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Performance of a protein extracted from potatoes for fining of white musts

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ABSTRACT

In this study, the potentiality of Patatin (P), a protein extracted from potato, as must fining agent was investigated on musts obtained from two South Italy grape *cultivars* (Falanghina and Greco). Besides P, fining agents as bentonite (B) and potassium caseinate (C) were assayed at different concentrations. The rate of sedimentation, the decline of turbidity during time, the absorbance at 420 nm, the GRP (grape reaction products) and hydroxycinnamic acids (HCA) concentrations were determined.

The comparative trials showed that P is a suitable fining agent to prevent browning and decrease haze during must settling because its effect on grape phenolics, brown pigments and turbidity is comparable and/or better than that detected for C. Its use as single fining agent or in combination with B depends on must characteristics.

1. Introduction

A clear appearance and the absence of haze are some of most important attributes for a white wine. A proper handling and settling of musts during pre-fermentative phases of winemaking can limit and/or avoid factors contributing to brown and haze of future white wine limiting oxidation and removing hazing material such as pectins, gums and proteins from musts. After grape crushing various reactions occurred, the most important are oxidations due to the enzyme-catalyzed oxidation of hydroxycinnamates (HCA) to correspondent quinones, which determine changes in tone and color intensity responsible for must browning (Cheynier, Basire, & Rigaud, 1989; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). HCA *o*-quinones can be trapped by natural occurring glutathione (GSH) to form 2-S-glutathionyl HCA usually referred to as GRP (grape reaction product) (Singleton, Salgues, Zaya, & Trousdale, 1985). GRP is colorless and limits reactions leading to browning competing with it. Therefore the sensitivity of musts to oxidative reactions of several white grape cultivars can be listed on the base of their HCA/GRP ratios (Cheynier, Souquet, & Moutounet, 1989). In a less extent also other phenolics (flavanols) constituting the whole pool of phenolics of musts are correlated with their browning potential (Cheynier, Rigaud, Souquet, Duprat, & Moutounet, 1990).

During winemaking the action of oxidative enzymes, as well as the presence of proteic and pectic material can be minimized by the early removal of suspended material from must. Besides limit the browning susceptibility and turbidity of future wine, the opportune removal of grape solids from must enhances ester production and limits the release of fusel alcohols during alcoholic fermentation, resulting in a global increase of wine aroma quality (Liu, Gallander, & Wilker, 1987; Moio, Ugliano, Gambuti, Genovese, & Piombino, 2004; Singleton, Sieberhagen, de Wet, & van Wyk, 1975). Several fining agents increase the efficiency with which must can be settled and make the precipitation of suspended solids easier. The fining agents most commonly added to the grape musts are bentonite, potassium caseinate and the synthetic polymer poly-viny-lpoly-pirrolidone PVPP. The main effect of bentonite is protein precipitation by adsorption and neutralization charge (Manfredini, 1989a), potassium caseinate mainly remove oxidizable and oxidized phenolic compounds by adsorption (Caillet, 1994) while PVPP is widely used in wine industry to remove phenolics and brown quinones (Sims, Eastridge, & Bates, 1995). For these reasons the color is more stable in white wine obtained from musts clarified using fining agents (Amati, Galassi, & Spinabelli, 1979; Manfredini, 1989b).

Due to the increasing problem of allergic reactions to foods and to the potential allergenicity of milk proteins (Asero et al., 2009), starting from July 1, 2013 the use of potassium caseinate during

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the production of wines must be labeled (Commission Regulation (EU) No. 1266/2010). Moreover, some strict vegetarians, such as vegans, do not accept any beverage treated with products of animal origin. These problems seem to be solvable by using plant origin proteins. Until now the vegetable proteins admitted for the fining of musts and wines are derived from wheat and pea (Reg. CE606/2009 Annex IA). Although the efficacy of wheat proteins in the removal of Chardonnay must turbidity (Marchal et al., 2002), their use is limited by the incidence of rare but severe allergic reactions when they are present in foods and cosmetics (Laurière et al., 2006; Pecquet, Bayrou, Vigan, Raison, & Laurière, 2004). Concerning pea proteins, it has been shown that they are less effective than potassium caseinate in reducing white wine browning potential (Cosme, Capão, Filipe-Ribeiro, Bennett, & Mendes-Faia, 2012). Therefore the search of different vegetable proteins to be used as alternative for potassium caseinate is still a necessity. Recently the Patatin (P), a protein extract from potatoes (*Solanum tuberosum*) has been shown a valid fining agent for the treatment of red wines to decrease their astringency (Gambuti, Rinaldi, & Moio, 2012). Patatin has chemical characteristics (an apparent molecular mass of about 40 kDa and an isoelectric point of 4.6) similar to that of animal proteins generally used as fining agent in enology (egg albumin and casein). However the potentiality of this protein as fining agent for white musts is still unknown. In this study the ability of this protein to facilitate must settling and decrease browning sensitivity of white musts obtained from two South Italy grape *cultivars* (Falanghina and Greco) was investigated.

