Treatment with different fining agents of white musts from spoiled wine grapes

Eric Meistermann,¹ Michel Pinsun²

¹Pôle Alsace, Institut Français de la Vigne et du Vin, Colmar; ²Chambre d'Agriculture d'Alsace, Colmar, France

Abstract

Fining agents have evolved significantly over the last twenty years. The need for winemakers to have objective references about the new products has prompted this study. The experiment consists in comparing the new fining products (charcoal, pea proteins, derivatives of chitin and composite products generally mixing PVPP + various proteins + bentonite) with reference products such as casein and PVPP and with non-treated controls. Fining agents were applied during clarification of 15 different musts obtained from wine grapes affected by different degrees and types of rot: fresh and dry *Botrytis cinerea* rot, powdery mildew and *Botrytis* contaminated with other fungi (*Aspergillus*, *Penicillium*, Basidiomycetes), giving the bunches off-flavours that were earthy and resembled fresh mushrooms. This study highlights the importance of good clarification of musts. The quality of control wines, without any treatment, increases with the clarity of the must. In most experiments, clarification of the must around 50 Nephelometric Turbidity Units (NTU) can eliminate or reduce any organoleptic defects in wine without fining. Use of pectolytic enzymes may be necessary in order to reach this level of clarity. The new allergen-free fining agents have the same effectiveness as reference products such as casein and polyvinylpyrrolidone (PVPP). Composite products are more efficient than pea protein alone and less than products including charcoal. However, the improvement of aromatic quality goes hand in hand with loss of body and persistence.

Introduction

Historical wine fining agents are all animal proteins (edible gelatin, isinglass, casein and potassium caseinates, egg albumin). Use of inorganic fining and stabilisation agents is more recent: bentonite and

kaolin in the 1950s, silicon dioxide allowed by the EU in 1979 (EEC n°337/79) and polyvinylpyrrolidone (PVPP) allowed in 1988 (EEC n°2253/88).

Several constraints (bovine spongiform encephalopathy in the 1990s and the requirement to include all ingredients with allergenic effect in the labelling in 2012) have led manufacturers of oenological products to develop new fining agents. Early research focussed on plant proteins^{1,2} and led to authorisation of wheat and pea proteins in 2005 (CE n°2165/2005). Wheat gluten is considered a common allergen and thus is subject to labelling requirements. Research into allergy-friendly products concentrated on study of non-protein compounds. Derivatives of chitin are natural polysaccharides extracted from *Aspergillus niger* mycelium which have found widespread applications in oenology.³ They have been authorised by the EU since 2011 (CE n°53/2011). In order to favour compounds that are naturally present in wine, research focussed on yeast derivative products including yeast proteins.⁴ The European Union added the use of yeast protein extracts to its permitted oenological practices for the treatment of musts and wines (CE n°144/2013) and also a potato glycoprotein called patatin (CE n°1251/2013) which can diminish the content of phenolics in wine.^{5,6}

Grapevine bunch rots can impact must and wine quality negatively by increasing susceptibility to oxidation and the risks of wine faults and organoleptic deficiencies. The treatment of these musts with casein, used alone or in combination with bentonite or PVPP, was usually recommended.^{7,8} Owing to the allergenic characteristics of casein, its use in wine processing will require a declaration on the label, so producers of oenological products have developed allergen-free alternatives to casein. Moreover, since 2005, treatment with charcoal for oenological use of must and new wine still in fermentation is allowed by the European Union (CE n°2165/2005). The offer of products intended to treat musts from spoiled grapes has increased significantly over the last ten years. Winemakers needed objective technical references about these new products.

The aim of this study is to assess the effectiveness of many allergen-free fining products as alternatives to casein for the treatment of musts from grapes affected by rots and to provide winemakers with objective references. The evaluation focussed on colour, phenolic content and sensory characteristics.

