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Wheat Gluten Used as a Clarifying Agent of Red Wines

R. MARCHAL,*,† L. MARCHAL-DELAHAUT,† A. LALLEMENT,‡ AND P. JEANDET†

Laboratoire d'Oenologie, URVVC, UPRES EA 2069, Faculté des Sciences, Université de Reims, BP 1039, 51687 Reims Cedex 2, France, and Institut Œnologique de Champagne, Z.I de Mardeuil, BP 25, 51201 Epernay Cedex, France

Bovine spongiform encephalopathy caused a situation of crisis leading the public and winemakers to lose their confidence in the use of gelatin as a fining agent and to reject animal proteins in general. Therefore, we started the search for a substitute for gelatin and egg protein by comparing gluten with these fining treatments currently used. This study concerned the fining of a Burgundy red wine (Rully, Controlled Appellation). For 6 g/hL, enzymatically hydrolyzed glutens (EHG) gave better efficiencies than deamidated glutens. The efficiency of the egg proteins treatment was situated between those of the hydrolyzed glutens and deamidated glutens. For 12 and 18 g/hL, turbidities of the wine treated by five glutens were 67 to 86% less than that of the control wine. Better results were obtained with egg proteins for short kinetics particularly. Wine fining with gluten was always better than gelatin treatments. The differences between the five glutens became very small when the dose incorporated in the wine increased. The volumes of lees generated by fining with gluten are situated between the values obtained with egg proteins and gelatin. After fining, immunodetection with gluten polyclonal antibodies failed to detect residual deamidated gluten.

KEYWORDS: Fining; clarifying; gluten; gelatin; egg albumin; Burgundy; red wine

INTRODUCTION

After alcoholic fermentation in a tank or a barrel, wine is a colloidal solution and suspension. In industrial wineries having high-volume tanks, the spontaneous clarifying is very long and insufficient because particle density is close to that of the wine. Moreover, fining softens the gustatory appraisal and can reduce the astringency of rough wines. In practice, these treatments also modify and stabilize the color of red wines.

For these reasons, organic fining agents were commonly used for a long time to clarify red wines (*1*−*3*). The influence of treatments with gelatin on the wine composition and the organoleptic perception have been largely reported in scientific and practical studies. These are often focused on the factors contributing to proteins−polyphenolic compound interactions, which are responsible for the expected flocculation and clarifying (*4*−*6*). But finings using gelatin are yet very empirical. The observed failures can be explained by an insufficient knowledge about tannins and the gelatin currently used (*6*), as well as an ignorance of the flocculation mechanism.

In addition, numerous cases of bovine spongiform encephalopathy really caused a situation of crisis, and winemakers have been encouraged to stop using bovine gelatin. In Europe, the fear of transmitting this disease to man led to the interdiction of the use of bovine plasma and blood cells (regulation EC no. 2087/97, Council of October 20, 1997). Some winemakers also hesitate to use egg albumin because of their animal origin.

Therefore, it was important to find out about treatments that could replace gelatin and egg-protein finings. We then started our investigation with wheat prolamins, commonly called gluten, to clarify red wines. Different treatments were established to compare the efficiencies of five glutens with those of the usual fining agents. We also quantified the lees heights generated by fining with animal or vegetal proteins (this parameter expresses the loss of wine; therefore, it is very important for winemakers). The aim of this work was also to estimate the effect of fining on the wine color and sensorial appraisal. The last point concerns the research of residual gluten proteins in the treated wine. Gluten indeed causes sometimes severe intolerance (celiac disease) and it is then very important to know if it is completely eliminated after clarifying. The results of these experiments have been reported in this study.

MATERIALS AND METHODS

Red Wine. The Burgundy wine (Rully, Controlled Appellation) used in this study was kindly offered by the Institut Oenologique des Vins de Bourgogne (Mecelley, France). The wine, elaborated with Pinot noir grapeberry variety, was racked five weeks after alcoholic and malolactic fermentations in a stainless steel tank. The usual wine analyses have been reported in Table 1. Sugar content was determined using an automated neocuproin hydrochloride method. Alcohol content was determined after distillation with a Dujardin−Salleron class II alcohometer 9 to 16% volume (accuracy: 1/10% v/v). pH was determined by using an Orion 420A pH meter. Total acidity was determined by

* Corresponding author. Phone and fax: 33 (0) 326 91 33 40. E-mail: richard.marchal@univ-reims.fr.
† Université de Reims.
‡ Institut Œnologique de Champagne, Z.I de Mardeuil.