2. Materials and methods

2.1. Fining experiments

Falanghina and Greco musts, provided by Cantina del Taburno (Foglianise, BN), were treated with casein (C), bentonite (B) and Patatin (P). These fining agents were used individually or combined at different concentrations. The doses (all in g/hL) are reported as dimensionless number near the letter indicating the fining agent used for the treatment (e.g. FALPB20 = Falanghina must + 20 g/hL Patatin + 20 g/hL bentonite). The fining treatments were: C20, C30 and C50 = treatments with 200, 300 and 500 mg/L of C; B20, B30 and B50 = treatments with 200, 300 and 500 mg/L of B; P20, P30 and P50 = treatments with 200, 300 and 500 mg/L of P; CB20, CB30 and CB50 = mixed treatments with 200 mg/L of C + 200 mg/L of B, 300 mg/L of C + 300 mg/L of B and 500 mg/L of C + 500 mg/L of B; PB20, PB30 and PB50 = mixed treatments with 200 mg/L of P + 200 mg/L of B, 300 mg/L of P + 300 mg/L of B and 500 mg/L of P + 500 mg/L of B. All fining experiments were done in duplicate in 500 mL cylinders. Just after crushing and pressing musts were added with 8 g/hL $K_2S_2O_5$ and with the fining agents. After 20 h at 10 ± 2 °C musts were separated from lees and the supernatant was analyzed. The chemical characteristics of musts used to perform the fining experiments were reported in Table 1. The two musts were chosen to perform the experiment because of Greco must is more sensitive to browning than Falanghina as showed by the differences in Abs 420 nm and ratio HCA/GRP (Table 1). Two control musts for each variety were considered. They differed for turbidity, Abs 420 nm and GRP content.

2.2. Enological products

Patatin P was supplied by Solanic (Veendam – The Netherlands). Producers guaranteed that the Patatin was not from genetically modified organism. The activated granular bentonite (Top Gran DC) was furnished by Dal Cin SPA (Concorezzo. MB, Italy), potassium caseinate were supplied by Oliver Ogar (San Giovanni Lupatoto, Verona, Italy). Bentonite was rehydrated 12 h before fining in a ratio 1:10 (w/v) with water.

2.3. Physical and spectrophotometric parameters

Turbidity was measured with a Hach turbidimeter before clarification and after 3, 6 and 20 h of treatment with fining agents. Flocculating rate was calculated as reported by Yokoi, Natsuda, Hirose, Hayashi, & Takasaki, 1995. Volume of the sediment during clarification was recorded at 3, 6 and 20 h from the beginning. Absorbance at 420 and 325 nm was detected before and after the treatment (0 time and after 20 h) through a Shimadzu UV-1800 spectrophotometer and then the reduction of Abs_{420} and Abs_{325} was calculated.

2.4. Total phenols content (TPC)

TPC was estimated by a colorimetric method (Folin–Ciocalteu) and referred to as milligrams per liter of gallic acid equivalents (GAE).

2.5. Polyphenolic composition by HPLC–DAD

Musts were analyzed by direct injection (Spanos & Wrolstad, 1990), after filtration through 0.45 μm filter (Durapore membrane filters. Millipore – Ireland). HCA and GRP were detected at 320 nm. Peaks were identified by comparing their retention time and UV–Vis spectra with the pure standard and they were quantified with the external standard method. An Agilent Eclipse XDB-C18, 4.6 × 150 mm, 5 μm was used for the separation. The column was thermostated at 40 C. All standards were dissolved in methanol, and results are expressed in mg/L of must.

Table 1
Chemical characteristics of musts used to perform the fining experiments.