Correspondence: Eric Meistermann. Biopôle, 28 rue de Herrlisheim, 68000 Colmar, France. Tel.: +33(0)3.89.30.22.72. E-mail: eric.meistermann@vignevin.com

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Materials and Methods

Characteristics of the musts

Experiments are set up after crushing non-clarified musts, either from mechanical harvesting and collected under the winepress in industrial conditions, or obtained in experimental winery from grapes selected in the vineyard. Fifteen trials were conducted during vintages 2011 to 2013. The sanitary state of the grapes was visually evaluated by determination of the botrytis severity (percentage of bunch area infected), the kind of botrytis rot (fresh or dry), the presence of other diseases (powdery mildew) and the presence of various fungi (*Aspergillus*, *Penicillium*, Basidiomycetes). The presence of off-flavours on bunches was also noted. These trials can be grouped in four categories according to the type of rot: i) *Botrytis low*: percentage of rot lower than 30%, fresh rot without other contamination. Five trials were made with the Pinot Gris grape variety and one with Riesling; ii) *Botrytis high*: more than 30% rot, fresh or

more dry rot. Two trials were made with the Riesling grape variety, one with Sylvaner and one with Pinot Blanc. iii) *Powdery mildew*: bunches with more than 50% of bunch area infected. Three trials were made with this kind of disease, one with the Chardonnay grape variety, one with Pinot Blanc and one with Pinot Gris. iv) *Other rots*: Botrytis contaminated with moulds, bunches with off-flavours that were earthy and recalled fresh mushrooms. These trials, on the Pinot Gris grape variety, were not representative of a normal harvest.

Experiments in the laboratory on a semi-industrial scale

Musts collected after crushing were added with sulphite (5-8 g/hl) and a preparation of pectolytic enzymes (with the exception of non-treated controls). Fining agents were added in must as recommended by the manufacturers. Musts were set to decantation at 11°C for a period of 18-20 hours. After racking, turbidity of the treated variants were adjusted to the same level by addition of fine deposit. Musts were put into fermentation with the addition of active dried yeasts. After alcoholic fermentation, a second racking was made and also an addition of sulphites (7-8 g/hl). On the laboratory scale, experiments were conducted in 1000 mL flasks, and wines were conserved in 750 mL bottles until they underwent sensory evaluation. On the experimental winery scale, experiments were conducted in 20

L glass containers and wines were stabilised and filtered on membrane cartridges to 3 µm and 0.65 µm before bottling and closure with screw caps.

Oenological products and treatments compared

All experiments, both on the laboratory and the experimental winery scale, included non-treated controls with two or three turbidity levels, in general 200, 100 and 50 NTU (Nephelometric Turbidity Unit); one variant was only treated with pectolytic enzymes (Enzym Must®, Oenofrance), one with casein (Siha Kasein®, Bergerow SIHA) and one other with PVPP (PolyEx®, Oenofrance). The following allergen-free fining agents were studied (all registered products): i) Oenological charcoal alone: GOTA (Oenofrance), Geosorb (Laffort), Flavoclean (IOC); ii) Oenological charcoal in association: GOTA + Formule 1-CF (Oenofrance), Polypresse AF (Martin Vialatte); iii) Pea proteins: Prov Green Pure Must (Martin Vialatte), LittoFresh Origine (La Littorale), Greenfine Must (Lamothe Abiet), Inofine V (IOC); iv) Derivatives of chitin: Qi'No[Ox] (IOC); v) Composite products, usually composed of PVPP, proteins and bentonite: Mostogel (Erbslöh); Colorprotect V and Viniprotect (IOC); LittoPur and LittoFresh Most (La Littorale); Polymust AF (Laffort); Polymix Natur (Lamothe Abiet); Polygreen (Martin Vialatte); Formule 1-CF and Altocase

(Oenofrance); Oenocer, Oenocil and Vegane Colle (Oenolia Conseil AEB).

Experiments included between 20 and 30 treatments on the laboratory scale and between 12 and 16 treatments on the experimental winery scale.

Analysis of conventional oenological parameters and colour

Turbidity was measured using a Hach Ratio x/R turbidimeter. Sugar content, pH, titratable acidity, free and total sulphur dioxide were measured according to the standards of the Organisation Internationale de la Vigne et du Vin.⁹ Colour was determined by measuring absorbance at 420 nm (10 mm cell) using a Shimadzu UVmini-1240 spectrophotometer. The phenolic content was determined by a spectrophotometric method and expressed as a total phenolic index (TPI = $A_{280nm} \times \text{dilution factor}$).¹⁰

Sensory evaluation

Sensory evaluation was performed at three stages during the winemaking. After must clarification and after alcoholic fermentation, all musts and wines were blind-tasted by three consultant oenologists (males aged 56, 51 and 36 years) and a global quality score was attributed to each variant with a detailed commentary.

Bottled wines from the experimental winery scale were presented three times to a panel of 12-15 trained winemakers, 2-4 months after bottling, and one and two

Table 1. Colour and total phenols of fined and unfined musts after racking and white wines after bottling (mean±SD).

