verde Region (North of Portugal) was used. The main characteristics of the wine were as follows: alcohol content 90.7 g/L, titratable acidity 7.0 g/L as tartaric acid, volatile acidity 0.18 g/L as acetic acid, pH 3.37, free sulphur dioxide 44 mg/L and total sulphur dioxide 128 mg/L.

2.2. Fining experiments at laboratory and semi-industrial scales

The first experiment involved the addition of the individual fining agents (potassium caseinate (CK – 0.4 g/L); pea protein (VP – 0.4 g/L); polyvinylpyrrolidone (PVPP – 0.25 g/L) and a commercial formulation fining agent (composed of pea protein and PVPP (VP/PVPP – 0.25 g/L)) at laboratory and semi-industrial scales to compare the effects of the allergen free fining agents with potassium caseinate.

A second experiment was done at laboratory scale using four different commercial formulation fining agents: 1.) bentonite and potassium caseinate (B/CK – 0.65 g/L), 2.) bentonite and pea protein (B/VP – 0.65 g/L), 3.) bentonite, PVPP and potassium caseinate (B/PVPP/CK – 0.55 g/L) and 4.) bentonite, PVPP and pea protein (B/PVPP/VP – 0.55 g/L) to evaluate the possibility of replacing the commercial formulations of potassium caseinate with pea protein.

Standard quantities of the fining agents were prepared to the manufacturer's specifications. The experiments were conducted in 1000 mL flasks at laboratory scale and in 500 L tanks at semi-industrial scale. Wine containing no fining agent was used as a control. The fining agents were thoroughly mixed, added to each treatment and allowed to remain in contact with the wines for 7 days at 20 °C. Samples were then centrifuged at 537.6 × g for 15 min before analysis. All experiments were run in duplicate.

2.3. Quantification of flavonoid phenols and non-flavonoid phenols

The phenolic content of the wines was determined using the absorbance at 280 nm before and after precipitation of the flavonoid phenols through reaction with formaldehyde, according to Kramling & Singleton (1969). Using this method, flavonoid, non-flavonoid and total phenols in the wines were quantified. The results were expressed as gallic acid equivalents by means of calibration curves with standard gallic acid.

The polyphenolic content was also determined by a spectrophotometric method and expressed as a total phenolic index (TPI = $A_{280\text{nm}} \times \text{dilution factor}$).

2.4. Turbidity

Turbidity was evaluated by measuring the optical density at 650 nm before and after centrifugation as described by Feuillat & Bergeret (1966).

2.5. Test for browning potential

Test tubes were filled with 20 mL of the wine to be tested. Control and test samples were sparged thoroughly with nitrogen and oxygen, respectively. All tubes were sealed hermetically and maintained at 55 °C for 5 days. The test was conducted on treated and untreated wine, and the browning value difference was calculated by measuring the increase in $A_{420\text{ nm}}$ as recommended by Singleton & Kramling (1976).

2.6. Chromatic characterisation

The absorption spectra of wine samples were recorded with a Shimadzu UV-2101 spectrophotometer (Shimadzu, Kyoto, Japan) scanned from a range of 380–770 nm using 1 cm path length quartz cells. Data were collected to determine a measure of L^* (lightness), a^* (redness), and b^* (yellowness) coordinates using the CIE Lab method according to Organisation Internationale de la Vigne et du Vin (OIV, 2006). The Chroma [$C^* = [(a^*)^2 + (b^*)^2]^{1/2}$] and hue-angle [$h^\circ = \tan^{-1}(b^*/a^*)$] values were also determined. To distinguish the colour more accurately, the difference was calculated using the following equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ and reported in CIE Lab units. This allows reliable quantification of the overall colour difference of a sample when compared to a reference sample (unfinned sample). Colour differences can be distinguished by the human eye when the difference between ΔE^* values are greater than two units (Spagna et al., 1996).

Table 2
Total phenolic index, non-flavonoids, flavonoids, total phenols, turbidity, browning potential, chromatic characteristics and colour of both fined and unfined white wine at a semi-industrial scale (mean ± SD).