	Falanghina must FALcontrol1	Falanghina must FALcontrol2	Greco must GREcontrol1	Greco must GREcontrol2
Soluble solids (°Brix)	23.03 ± 0.15	23.13 ± 0.06	22.70 ± 0.10	22.93 ± 0.15
Titrable acidity (g/L tartaric acid)	7.72 ± 0.13	7.76 ± 0.15	8.4 ± 0.08	8.35 ± 0.05
pH	3.30 ± 0.01	3.24 ± 0.02	3.18 ± 0.02	3.10 ± 0.02
Turbidity (NTU)	525 ± 3	691 ± 1	1450 ± 19.8	463 ± 16
Abs_{420nm}	0.454 ± 0.007	0.226 ± 0.001	1.553 ± 0.011	1.096 ± 0.001
HCA[a] (mg/L)	52.769 ± 6.234	66.370 ± 2.159	36.915 ± 2.584	43.242 ± 0.837
TPC[b] (mg/L GAE)	510 ± 47	466 ± 17	532 ± 27	525 ± 34
GRP[c] (mg/L)	3.883 ± 0.320	6.391 ± 1.541	0.441 ± 0.015	1.382 ± 0.009
HCA/GRP	13	10	84	31

[a] HCA = total HCA (sum of caftaric acid + coutaric acid + fertaric acid + caffeic acid).
[b] TPC = total phenolic compounds.
[c] GRP = grape reaction product.

2.6. GRP identification and quantification

GRP was identified at HPLC–DAD comparing the spectrum with that reported by Cheynier, Trousdale, Singleton, Salgues, and Wylde (1986) and it was quantified as caftaric acid. Its identification, however, was confirmed by injection to LC–MS with the same conditions of HPLC–DAD method. The presence of the pseudo-molecular ion (m/z = 618) at the same retention time of GRP peak at HPLC–DAD gave a further certainty of its identity.

2.7. Statistical analysis

All determinations were performed in duplicate and the present results are the average values of four determinations (two experimental × two analytical replicates). Analysis of Variance (ANOVA) was carried out using XLSTAT, version 2012.4.01 to compare data obtained from different musts. Fisher's Least Significant Differences (LSD) procedure was used to discriminate among the means of the variables when necessary. Differences at $P \leqslant 0.05$ were considered significant.

3. Results and discussion

The evolution of turbidity (Nephelometric Turbidity Units, NTU) of musts was monitored during 20 h of treatment (Figs. 1 and 2). For all musts analyzed, the decrease of haze observed after each treatment showed a promoting effect of P on must colloids flocculation. The flocculation of must colloids is due to decline of charge density by supplied fining agent. In agreement with previous studies (Watanabe, Suzuki, Sasaki, Nakashimada, & Nishio, 1999), the phenomenon is highly dependent on both the concentration and kind of proteic agent used as well as on the contemporary presence of bentonite. Fining is a process involving first coagulation of particles responsible for hazing, then their flocculation and, finally their sedimentation. The nature of proteins and/or bentonite (ionic charges, hydrophobicity, solubility etc.) used for fining is fundamental to induce or not flocculation and sedimentation and, as a consequence, result in the removal of potential haze precursors from musts and wines. The efficacy of this process depends on nature of colloids in matrix. Low concentration of fining agents or the absence of bentonite can leave coagulation and flocculation step incomplete. In contrast excessive fining can be detrimental as this may result in the introduction of potential haze precursors, such as proteins, in the juice and in the impoverishment of must in aroma precursors and yeasts nutritional factors. P was an efficient fining agent because, apart the sample GRE PB20, turbidity resulted always lower than that observed with other treatments. Because the rapidity of sedimentation is one of the most useful parameter for an enologist as it highly affects production costs, the flocculating rate was also calculated. The flocculating rate was higher for Greco (96.5%, 98.1% and 99.4% for P20, P30 and P50 respectively) than for Falanghina musts (76.2%, 85.3% and 96.3% for P20, P30