Fining treatment	n.	Musts after racking		n.	Wines after bottling	
		Colour ΔDO_{420}	Δ Total Phenol Index		Colour ΔDO_{420}	Δ Total Phenol Index
Unfined must						
Turbidity $\approx$ 50 NTU	11	—	—	6	—	—
Turbidity $\approx$ 100 NTU	15	-0.006 ± 0.009 a	-0.12 ± 0.13 ab	5	-0.006 ± 0.004	-0.30 ± 0.19
Turbidity $\approx$ 200 NTU	12	0.007 ± 0.009 a	-0.07 ± 0.13 a	7	-0.001 ± 0.003	-0.09 ± 0.16
Casein	16	-0.029 ± 0.007 a	-0.34 ± 0.11 ab	6	-0.004 ± 0.004	-0.05 ± 0.17
PVPP	11	-0.041 ± 0.008 a	-1.07 ± 0.11 c	6	-0.012 ± 0.004	-0.48 ± 0.17
Products with charcoal						
Charcoal alone	12	-0.021 ± 0.009 a	-0.74 ± 0.13 bc	3	-0.003 ± 0.005	-0.27 ± 0.24
Formule 1-CF + Gota	7	-0.017 ± 0.011 a	-1.11 ± 0.17 c	4	-0.018 ± 0.005	-0.65 ± 0.21
Polypresse AF	6	-0.025 ± 0.011 a	-1.13 ± 0.16 c	4	-0.011 ± 0.005	-0.60 ± 0.21
Pea proteins	8	-0.038 ± 0.009 a	-0.59 ± 0.13 abc	3	-0.006 ± 0.005	-0.03 ± 0.24
Chitin (Qi'No[Ox])	4	-0.022 ± 0.015 a	-0.41 ± 0.22 abc	2	-0.011 ± 0.006	-0.25 ± 0.29
Composite products						
Altocase	10	-0.014 ± 0.009 a	-0.64 ± 0.14 abc	5	-0.007 ± 0.004	-0.34 ± 0.19
Colorprotect V	10	-0.031 ± 0.009 a	-0.58 ± 0.14 abc	5	-0.007 ± 0.004	-0.40 ± 0.19
Formule 1-CF	5	-0.004 ± 0.013 a	-0.61 ± 0.2 abc	0		
LittoPur	6	-0.020 ± 0.012 a	-0.50 ± 0.18 abc	2	0.002 ± 0.006	0.00 ± 0.29
MostoGel	10	-0.008 ± 0.009 a	-0.08 ± 0.14 ab	4	-0.002 ± 0.005	0.18 ± 0.21
Oenocil	7	-0.010 ± 0.011 a	-0.51 ± 0.17 abc	2	-0.011 ± 0.006	-0.35 ± 0.29
Polygreen	15	-0.013 ± 0.011 a	-0.60 ± 0.17 abc	2	-0.017 ± 0.006	-0.55 ± 0.29
Polymix Natur	7	-0.002 ± 0.01 a	-0.53 ± 0.15 abc	4	-0.005 ± 0.005	0.07 ± 0.21
Polymust AF	9	0.002 ± 0.012 a	-0.25 ± 0.18 ab	2	0.002 ± 0.006	-0.30 ± 0.29
		P=0.003	P=<0.0001		P=0.170 (NS)	P=0.188 (NS)

n = number of samples analysed

years later. Samples were presented to the panel in tasting glasses in a randomised order. Eleven attributes were selected: visual (colour intensity), aroma (intensity and quality) and taste (smoothness, warmth, acidity, bitterness, body, balance, persistence) and a global quality rating. The attributes were quantified using a five-point intensity scale.

Statistical analysis

The data are presented as mean±standard deviation. They were statistically tested by analysis of variance (ANOVA) using XLSTAT Base 19.4 software (Addinsoft, France, 2017). Fisher's least significant difference test (LSD, 5% level) was applied to determine significant differences between the treatments. The model was statistically significant when P values were less than 0.05.

Results and Discussion

Effect of fining agents on conventional oenological parameters, colour and total phenol index

Sugar content, total acidity and pH values did not differ much between treated and untreated musts. Turbidity of musts from each trial was adjusted at the same level by the addition of fine deposit. It ranged between 50 and 100 NTU. Grape rot

impacts colour and phenol compounds of musts and wines.¹¹ To overcome these effects, results are presented in terms of difference as compared to unfined must (50 NTU) from each trial (Table 1). Means within a column followed by the same letter are not significantly different (LSD, 5%).