Fining agents	TPI	Non-flavonoids (mg/L gallic acid)	Flavonoids (mg/L gallic acid)	Total phenols (mg/L gallic acid)	Turbidity	Browning potential ^{II}	$L^*(\%)$	a^*	Chromatic b^*	Characteristics C^*	h°	ΔE^*	Colour ^I
T	5.49 ± 0.00 ^a	94 ± 1 ^a	112 ± 4 ^a	206 ± 1 ^a	1.25 ± 0.07 ^a	0.021 ± 0.002 ^a	90.5 ± 0.3 ^a	-0.54 ± 0.01 ^a	3.10 ± 0.04 ^a	3.14 ± 0.03 ^a	99.90 ± 0.36 ^a	–	0.071 ± 0.000 ^a
CK	4.78 ± 0.07 ^b	89 ± 1 ^b	94 ± 3 ^b	182 ± 2 ^b	0.85 ± 0.07 ^a	0.002 ± 0.001 ^c	95.2 ± 0.0 ^b	-0.54 ± 0.04 ^a	2.16 ± 0.01 ^c	2.23 ± 0.02 ^c	104.65 ± 0.86 ^b	4.87 ± 0.27 ^a	0.065 ± 0.000 ^b
PVPP	4.72 ± 0.34 ^c	87 ± 2 ^c	88 ± 9 ^c	173 ± 1 ^c	1.80 ± 0.28 ^b	0.006 ± 0.000 ^b	90.2 ± 0.0 ^a	-0.63 ± 0.04 ^b	2.41 ± 0.01 ^b	2.49 ± 0.02 ^b	103.30 ± 1.38 ^b	0.77 ± 0.29 ^b	0.060 ± 0.001 ^c
VP/PVPP	5.35 ± 0.02 ^a	89 ± 1 ^b	110 ± 2 ^a	201 ± 1 ^a	0.95 ± 0.07 ^a	0.007 ± 0.000 ^b	94.9 ± 0.6 ^b	-0.55 ± 0.01 ^a	2.31 ± 0.01 ^b	2.37 ± 0.09 ^b	104.04 ± 0.26 ^b	4.15 ± 0.30 ^a	0.065 ± 0.000 ^b

Unfinned wine (T), potassium caseinate (CK), pea protein and PVPP (VP/PVPP), TPI – total phenol index, ^I – total phenol index, ^{II} – Absorbance unit; – Difference between with and without nitrogen; $L^*(\%)$ – lightness, a^* – redness, b^* – yellowness, ΔE^* – total colour difference. The values corresponding to ΔE^* were obtained taking as a reference the unfined wine (T). Means within a column followed by the same letter are not significantly different (LSD, 5%).

Table 3
Total phenolic index, non-flavonoids, flavonoids, total phenols, turbidity, browning potential, chromatic characteristics and colour of both fined and unfined white wine at a laboratory scale (mean $\pm$ SD).

Fining treatment	TPI	Non-flavonoids (mg/L gallic acid)	Flavonoids (mg/L gallic acid)	Total phenols (mg/L gallic acid)	Turbidity	Browning Potential ^{II}	L* (%)	a*	b*	Chromatic Characteristics C*	h°	ΔE^*	Colour ^I
T	7.50 $\pm$ 0.02 ^a	89 $\pm$ 1 ^a	178 $\pm$ 0 ^a	267 $\pm$ 1 ^a	0.45 $\pm$ 0.07 ^a	0.012 $\pm$ 0.003 ^a	90.7 $\pm$ 0.1 ^a	-0.72 $\pm$ 0.03 ^a	2.67 $\pm$ 0.12 ^a	2.77 $\pm$ 0.12 ^a	104.99 $\pm$ 0.65 ^{ab}	—	0.063 $\pm$ 0.001 ^a
B/CK	6.65 $\pm$ 0.02 ^c	87 $\pm$ 0 ^b	154 $\pm$ 1 ^d	241 $\pm$ 1 ^c	0.18 $\pm$ 0.04 ^b	0.004 $\pm$ 0.001 ^b	96.2 $\pm$ 0.9 ^c	-0.55 $\pm$ 0.03 ^b	2.05 $\pm$ 0.11 ^b	2.12 $\pm$ 0.10 ^b	106.03 $\pm$ 1.32 ^b	5.45 $\pm$ 0.96 ^c	0.060 $\pm$ 0.001 ^b
B/VP	6.55 $\pm$ 0.03 ^c	82 $\pm$ 0 ^d	156 $\pm$ 1 ^c	238 $\pm$ 1 ^d	0.15 $\pm$ 0.07 ^b	0.006 $\pm$ 0.001 ^{ab}	98.2 $\pm$ 0.3 ^d	-0.59 $\pm$ 0.06 ^b	2.14 $\pm$ 0.11 ^b	2.22 $\pm$ 0.10 ^b	104.33 $\pm$ 0.88 ^{ab}	7.46 $\pm$ 0.38 ^d	0.054 $\pm$ 0.001 ^d
B/PVPP/CK	6.65 $\pm$ 0.02 ^c	87 $\pm$ 0 ^b	155 $\pm$ 0 ^d	241 $\pm$ 1 ^c	0.45 $\pm$ 0.03 ^a	0.001 $\pm$ 0.001 ^b	91.2 $\pm$ 0.6 ^a	-0.52 $\pm$ 0.02 ^b	1.61 $\pm$ 0.18 ^c	1.69 $\pm$ 0.16 ^c	112.97 $\pm$ 2.69 ^c	1.26 $\pm$ 0.35 ^a	0.060 $\pm$ 0.001 ^b
B/PVPP/VP	6.69 $\pm$ 0.03 ^b	83 $\pm$ 0 ^c	159 $\pm$ 1 ^b	242 $\pm$ 1 ^b	0.25 $\pm$ 0.00 ^b	0.007 $\pm$ 0.001 ^{ab}	94.0 $\pm$ 0.4 ^b	-0.69 $\pm$ 0.09 ^a	2.21 $\pm$ 0.20 ^b	2.32 $\pm$ 0.18 ^b	103.36 $\pm$ 1.40 ^a	3.33 $\pm$ 0.76 ^b	0.057 $\pm$ 0.001 ^c