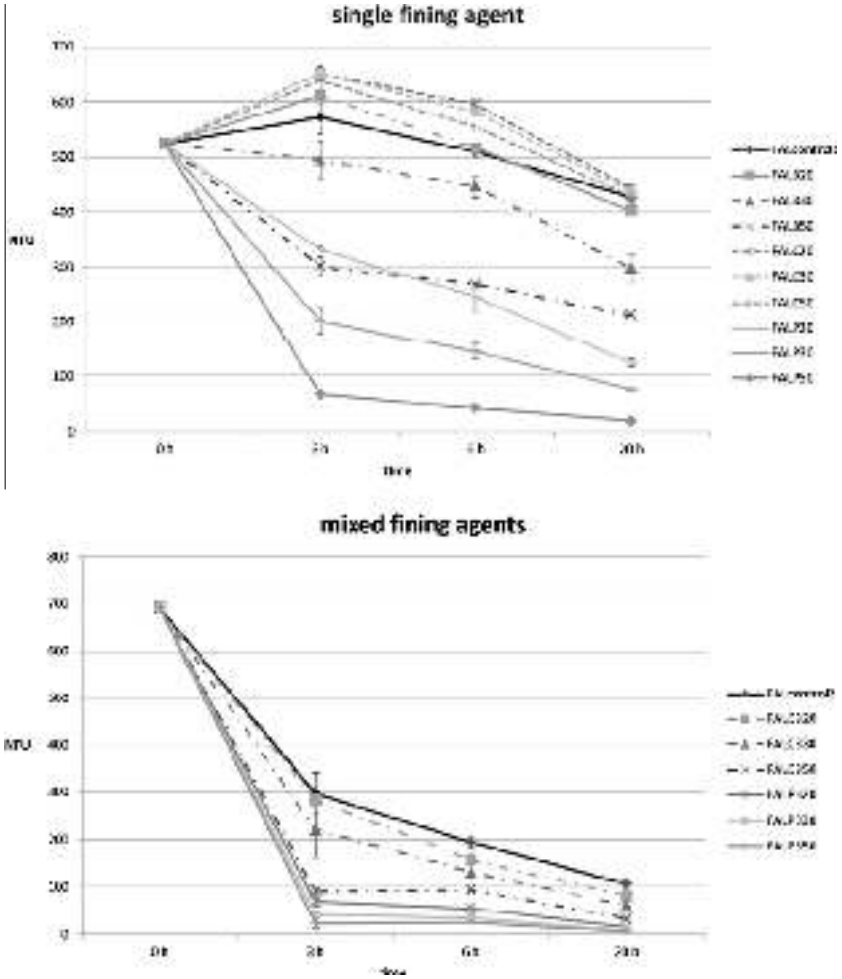

Fig. 1. Turbidity (NTU) decreasing during settling of Falanghina musts. The fining treatments were: C20, C30 and C50 = treatments with 200, 300 and 500 mg/L of C; B20, B30 and B50 = treatments with 200, 300 and 500 mg/L of B; P20, P30 and P50 = treatments with 200, 300 and 500 mg/L of P; CB20, CB30 and CB50 = mixed treatments with 200 mg/L of C + 200 mg/L of B, 300 mg/L of C + 300 mg/L of B and 500 mg/L of C + 500 mg/L of B; PB20, PB30 and PB50 = mixed treatments with 200 mg/L of P + 200 mg/L of B, 300 mg/L of P + 300 mg/L of B and 500 mg/L of P + 500 mg/L of B.