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Table 1. Analytical Characteristics of the Burgundy Red Wine before Fining

sugar content (g/L)	1.1
total acidity (g/L H_2SO_4)	4.0
pH	3.32
volatile acidity (g/L H_2SO_4)	0.31
alcohol content (%v/v)	12.25
mailic acid (g/L)	0.9
free SO_2 (mg/L)	12
total SO_2 (mg/L)	34
iron (mg/L)	3
turbidity (NTU)	247

M/64 NaOH additions using blue bromothymol as a colorimetric indicator. Malic acid (enzymatic), iron, volatile acidity (KI/KIO_3), free SO_2, and total SO_2 (pararozaniline) contents were quantified using automated methods (Intégral Plus apparatus, Alliance Instrument, France). Malolactic fermentation had not been completely finished because of the decrease in temperature in the cellar.

Enological Products. A vital gluten, which was not hydrolyzed at all, rapidly forms a viscoelastic network which is quite impossible to use if prepared in advance. Although its good fining effect was shown in preliminary tests, its enological interest is quite limited because the suspension has to be freshly prepared just before addition into the wine. Therefore, we discontinued experiments with vital gluten. Four glutens (noted gluten 1 to gluten 4) were supplied by French companies, and gluten 5 came from Australia. Producers gave us their guarantee that these glutens did not come from genetically modified organisms. Gluten 1 (Chamtor, Bazancourt, France) and gluten 3 (Roquette, Lestrem, France) were obtained from enzymatically hydrolyzed glutens (EHG). The pHi values of these proteins were between 6 and 8. Gluten 2 (Chamtor, Bazancourt, France) and gluten 5 (Manildra Group, Australia) were obtained from vital gluten deamidation. Glutens 2 and 5 gave very good suspensions without sedimentation of particles; their pHi values were between 3.5 and 4.5. Gluten 4 (Roquette, Lestrem, France) was a thermally treated product.

Two gelatins and hen egg proteins (commonly called "egg albumin" by winemakers) were supplied by the Institut Oenologique de Champagne (Epernay, France). Two gelatins (noted G_1 and G_2) came from porcin hydrolyzed collagen and were supplied as aqueous solutions (50 and 100 g/L respectively) and diluted at 10 g/L with a 1 g/L tartaric acid aqueous solution adjusted at pH 3.2 with 1 M NaOH. The gelatins were completely soluble. The hen egg albumin (noted HEP) and the five glutens were supplied as powders, and were only partially soluble (solution/suspension systems). They were also prepared diluted at 10 g/L in a 1 g/L tartaric acid aqueous solution adjusted at pH 3.2 with 1 M NaOH. Sulfur dioxide (SO_2) was added, at a concentration of 1 g/L, to all the solutions of enological products which were kept at 4 °C for ideal preserving conditions. Solutions were prepared 12 h before being used for wine fining. For all fining agents solutions, the concentration was 10 g/L.

Fining Experiments. Fining tests were realized in plastic graduated cylinders (volume, 500 mL; internal diameter, 52 mm) which were filled up to 260 mm height (500 mL) with wine. Experiments were realized six weeks after the end of the malolactic fermentation. Enological products were introduced with automatic Biohit pipettors, and sulfur dioxide was added to the wine (60 mg/L) to avoid an eventual acetic disease. Graduated cylinders were turned over twice to homogenize the fining agents and wine. As gluten clarifying activity was totally unknown, doses used for the experiments were 6, 12, and 18 g/hL. These values are the minimum and the maximun doses commonly used for gelatin and egg proteins.

Clarifying Kinetics. A 15-mL aliquot was taken from the graduated cylinder with a 25-mL plastic pipet. As liquid from the top is always a bit clearer than that from the bottom, it does not reflect the real turbidity. To avoid this problem, the pipet, locked on top with a finger, was introduced down to 200-mL liquid level and then unlocked to allow the sample to enter it. Kinetics were done at room temperature (20 ± 2 °C). Turbidities were measured 48, 72, 96, and 156 h after the addition of fining agents. Turbidities were measured with a turbidimeter Hach 2100 AN calibrated with the GELEX secondary turbidity standards kit, and expressed in nephelometric turbidity units (NTU). A preliminary study was made with a control wine for the determination of standard deviations. Three measures were made each day (in three graduated cylinders), during 5 days. Standard deviations were calculated for two and three measures (Table 2). Values were always very small; therefore we decided to make only one assay for the clarifying experiments.