Unfined wine (T), bentonite/potassium caseinate (B/CK), bentonite/pea protein (B/VP), bentonite/PVPP/potassium caseinate (B/PVPP/CK), and bentonite/PVPP/pea protein (B/PVPP/VP). TPI – total phenol index, ^I – absorbance unit; ^{II} – difference between with and without nitrogen; L* (%) – lightness, a* – redness, b* – yellowness, ΔE^* – total colour difference. The values corresponding to ΔE^* were obtained taking as a reference the unfined wine (T). Means within a column followed by the same letter are not significantly different (LSD, 5%).

Table 4

Sensory analysis of unfined and fined wines (mean $\pm$ SD).

	Visual	Aroma	Taste
<i>Laboratorial scale trial</i>			
T	13 $\pm$ 3 ^a	30 $\pm$ 3 ^a	35 $\pm$ 2 ^a
VP	13 $\pm$ 3 ^a	28 $\pm$ 5 ^a	33 $\pm$ 5 ^a
CK	13 $\pm$ 2 ^a	31 $\pm$ 2 ^a	33 $\pm$ 3 ^a
PVPP	13 $\pm$ 3 ^a	32 $\pm$ 5 ^a	35 $\pm$ 1 ^a
VP/PVPP	14 $\pm$ 2 ^a	32 $\pm$ 3 ^a	34 $\pm$ 4 ^a
<i>Semi-industrial scale trial</i>			
T	14 $\pm$ 3 ^a	33 $\pm$ 3 ^a	35 $\pm$ 2 ^a
CK	13 $\pm$ 3 ^a	32 $\pm$ 4 ^a	36 $\pm$ 3 ^a
PVPP	14 $\pm$ 3 ^a	30 $\pm$ 5 ^a	36 $\pm$ 4 ^a
VP/PVPP	14 $\pm$ 3 ^a	33 $\pm$ 3 ^a	36 $\pm$ 3 ^a
<i>Laboratorial scale trial</i>			
T	13 $\pm$ 2 ^a	32 $\pm$ 2 ^a	35 $\pm$ 3 ^a
B/CK	14 $\pm$ 3 ^a	33 $\pm$ 4 ^a	37 $\pm$ 3 ^a
B/VP	13 $\pm$ 2 ^a	27 $\pm$ 3 ^b	32 $\pm$ 5 ^a
B/PVPP/CK	13 $\pm$ 3 ^a	30 $\pm$ 3 ^{ab}	34 $\pm$ 4 ^a
B/PVPP/VP	14 $\pm$ 3 ^a	32 $\pm$ 5 ^{ab}	35 $\pm$ 4 ^a

T – Unfined wine, VP – pea protein, VP/PVPP – pea protein and PVPP, CK – potassium caseinate, B/CK – bentonite/potassium caseinate, B/VP – bentonite/pea protein, B/PVPP/CK – bentonite/PVPP/potassium caseinate, B/PVPP/VP – bentonite/PVPP/pea protein. Means within a column followed by the same letter are not significantly different (Duncan, 5%).