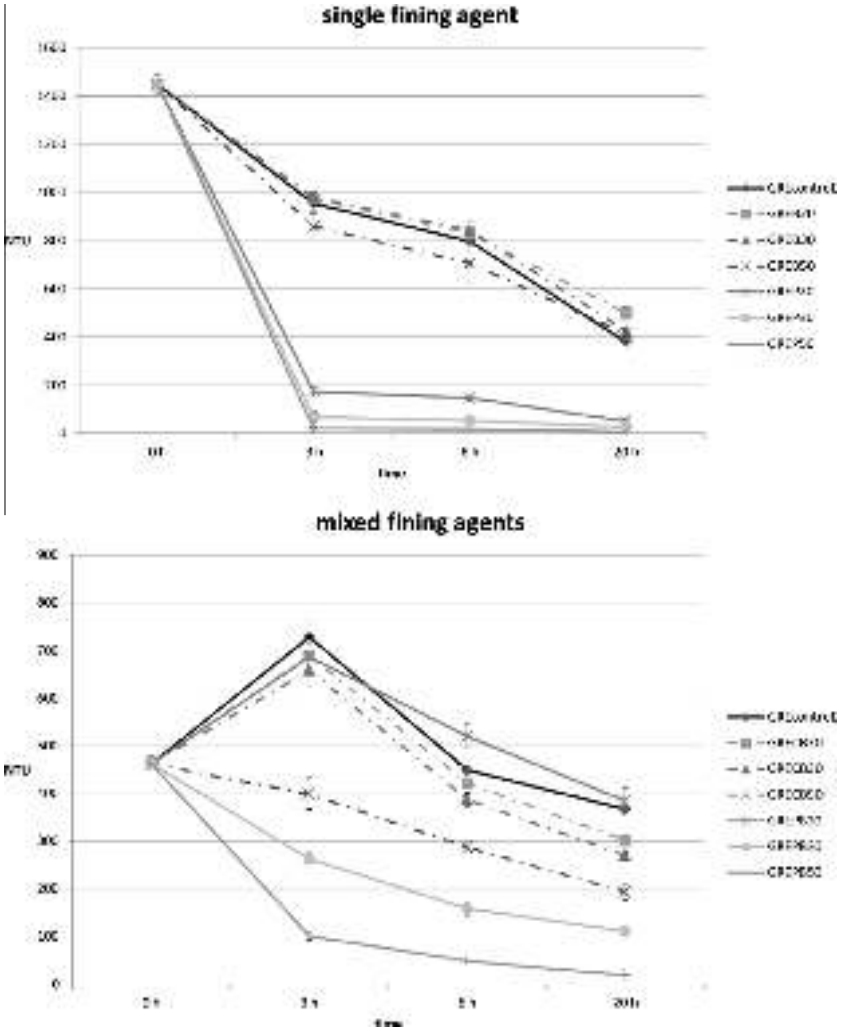

Fig. 2. Turbidity (NTU) decreasing during settling of Greco musts. Must treatments: see Fig. 1.

and P50 respectively) treated with P. Comparing the fining agents used, after 3 h of treatment, the flocculating rate of P and PB was always the highest: it was higher than 85% for Falanghina musts treated with P30 and P50 and for all Greco musts treated with P. In agreement with literature (Watanabe et al., 1999), both the effectiveness and the rate of settling depend on concentration used for the treatment. At the same time, this efficacy was also evident observing the volume of the formed sediment, which was always higher for Patatin treatments.

The absorbance at 420 nm (Abs_{420nm}) is considered an indicator of the degree of browning of musts and white wines during storage (Mayén, Barón, Mérida, & Medina, 1997). In spite of the fining treatment applied a decrease of Abs_{420nm} ($Abs_{420nmt=0} - Abs_{420nmt=20h}$) of Falanghina and Greco musts was observed after 20 h of settling (Table 2). The reduction of Abs_{420nm} was particularly high for Greco must treated whit Patatin. The different behaviors observed between musts may be related to differences in the amount of brown quinones in row material and/or into a different capability to absorb these substances in colloidal network originated by protein and bentonite addition. In view of the evidence that wines obtained from Greco grape cultivar are often characterized by a brown color (Moio, 2012), this result is of particular interest for enologists working with grape cultivars with similar characteristics.

For both parameters, the NTU and the Abs_{420nm}, as well as for flocculating rate, a varietal effect was observed: the best treatments were the coupled treatments (P + B) for Falanghina, while the treatments with P were the best for Greco musts. Coupled treatment with bentonite is usually performed on white musts rich in native proteins to reduce turbidity. This inorganic fining agent is capable of adsorbing haze active protein, but a very little effect on free and haze active polyphenols has been reported (Siebert & Lynn, 1997). Its use in mix with organic proteins during fining allows to obtain a network of colloids among proteins, phenolic compounds and the inorganic fining agent that can help flocculation and decreases musts haze. A difference between native colloids of the two musts considered, richer in proteins Falanghina and in polyphenols Greco (Minussi et al., 2003), could justify the differences observed.

Falanghina musts treated with Patatin had less TPC (287, 367, 382 mg/L GAE, corresponding to the doses 50, 30, and, 20 g/hL) than musts treated with other agents. The results clearly indicate that P is more effective in removing phenolics from medium than C. Potassium caseinate is usually used for fining of musts owing to the great ability to increase the adsorption of TPC with respect to the other fining agents used on white wine (Spagna, Barbagallo, & Pifferi, 2000). As C and P have a similar isoelectric point (Pots, de Jongh, Gruppen, Hessing, & Voragen, 1998) it is likely that, as occurs for caseinate, the precipitate formed after the aggregation of the colloids is able of taking the polyphenols into its protein structure by means of hydrogen bonds (Siebert, Troukhanova, & Lynn, 1996). In addition, due to the presence of

Table 2
Decrease of Abs_{420nm} ($Abs_{420nmt=0} - Abs_{420nmt=20h}$) of Falanghina and Greco musts after the treatment with fining agents.