Determination of Lees Volume after Fining. Graduated cylinders, with their 52-mm internal diameter, do not allow a precise measurement of lees height. Therefore, we used white glass bottles from Champagne for the fining experiments. The bottle was filled with wine and the fining agent (12 g/hL), closed with a cap, and kept still with the neck down. Three bottles were made for each treatment for the calculation of the average value and standard deviations. Bottles were kept at room temperature and protected from draft and light. After 20 days in that position (i.e., the time required for a total spontaneous clarification of the untreated wine), the bottles were rotated twice according to the vertical axis in order to let the flocculates slide down from the bulge onto the neck of the bottle. Thus, lees were gathered on a small surface permitting an easy measurement of their height. Lees height was then measured with a special ruler adapted to the bottle neck, converted to mL, and finally converted to percentage lees/liquid (v/v). A calibration was preliminarily realized with four bottles closed with a cap.

Characterization of Proteic Fining Agents by SDS−PAGE. Molecular weight ranges of enological proteins (glutens, gelatins, and egg proteins) were determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE in reducing conditions) according to the Laemmli method (*7*) using slab gels (0.75 mm thick). The stacking gels consisted of T = 5% and C = 2.7%, and the separating gels consisted of T = 13.5% and C = 2.7%. A vertical electrophoresis apparatus (Mini-Protean, Bio-Rad) was used to run the gel at a constant voltage setting at 150 V until the bromophenol blue tracker dye reached the bottom of the gel (usually 65 min at room temperature). Standard proteins from 14,000 to 94,000 au were used as molecular-weight markers (LMW Pharmacia). Fining agents and standard proteins were treated in the same way with Laemmli buffer (v/v) containing β-mercaptoethanol, and 20 μL was loaded in each well. Molecular weights of protein bands were calculated from the linear regression equation of log MW vs mobility. After electrophoresis, separated proteins were stained with 1.5% Coomassie brilliant blue in 50% (v/v) methanol, and destained in acetic acid/methanol/water (1:2:7).

Production of Polyclonal Antisera. Preimmune sera (PIS) were obtained from a New Zealand white female rabbit before immunization. Soluble deamidated gluten 5 (antigen) was incorporated in 0.3 mL of 3% polyacrylamide and mixed with 0.8 mL of Freund's complete adjuvant (Sigma-Aldrich, France) (*8*) to give a final protein concentration of approximately 1 mg/mL. The rabbit was intradermally immunized on the back at 6 sites (6 × 0.15 mL of the immunogen). Subsequent injections with the same antigen preparation were made at weeks 3 and 6 in Freund's incomplete adjuvant. The rabbit was bled at week 8. Blood was left 1 h at 30 °C before being centrifuged (10 min at 6000*g*). The antisera, containing deamidated gluten polyclonal antibodies, was stored at −20 °C.

Cross-Reactivity and Dot-Blot Staining. The reactivity of the antibodies raised against the Australian deamidated gluten 5 was tested using a noncompetitive dot blot technique. Aqueous deamidated gluten 5 solutions were prepared between 0.25 and 2 g/hL (Figure 7, strips A and B). Nondiluted control wine (not treated with gluten 5), nondiluted wine treated with gluten 5, and PIS were spotted (3 μL) in duplicate onto nitrocellulose membranes (Sartorius, Göttingen-Germany) and air-dried at room temperature (Figure 7, strips C and D). Nonspecific binding was blocked with Tris buffer saline (TBS: 25 mM Tris, 0.5 M NaCl, adjusted to pH 7.5 with HCl) plus 2% nonfat dry milk. The membranes were rinsed 3 times in TBS and incubated 3 h in the presence of deamidated gluten antibodies (antisera dilution, 1/15,000). The membranes were successively washed with TBS, TBS + 0.05% Tween 20, and TBS again, before being incubated with goat anti-rabbit IgG (1/1500 dilution in TBS plus 1% nonfat dry milk) during 3 h at room temperature, and washed again with TBS, TBS + 0.05% Tween 20, and TBS again, successively. Goat anti-rabbit horseradish peroxi-

Table 2. Determination of Turbidity Standard Deviations for Static Clarifying Kinetics using a Control Wine (no fining)

	NTU after 22 hours	NTU after 51 hours	NTU after 78 hours	NTU after 96 hours	NTU after 115 hours
cylinder 1	79.1	44.1	27.1	22.1	16.7
cylinder 2	79.3	43.2	26.1	21.0	16.2
cylinder 3	77.6	44.4	27.3	21.5	15.8
Average value and standard deviation for $n = 2$	79.2 ± 0.1	43.7 ± 0.45	26.6 ± 0.5	21.5 ± 0.55	16.4 ± 0.25
Average value and standard deviation for $n = 3$	78.7 ± 0.54	43.9 ± 0.36	26.8 ± 0.36	21.5 ± 0.32	16.2 ± 0.26