2.7. Analysis of conventional oenological parameters and colour

Alcohol content, pH, titratable acidity, volatile acidity, free and total sulphur dioxide were measured according to the Organisation Internationale de la Vigne et du Vin methods (OIV, 2006).

Colour was determined by measuring absorbance at 420 nm (10 mm cell) using a Shimadzu UV-2101 spectrophotometer (Shimadzu, Kyoto, Japan) in line with the Organisation Internationale de la Vigne et du Vin methods (OIV, 2006).

2.8. Sensory evaluation

The sensory analysis was performed by a trained panel with extensive wine tasting experience. The samples were stored at appropriate light and temperature conditions (20 °C). The wines were presented in three sessions; two for fining trials at laboratory scale and another for a fining trial at semi-industrial scale. Samples were presented to the panel in tasting glasses marked with three digit numbers and in a randomised order. Fifteen attributes were selected: visual (limpidity, ton and colour intensity), aroma (limpidity, intensity, finesse, harmony and vegetable) and taste (flavour limpidity, flavour intensity, body, flavour harmony, persistence, mouth end and vegetable flavour). The attributes were quantified using a five-point intensity scale (ISO 4121, 2003). A total sensory score was calculated for each wine as the sum of an average score of visual, aroma and taste attributes. All evaluations were conducted from 10:00 to 12:00 A.M. in an individual booth (ISO 8589, 2007) and according to standardised procedures (ISO 3591, 1977).

2.9. Statistical analysis

The data are presented as mean $\pm$ standard deviation. Both physicochemical and sensory data were statistically tested by analysis of variance (ANOVA) using the Statistica 7 software (Statsoft, OK, USA). Fisher's least significant difference (LSD, 5% level) test was applied to physicochemical data to determine significant differences between the fining treatments. Duncan's multiple range test (MRT) was applied to sensory data to determine significant differences between the fining treatments. The model was statistically significant when *p* values were less than 0.05.

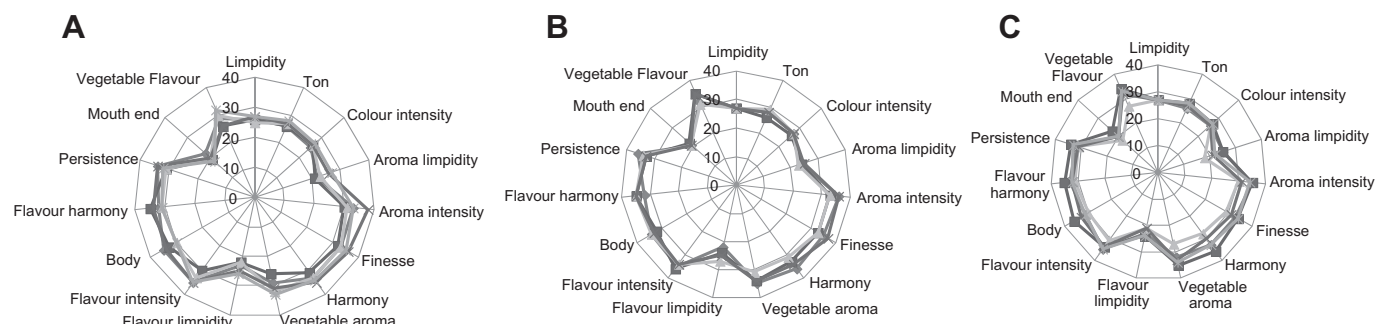


Fig. 1. Sensory profiles of white wine obtained by the mean of the scores given by the panellists at (A) laboratory scale, (◆) – T, (■) – VP, (▲) – CK, (×) – PVPP, (*) – VP/PVPP (B) semi-industrial scale, (◆) – T, (■) – CK, (▲) – PVPP, (×) – VP/PVPP and (C) laboratory scale, (◆) – T, (■) – B/CK, (▲) – B/VP, (×) – B/PVPP/CK, (*) – B/PVPP/VP. T – (unfined wine), VP – pea protein, VP/PVPP – pea protein and PVPP, CK – potassium caseinate, B/CK – bentonite and potassium caseinate, B/VP – bentonite and pea protein, B/PVPP/CK – bentonite, PVPP and potassium caseinate, B/PVPP/VP – bentonite, PVPP and pea protein.