$Abs_{420nmt=0} - Abs_{420nmt=20h}$	
Must sample	Mean ± sd
FALANGHINA single fining agents	
FALcontrol1	0.107 ± 0.008 a
FALC20	0.084 ± 0.007 a
FALC30	0.109 ± 0.001 a
FALC50	0.101 ± 0.026 a
FALB20	0.105 ± 0.003 a
FALB30	0.105 ± 0.001 a
FALB50	0.065 ± 0.025 a
FALP20	0.119 ± 0.014 a
FALP30	0.094 ± 0.004 a
FALP50	0.121 ± 0.004 a
GRECO single fining agents	
GREcontrol1	0.649 ± 0.093 b
GREB20	0.679 ± 0.089 b
GREB30	0.649 ± 0.137 b
GREB50	0.750 ± 0.054 b
GREP20	1.012 ± 0.003 a
GREP30	1.113 ± 0.003 a
GRE P50	1.220 ± 0.006 a
FALANGHINA mixed fining agents	
FALcontrol2	0.089 ± 0.016 e
FALPB20	0.273 ± 0.015 b
FALPB30	0.305 ± 0.003 a
FALPB50	0.328 ± 0.002 a
FALCB20	0.178 ± 0.010 d
FALCB30	0.224 ± 0.018 c
FALCB50	0.255 ± 0.008 b
GRECO mixed fining agents	
GREcontrol2	0.135 ± 0.008 f
GREPB20	0.224 ± 0.009 ef
GREPB30	0.659 ± 0.041 b
GREPB50	0.880 ± 0.002 a
GRECB20	0.349 ± 0.151 de
GRECB30	0.619 ± 0.120 bc
GRECB50	0.446 ± 0.212 cd

For each fining agent values followed by different letters on the column are significantly different ($p < 0.05$). Must treatments: see Fig. 1.

aromatic groups both in the proteins and in the polyphenol and to the high hydrophobicity of P (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2011), the formation of π–π type bonds is likely. Since a relationship between browning susceptibility and the amount of phenolic compounds in musts and wines (Barroso, Sánchez, Otero, Cela, & Pérez-Bustamante, 1989; Li, Guo, & Wang, 2008) has been shown, the removal of polyphenols would stabilize future white wine, resulting in diminished potential for browning.

Data on caftaric, coutaric, fertaric, caffeic, total HCA and GRP in treated musts are reported in Tables 3 and 4. It is well known that must browning is essentially due to the enzymatic oxidation of the hydroxycinnamates, which derived from mesocarp and placental cells of the pulp (Adams, 2006). The level of hydroxycinnamates detected for Fanghina and Greco musts fall in the range detected for *vinifera* varieties (Singleton, Zaya, & Trousdale, 1986). In agreement with literature (Rodríguez Montealegre, Romero Peces, Chacón Vozmediano, Martínez Gascueña, & García Romero, 2006; Singleton et al., 1986) caftaric acid (caffeoyl–tartaric) was the predominant HCA in both musts considered while low quantities of caffeic acid derive from hydrolysis of tartaric ester. A great difference in the content of HCA and GRP of untreated must was observed, higher HCA in Greco musts, higher GRP in Falanghina musts. According to Cheynier, Basire, et al. (1989a) and Cheynier, Souquet, et al. (1989b) musts samples can be separated into three classes: (1) light colored oxidized musts with low HCA concentrations, (2) intermediate musts and (3) dark oxidized musts with high HCA concentrations. In the third class, the maximum level of GRP was much lower and that of HCA higher. Therefore, in accordance with their more intense brown colors (Table 1) Greco musts can be classified as dark oxidized musts meaning that, probably, this cultivar had a lower content of glutathione in grape determining a weak protection over oxidation during first phases of winemaking (destemming, crushing and pressing). The evidence that the ratio HCA/GRP was higher in Greco musts (from 3 to 8-fold) than Falanghina ones (Table 1) supports this hypothesis.

Data on HCA and GRP in treated musts support the finding that P is capable to prevent future browning of wine (Tables 3 and 4). On Falanghina musts only the mixed treatments were effectiveness in decreasing caftaric acid and total HCA (Table 3). The effectiveness of PB mix on caftaric acid and total HCA were always lower than that of CBs mix. No significant effect was detected on GRP content of Falanghina musts. In the case of Greco musts (Table 4) P20 and P50 treatments decreased caftaric acid and total HCA while the mix PBs only decreased the content of caftaric acid. In contrast with data observed for Falanghina musts, all P and PBs

Table 3
Content of cinnamic acids (mg/L) and GRP (mg/L) of Falanghina musts after the treatment with fining agents.