Most fining agents reduce colour and the total phenolic index. Effect on the colour of musts is greater with pea proteins, PVPP, casein and products containing charcoal than with composite products. These differences are minimised by alcoholic fermentation and the colour of bottled wines

Table 2. Sensory analysis of fined and unfined musts after racking and white wines after fermentation (mean±SD). Global quality score out of 5 points.

Fining treatment	n.	Musts after racking (score/5)	Wines after fermentation (score/5)
Unfined must			
Turbidity ≈ 50 NTU	17	2.7±0.3 b	3.5±0.3 c
Turbidity ≈ 100 NTU	15	2.2±0.3 ab	2.0±0.3 abc
Turbidity ≈ 200 NTU	17	1.1±0.3 a	1.5±0.3 a
Casein	19	3.1±0.2 b	2.6±0.2 abc
PVPP	17	2.8±0.3 b	2.5±0.3 abc
Products with charcoal			
Charcoal alone	15	3.1±0.3 b	2.9±0.3 abc
Formule 1-CF + Gota	14	2.8±0.3 b	2.9±0.3 abc
Polypresse AF	8	3.3±0.3 b	2.4±0.4 abc
Pea proteins	17	2.3±0.3 ab	2.4±0.3 abc
Chitin (Qi'No[Ox])	10	3.3±0.4 b	2.7±0.3 abc
Composite products			
Altocase	14	3.2±0.3 b	3.2±0.3 bc
Colorprotect V	11	3.0±0.3 b	3.0±0.3 bc
Formule 1-CF	6	3.0±0.4 b	3.0±0.4 abc
LittoPur	7	2.9±0.4 b	2.4±0.4 abc
MostoGel	10	2.7±0.3 b	2.7±0.3 abc
Oenocil	8	1.9±0.3 ab	2.6±0.4 abc
Polygreen	14	2.6±0.3 b	2.5±0.3 abc
Polymix Natur	10	2.9±0.3 b	2.5±0.3 abc
Polymust AF	7	2.7±0.4 b	3.0±0.4 abc
		P=<0.0001	P=0.001

n = number of samples analysed

Table 3. Global sensory quality (score/5) of fined and unfined white wines after bottling (mean±SD).

Fining treatment	Botrytis low (11.F3)	Botrytis low (11.F4)	Botrytis high (12.F4)	Botrytis high (13.F5)	Powdery mildew (12.F2)	Other rots (13.F4)
Unfined must						
Turbidity ≈ 50 NTU	3.1±0.2 c	3.4±0.3 c	3.1±0.2 c	3.0±0.3 c	3±0.1 c	1.8±0.2 abc
Turbidity ≈ 100 NTU	2.9±0.2 abc	2.6±0.3 ab	2.7±0.2 c	2.8±0.3 bc	-	2.0±0.2 abc
Turbidity ≈ 200 NTU	2.5±0.2 ab	2.2±0.3 a	2.1±0.2 ab	1.8±0.2 a	2.3±0.2 a	1.7±0.1 a
Casein	2.9±0.3 abc	2.8±0.2 abc	2.1±0.3 ab	2.8±0.4 bc	2.5±0.2 abc	2.2±0.2 bc
PVPP	2.8±0.2 abc	2.9±0.3 bc	2.8±0.3 c	2.7±0.4 bc	2.6±0.2 abc	2.3±0.2 cd
Products with charcoal						
Charcoal alone			1.9±0.2 a		2.6±0.2 abc	2.3±0.2 cd
Formule 1-CF+Gota	2.5±0.2 ab	2.9±0.2 bc		2.5±0.3 bc		2.7±0.2 d
Polypresse AF	2.7±0.2 abc	2.5±0.2 ab	2.6±0.2 bc		2.6±0.2 ab	
Pea proteins (Greenfine)				2.7±0.4 bc		2.1±0.2 abc
Chitin (Qi'No[Ox])				2.5±0.2 bc		2.2±0.2 bc
Composite products						
Altocase	2.7±0.2 abc	2.7±0.3 abc	2.6±0.2 bc	2.2±0.3 ab	2.8±0.2 bc	
Colorprotect V	2.8±0.2 abc	2.6±0.3 ab	2.8±0.3 c		2.6±0.1 abc	2.3±0.3 cd
LittoPur			2.9±0.2 c		2.7±0.2 abc	
MostoGel	2.8±0.2 abc	2.9±0.3 bc	2.8±0.2 c		2.5±0.2 abc	
Oenocil	2.4±0.2 a	2.9±0.3 bc				
Polygreen				2.7±0.4 bc		2.1±0.2 abc
Polymix Natur	3±0.2 bc	2.9±0.2 bc	2.6±0.3 bc		2.7±0.2 abc	
Polymust AF			2.8±0.2 c		2.4±0.2 abc	
LittoFresh Most				2.5±0.2 bc		1.9±0.2 abc
Oenocel				2.2±0.3 ab		2.2±0.2 bc
	P=<0.0001	P=<0.0001	P=<0.0001	P=<0.0001	P=<0.0001	P=<0.0001