3. Results and discussion

3.1. Effect of the fining agents on the total phenols, non-flavonoid and flavonoid compounds

The results indicate that the fining agents tested decreased the amount of total phenols (1–11%), flavonoid (3–15%) and non-flavonoid (3–7%) compounds significantly ($p < 0.05$), with the exception of the formulation VP/PVPP (Table 1). The fining agents have a greater influence on flavonoid phenols than on other polyphenols as previously observed (Amati et al., 1979; Cosme et al., 2008; Marais & Allen, 1994; Puig-Deu, López-Tamames, Buxaderas, & Torre-Boronat, 1996). The results obtained in the fining trials conducted at semi-industrial scale (Table 2) are in accordance with the results from the trial at laboratory scale (Table 1). When comparing classic fining agent formulations containing potassium caseinate with alternative fining agent formulations containing pea protein, the alternative formulation had a greater effect on the non-flavonoid phenols; however, all formulations reduced non-flavonoid, flavonoid and total phenols significantly ($p < 0.05$) when compared with the untreated wine (Table 3).

3.2. Effects of the fining agents on the colour, chromatic characteristics, clarity and browning potential

The main objective of white wine fining is to clarify and eliminate, by adsorptive precipitation, those compounds that could lead to turbidity or changes in colour. Potassium caseinate added alone or in a commercial fining agent formulation was more effective at reducing the browning potential ($p < 0.05$) in all experiments (Tables 1–3), leading to more stable wines in regard to oxidation. The browning of white wine is directly related to oxidation and represents one of the main stability problems. Phenolic compounds are known to be responsible for wine browning. Barroso, López-Sánchez, Otero, Cela, and Pérez-Bustamente (1989) recognised a relationship between browning susceptibility and the amount of phenolic compounds. Spagna, Barbagallo, & Pifferi (2000) suggested that the removal of polyphenols would stabilise white wine, resulting in diminished potential for browning. Browning results from the oxidation of (poly)phenols to quinones, which polymerise to form macromolecules with a yellow-brown colour (Baron, Mayen, Merida, & Medina, 2000). Therefore, the reduction of the wine browning potential is essential to the wine producers because the visual characteristics of the white wine are important for the market.

Wine colour (expressed as the absorbance at 420 nm) was significantly reduced ($p < 0.05$) by each fining agent tested in all experiments (Tables 1–3).

The clarification capacity was higher for pea protein alone, followed by the formulations with PVPP or with bentonite, as observed in the two laboratory scale experiments (Tables 1 and 3). These results are in agreement with the study by Walker et al. (2007), who observed a good clarification in beer fined with pea protein. In this work, PVPP was not effective in increasing wine clarity in either laboratory (Table 1) or semi-industrial experiments (Table 2), suggesting that sedimentation of the small particles that compose PVPP powder could be difficult by gravity alone.

The results obtained with the CIELab method for determining the chromatic characteristics of the unfined and fined wine at laboratory and semi-industrial scale are presented in Tables 1–3. After application of PVPP, lightness (L^*) was unchanged, suggesting no clarifying action (Tables 1 and 2). These results are in agreement with the turbidity data. The value for b^* (yellowness) decreased significantly ($p < 0.05$) with all fining agents assayed; however, the decrease was greater in all experiments fined with potassium caseinate, indicating a higher reduction in the yellow intensity of the fined wine (Tables 1–3). Chroma (C^*) is a parameter that indicates the contribution of a^* (redness) and b^* (yellowness). The value of C^* decreased significantly after addition of pea protein, potassium caseinate and formulations of pea protein with PVPP. The hue-angle (h°) values increased after addition of pea protein, potassium caseinate, PVPP and the formulations of pea protein with PVPP; however, the h° values were greater in potassium caseinate and formulations of pea protein with PVPP. This observation could indicate that some yellow pigments were removed. According to Spagna et al. (1996) colour variation (ΔE^*), which is the geometric mean of ΔL^* , Δa^* and Δb^* , can be visually discriminated by the human eye when it is greater than 2 CIELab units. Between each wine and the unfined wine, the greatest values obtained for the ΔE^* occurred for potassium caseinate and pea protein, followed by the formulations of pea protein with PVPP or pea protein with bentonite (Tables 1–3).