	Caftaric acid Mean ± sd	Coutaric acid Mean ± sd	Fertaric acid Mean ± sd	Caffeic acid Mean ± sd	Total HCA Mean ± sd	GRP Mean ± sd
FALcontrol1	43.787 ± 0.743 a	4.075 ± 0.420 a	1.284 ± 0.115 a	0.123 ± 0.006 ab	49.269 ± 1.284 a	3.983 ± 0.178 a
FALC20	36.493 ± 0.148 b	3.544 ± 0.093 abc	1.155 ± 0.025 a	0.143 ± 0.028 a	41.335 ± 0.058 b	3.625 ± 0.008 ab
FALC30	36.536 ± 0.121 b	3.477 ± 0.005 abc	1.160 ± 0.006 a	0.099 ± 0.004 ab	41.272 ± 0.129 b	3.741 ± 0.158 ab
FALC50	39.407 ± 0.466 ab	3.696 ± 0.102 abc	1.201 ± 0.057 a	0.111 ± 0.035 ab	44.416 ± 0.272 ab	3.782 ± 0.017 ab
FALB20	37.053 ± 1.113 b	3.357 ± 0.068 bc	1.145 ± 0.029 a	0.085 ± 0.002 ab	41.640 ± 1.208 b	3.789 ± 0.088 ab
FALB30	35.657 ± 1.410 b	3.279 ± 0.091 c	1.110 ± 0.033 a	0.088 ± 0.005 ab	40.134 ± 1.529 b	3.493 ± 0.157 b
FALB50	37.945 ± 0.765 b	3.549 ± 0.057 abc	1.159 ± 0.046 a	0.091 ± 0.007 ab	42.745 ± 0.875 b	3.649 ± 0.083 ab
FALP20	42.864 ± 1.870 a	3.898 ± 0.137 abc	1.126 ± 0.053 a	0.097 ± 0.001 ab	47.985 ± 2.062 a	3.922 ± 0.061 ab
FALP30	43.653 ± 0.409 a	3.983 ± 0.001 ab	1.125 ± 0.000 a	0.097 ± 0.007 ab	48.857 ± 0.416 a	3.867 ± 0.065 ab
FALP50	43.564 ± 2.029 a	3.959 ± 0.186 ab	1.103 ± 0.048 a	0.080 ± 0.000 b	48.705 ± 2.263 a	3.873 ± 0.160 ab
FALcontrol2	48.022 ± 0.483 a	7.094 ± 0.372 a	8.215 ± 0.493 a	3.040 ± 0.812 a	66.370 ± 2.159 a	0.539 ± 0.013 ab
FALPB20	40.402 ± 1.628 b	6.627 ± 0.019 a	7.434 ± 0.279 a	2.319 ± 0.057 a	56.782 ± 1.310 b	0.557 ± 0.032 a
FALPB30	42.323 ± 0.475 b	7.251 ± 0.609 a	7.311 ± 0.294 a	0.573 ± 0.054 b	57.458 ± 0.481 b	0.533 ± 0.003 ab
FALPB50	41.237 ± 0.173 b	6.906 ± 0.029 a	7.354 ± 0.014 a	0.608 ± 0.049 b	56.105 ± 0.109 b	0.533 ± 0.001 ab
FALCB20	33.186 ± 0.829 c	6.333 ± 0.261 a	8.292 ± 0.185 a	0.719 ± 0.165 b	48.530 ± 0.918 c	0.489 ± 0.003 bc
FALCB30	33.858 ± 0.295 c	6.242 ± 0.031 a	8.316 ± 0.146 a	0.783 ± 0.211 b	49.199 ± 0.031 c	0.394 ± 0.006 d
FALCB50	32.193 ± 0.569 c	6.455 ± 0.215 a	7.753 ± 0.268 a	0.755 ± 0.118 b	47.156 ± 0.933 c	0.453 ± 0.016 c
FALcontrol2	48.022 ± 0.483 a	7.094 ± 0.372 a	8.215 ± 0.493 a	3.040 ± 0.812 a	66.370 ± 2.159 a	0.539 ± 0.013 ab

Total HCA = (caftaric acid + coutaric acid + fertaric acid + caffeic acid). For each fining agent values followed by different letters on the column are significantly different ($p < 0.05$). Must treatments: see Fig. 1.