shows fewer differences. Anneraud *et al.*¹² also showed that the efficiency of pea proteins is comparable to casein, but according to Cosme *et al.*¹³ they are less effective in reducing the white wine browning potential. The reduction of phenolic compounds in musts is considerable after fining with PVPP, products containing charcoal and composite products which all contain PVPP. It is lower with casein and pea proteins. The reduction is still perceptible after bottling but differences are not significant.

Effects of the fining agents on sensory evaluation

Results of the sensory evaluation of musts and after fermentation (Table 2) at first highlight the importance of must clarification on unfined wine quality. The clearest unfined control presents on average the best sensory quality for both musts and wines as opposed to less clarified musts. There is an obvious link between the turbidity of unfined musts and the global quality of wines after fermentation (Figure 1). Only in the case of off-flavours does clarification have no effect on wine quality (trial 13.F4).

All fining products improve the organoleptic quality of must with the exception of Oenocil® because this product is not neutral in terms of aroma. It develops a rancid odour that is perceptible in must but much less so in wine. After alcoholic fermentation, the clearest unfined control presented a better overall sensory quality than fined variants with turbidity that is higher after must clarification. The positive effect of fining is less pronounced on wine than on must. This may probably be explained by the fact that some molecules responsible for off-flavours in musts are degraded during alcoholic fermentation as is the case with 2-Methylisoborneol.¹⁴ Sensory improvement after fining is lower with pea proteins than with other products for both musts and wines.

Wines from the experimental winery scale were presented to a trained panel of 12-15 winemakers. The results of the first sensory evaluation after bottling as a global sensory score are reported in Table 3.

The importance of good clarification of musts before fermentation is confirmed. Only in the case of off-flavours due to rots

other than Botrytis is clarification incapable of improving the wine quality.

For the grape harvest with little Botrytis (category *Botrytis low*), the aromatic quality enhancement after fining is not enough to compensate for the loss of persistence in the mouth resulting in lower global wine quality. This effect is perceptible with charcoal products. The negative impact of the product Oenocil® is clear in trial 11.F3 but less pronounced in trial 11.F4. Even when the Botrytis rate is higher (category *Botrytis high*), the clearest unfined control was still preferred by the panel of tasters. The same results were observed in the case of grapes infected with powdery mildew. Moreover, during the aging process of bottled wines, differences between treatments tend to decrease. If grapes are affected with other rots and earthy or mushroomy off-flavours, all fining products, especially charcoal products, have a positive effect on the wine quality. However, pea proteins are of little interest for enhancing the aromatic quality of wines.

Conclusions

This study shows that in the case of wine grape rot without off-flavours such as earthy or mushroomy odours, meticulous clarification of must after crushing can enhance the wine quality and avoid the consequences of grape rot. Fining products are not necessary. The turbidity of must after clarification has to be around 50 NTU. Use of pectolytic enzymes may be required in order to achieve this degree of clarification. However, excessive trituration of bunches can render must clarification impossible.

The effectiveness of fining products to improve sensory quality is more evident when tasting musts after clarification than in wines after alcoholic fermentation, probably because some off-flavour molecules are broken down during fermentation. The need for treatment is less frequent than generally accepted.

New allergen-free fining products used during clarification of musts from spoiled wine grapes are at least as effective for improving wine quality as reference products such as casein and PVPP alone. The efficiency of composite products including PVPP, proteins and fining agents is better than that of pea proteins and lower than charcoal products. However, fining musts during clarification can enhance the quality of the wine after fermentation, yet this is always accompanied to a greater or lesser extent by a loss of persistence and mouth-feel. The impact of fining on global wine quality depends on the balance of these two

Figure 1. Relationship between turbidity (NTU) of unfined musts after racking and global sensory quality of wines after alcoholic fermentation for the different trials carried out with different types of wine grape rots: Botrytis low (▲), Botrytis high (■), powdery mildew (○) and other rots (*).

opposite effects.

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