3.3. Effect of the fining agents on sensory evaluation

No sensory differences were detected among the wines fined at laboratory or semi-industrial scales (Table 4). Previously, Walker et al. (2007) found few discernible differences among assays using pea protein. These data, combined with previous findings, suggest that the addition of pea protein has no sensorial impact on alcoholic beverages (Fig. 1).

4. Conclusions

Potassium caseinate, pea protein and PVPP decreased the concentration of total phenolics, non-flavonoids and flavonoids.

However, only potassium caseinate alone, or mixed in commercial formulations with other fining agents, was effective at reducing the wine browning potential. These results are in agreement with the b^* value from the CIELab. No sensory differences were detected among the wines subjected to the different fining treatments.

These results suggest that the use of pea protein could be an alternative for potassium caseinate, but it is less effective at reducing the white wine browning potential. However, due to the risk of allergic reactions, the search for an allergen free alternative for potassium caseinate with equal or greater browning inhibition activity is essential for the wine industry. Therefore, more detailed research is needed in order to identify and formulate an alternative and cost-effective fining agent.

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References

- Amati, A., Galassi, S., & Spinabelli, U. (1979). Sull'impiego del "Caseinato potassico" nella stabilizzazione dei vini bianchi. II – Effetti dell'aggiunta al vino. *Vignevini*, 6, 27–33.
- Baron, R., Mayen, M., Merida, J., & Medina, M. (2000). Comparative study of browning and flavan-3-ols during the storage of white sherry wines treated with different fining agents. *Journal of the Science of Food and Agriculture*, 80, 226–230.
- Barroso, C. G., López-Sánchez, L., Otero, J. C., Cela, R., & Pérez-Bustamente, J. A. (1989). Studies on the browning of fino sherry and its relation to polyphenolic compounds. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 189, 322–325.
- Braga, A., Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2007). Gelatine, casein and potassium caseinate as wine fining agents: effect on colour, phenolic compounds and sensory characteristics. *Journal International des Sciences de la Vigne et du Vin*, 4, 203–214.
- Caillat, M. M. (1994). Stabilisation et clarification des vins par le collage. *Revue des Oenologues*, 74, 15–18.
- Cattaneo, A., Ballabio, C., Bernardini, R., Bertelli, A. A. E., Novembre, E., Vierucci, A., et al. (2003). Assessment of residual immunoreactivity in red or white wines clarified with pea or lupin extracts. *International Journal on Tissue Reactions*, 4, 159–165.
- Cosme, F., Ricardo-da-Silva, J. M., & Laureano, O. (2008). Interactions between protein fining agents and proanthocyanidins in white wine. *Food Chemistry*, 106, 536–544.
- Deutscher Weinverband. (2010). www.dvw-online.de.
- Donel, L. W., Bécard, G., & Irwin, P. L. (1993). Binding of flavonoids by polyvinylpyrrolidone. *Journal of Agricultural and Food Chemistry*, 41, 753–757.
- Feuillat, M., & Bergeret, J. (1966). Détermination de la limpidité des mouts et vins. In J. Blouin (Ed.), *Manuel Pratique d'Analyse des Mouts et des Vins*. Gironde: Chambre d'Agriculture de la Gironde.
- Fischerleitner, E., & Eder, R. (2007). Nachweis von Caseinrückständen im Wein mittels Elektrophorese und western blotting. *Mitteilungen Klosterneuburg*, 57, 23–28.
- 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, 191–211.
- ISO 3591. (1977). *Sensory analysis-apparatus—Wine-tasting glass*. Retrieved November 20 2008 from <http://www.iso.org/iso/rss.xml?3Fcsnumber%3D9002%26rss%3Ddetail>.
- ISO 4121. (2003). *Sensory analysis—Guidelines for the use of quantitative response scales*. Retrieved November 20 2008 from http://www.iso.org/iso/catalogue_detail.htm?3Fcsnumber%3D33817.
- ISO 8589. (2007). *Sensory analysis—General guidance for the design of test rooms*. Retrieved November 20 2008 from http://www.iso.org/iso/catalogue_detail.htm?3Fcsnumber%3D36385.