Table 4
Content of cinnamic acids (mg/L) and GRP (mg/L) of Greco musts after the treatment with fining agents.

	Caftaric acid Mean ± sd	Coutaric acid Mean ± sd	Fertaric acid Mean ± sd	Caffeic acid Mean ± sd	Total HCA Mean ± sd	GRP Mean ± sd
GREcontrol1	30.390 ± 0.798 a	2.765 ± 0.230 bc	2.053 ± 0.000 a	0.707 ± 0.142 a	35.915 ± 1.170 a	0.441 ± 0.015 a
GREB20	26.710 ± 0.095 b	2.612 ± 0.022 ab	2.180 ± 0.005 a	0.580 ± 0.006 a	32.083 ± 0.118 b	0.385 ± 0.004 b
GREB30	26.477 ± 0.100 b	2.620 ± 0.004 ab	2.163 ± 0.013 a	0.638 ± 0.025 a	31.899 ± 0.142 b	0.456 ± 0.016 a
GREB50	22.304 ± 0.235 d	2.197 ± 0.061 c	1.842 ± 0.094 a	0.418 ± 0.132 a	26.762 ± 0.212 c	0.434 ± 0.002 a
GREP20	24.502 ± 0.736 bc	2.772 ± 0.056 bc	2.010 ± 0.055 a	0.546 ± 0.011 a	29.830 ± 0.859 bc	0.314 ± 0.005 c
GREP30	31.849 ± 1.401 a	3.908 ± 0.108 a	2.340 ± 0.078 a	0.507 ± 0.029 a	38.604 ± 1.616 a	0.325 ± 0.011 c
GREP50	24.934 ± 0.496 b	3.142 ± 0.051 ab	2.157 ± 0.094 a	0.695 ± 0.054 a	30.928 ± 0.587 b	0.296 ± 0.005 c
GREcontrol2	40.864 ± 0.212 a	2.338 ± 0.012 a	2.411 ± 0.052 a	0.643 ± 0.037 a	46.257 ± 0.209 a	1.822 ± 0.033 a
GREPB20	36.784 ± 1.493 b	2.329 ± 0.092 a	2.151 ± 0.070 ab	0.638 ± 0.063 a	41.902 ± 1.718 b	1.431 ± 0.041 b
GREPB30	36.333 ± 0.050 b	2.201 ± 0.068 ab	2.279 ± 0.111 ab	0.620 ± 0.028 a	41.433 ± 0.101 b	0.085 ± 0.000 e
GREPB50	34.020 ± 1.809 bc	2.124 ± 0.120 ab	2.360 ± 0.137 a	0.508 ± 0.090 a	39.012 ± 1.975 bc	0.850 ± 0.031 c
GRECB20	31.855 ± 1.032 c	1.894 ± 0.005 b	2.122 ± 0.100 ab	0.564 ± 0.014 a	36.435 ± 0.951 c	0.718 ± 0.043 d
GRECB30	33.306 ± 0.288 bc	1.918 ± 0.161 b	2.172 ± 0.111 ab	0.581 ± 0.023 a	37.977 ± 0.039 bc	0.799 ± 0.005 cd

Total HCA = (caftaric acid + coutaric acid + fertaric acid + caffeic acid). For each fining agent values followed by different letters on the column are significantly different ($p < 0.05$). Must treatments: see Fig. 1.

treatments significantly decreased the GRP content of Greco. Because of the scavenging of *o*-quinones by glutathione to produce colorless GRP can result in wines that have less propensity to brown enzymatically (Rigaud, Cheynier, Souquet, & Moutounet, 1991), it is interesting to find a fining treatment that, especially for easy-to-brown musts, decreases HCA limiting the removal of GRP. On the base of data only for Falanghina musts the PB treatments did not determine a significant decrease of GRP, in spite of the effectiveness in decreasing HCA (Table 3).

4. Conclusions

This study highlighted Patatin as a potential replacement of conventional fining agents used to improve the settling of white musts because of: (i) good flocculating activity (higher than 85% after 3 h of contact at the dose of 30 g/hL), (ii) a browning inhibition activity higher than potassium caseinate and (iii) the feature of being less allergic for human. Patatin is also economically attractive because its production allows to exploit waste water from potato processing that is a waste by-product. Therefore, more detailed researches are needed in order to evaluate the effect of important parameters such as pH and temperature on flocculating activity of P, as well as the capacity to retain important components of musts as aroma precursors or pest residues during settling.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.05.067.

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