- Jouve, C., Cabanis, J. C., Bourzeix, M., Heredia, N., Rosec, J. P., & Vialatte, C. (1989). Teneurs en catéchines et procyanidols de vins blancs et rose; Effets du collage par la caséine. *Revue Française d'Oenologie*, 117, 14–20.
- Kramling, T. E., & Singleton, V. L. (1969). An estimate of the nonflavonoid phenols in wines. *American Journal of Enology and Viticulture*, 20, 86–92.
- Kirschner, S., Belloni, B., Kugler, C., Ring, J., & Brockoe, K. (2009). Allergenicity of wine containing processing aids: a double-blind, placebo-controlled food challenge. *Journal of Investigational Allergology and Clinical Immunology*, 19, 210–217.
- 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, 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, 4383–4389.
- Lefebvre, S., Restani, P., & Scotti, B. (2003). L'utilisation des protéines végétales en oenologie: le point sur l'autorisation et les risques d'allergie. *Revue Française d'Oenologie*, 202, 10–14.
- Lefebvre, S., Sieczkowski, N., & Vidal, F. (2005). La sécurité alimentaire en oenologie: cas des protéines végétales. *Revue Française d'Oenologie*, 210, 23–30.
- Lifrani, A., dos Santos, J., Dubarry, M., Rautureau, M., Blachier, F., & Tome, D. (2009). Development of animal models and sandwich-ELISA tests to detect the allergenicity and antigenicity of fining agents residues in wines. *Journal of Agricultural and Food Chemistry*, 57, 525–534.
- Marais, J., & Allen, C. (1994). Effect of PVPP on total polyphenol and flavanoid concentration in white wine. *The Australian Grapegrower & Winemaker*, 12, 74–75.
- Marchal, R., Marchal-Delahaut, L., Michels, F., Parmentier, M., Lallement, A., & Jeandet, P. (2002). Use of wheat gluten as clarifying agent of musts and white wines. *American Journal of Enology and Viticulture*, 53, 308–314.
- Rawel, H. M., Kroll, J., & Kulling, S. (2007). Effect of non-protein components on the degradability of proteins. *Biotechnology Advances*, 25, 611–613.
- 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.
- OIV (Organisation International de la Vigne et du Vin). (2006). *Récueil de Méthodes Internationales d'Analyse des Vins et des Moûts*. Paris: Edition Officielle.
- Sims, C. A., Eastridge, J. S., & Bates, R. P. (1995). Changes in phenols, color, and sensory characteristics of muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. *American Journal of Enology and Viticulture*, 46, 155–158.
- Singleton, V. L., & Kramling, T. E. (1976). Browning of white wines and an accelerated test for browning capacity. *American Journal of Enology and Viticulture*, 27, 157–160.
- Spagna, G., Pifferi, P. G., Rangoni, C., Mattivi, F., Nicolini, G., & Palmonari, R. (1996). The stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food Research International*, 29, 241–248.
- Spagna, G., Barbagallo, R. N., & Pifferi, P. G. (2000). Fining treatments of white wines by means of polymeric adjuvants for their stabilization against browning. *Journal of Agricultural and Food Chemistry*, 48, 4619–4627.
- Teissedre, P. L. (2011). Allergènes en usages dans les pratiques oenologiques. *Revue des Oenologues*, 138, 7–8.
- Puig-Deu, M., López-Tamames, E., Buxaderas, S., & Torre-Boronat, M. C. (1996). Influence of must racking and fining procedures on the composition of white wine. *Vitis*, 35, 141–145.
- UE Directive 2007/68/EC dated November 28. (2007). Amending DIRECTIVE 2000/13/EC as regards certain food ingredients. *Official Journal of the European Union* L 310–L 311, November 28, 2007.
- Verza, S. G., Pavei, C., & Ortega, G. G. (2008). Study of the specificity of cross-povidone (PVPP) as binding agent in the quantification of polyphenolic compounds. *Journal of the Brazilian Chemical Society*, 19, 1627–1633.
- Walker, S. L., Camarena, M. C. D., & Freeman, G. (2007). Alternatives to isinglass for beer clarification. *Journal of the Institute of Brewing*, 113, 347–354.
- Weber, P., Steinhart, H., & Paschke, A. (2007). Investigation of the allergenic potential of wines fined with various proteinogenic fining agents by ELISA. *Journal of Agricultural and Food Chemistry*, 55, 3127–3133.
- Weber, P., Steinhart, H., & Paschke, A. (2009). Determination of the bovine food allergen casein in white wines by quantitative indirect ELISA, SDS-PAGE, western blot and immunostaining. *Journal of Agricultural and Food Chemistry*, 57, 8399–8405.