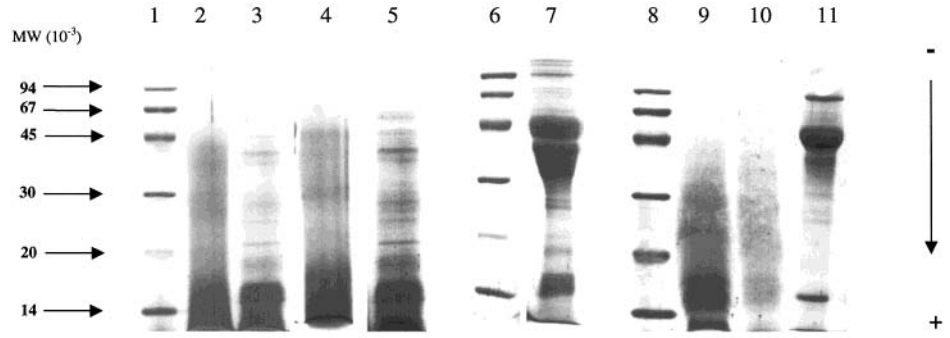


Figure 1. SDS–PAGE analysis of wheat glutens and animal proteins used for the Burgundy wine finings. Proteins were stained with Coomassie brilliant blue. Lanes 1, 6, and 8, MW markers; lane 2, deamidated gluten 2 (Chamtor); lane 3, hydrolyzed gluten 1 (Chamtor); lane 4, deamidated gluten 5 (Manildra); lane 5, hydrolyzed gluten 3 (Roquette); lane 6, thermally treated gluten 4 (Roquette); lane 9, gelatin 1; lane 10, gelatin 2; lane 11, hen egg proteins (HEP). Relative MW ($\times 10^{-3}$) of standard proteins are given on the left side of the gel.

dase-conjugated polyclonal antibodies were obtained from Sigma-Aldrich (France). After further washing in PBS, peroxidase activity was stained using a chemiluminescent substrate (Supersignal Western Blotting, Pierce); the nitrocellulose membrane was incubated with equal parts of luminol/enhancer solution and peroxide solution for 8 min with a sufficient volume to ensure that the blot was completely wetted with the substrate. The membrane was then placed in a plastic sheet protector and exposed against a standard autoradiographic film (Biomax ML, Kodak) for 30 s. Finally, the film was developed and fixed using GBX solutions (Kodak, France). The ability of the antisera to bind to wine spot (after dot-blots) was assessed by visually comparing the color of each spot stained with the antiserum test with that of the nonimmune serum.

Sensorial Appraisal. A preliminary test realized by two trained assessors indicated the absence of easily detectable differences among the wines. For this reason, wines were compared using triangular tests. This test is used to determine an unspecified sensory difference between two wines. This method of difference testing involves the simultaneous presentation of three coded samples, two of which are identical. The 15 assessors were asked to select the sample perceived as different. For each comparison, they made olfactive and gustative tests. The panel was composed of enologists, enology students, and professors. Each order of glass presentation was used an equal number of times. The forced-choice option was used. To have a statistically significant perceivable difference between the samples, the number of correct responses had to be equal to or higher than nine (*9*). The comparisons tested were: control wine vs gelatin-treated wine, control wine vs deamidated (gluten 5) treated wine, gelatin-treated wine vs deamidated (gluten 5) treated wine, and deamidated (gluten 5) treated wine vs egg-protein treated wine. All wines compared were treated with 12 g/hL proteins.

RESULTS AND DISCUSSION

Electrophoretic Characteristics of Fining Agents. Molecular weight ranges of proteins from glutens and animal proteins can be observed in Figure 1. The electrophoretic profile of gluten 4 (lane 7) shows a classical profile of a vital gluten. Proteins have molecular weights mainly between 30,000 and 45,000 and at 16,000. Vital gluten contains as well numerous minor proteins between 10,000 and 100,000. Using SDS−PAGE, glutenin subunits with high MW are situated between 100,000 and 160,000 (*10, 11*). Glutenin subunits with low MW are situated between 30,000 and 70,000 (*12*, *13*). With SDS−PAGE, gliadin MW are between 30,000 and 40,000 for α/β and γ gliadins, and 60−80,000 for ω gliadins. In reducing conditions, glutenin subunits with low MW have the same mobilities in SDS−PAGE as gliadins (*14*). This makes the explanation of the electrophoresis very difficult. Although glutens 1 and 3 (lanes 3 and 5) have been partially hydrolyzed, it is possible to observe a few distinctive bands. One can also observe that gluten 2 (lane 2) and gluten 5 (lane 4), two deamidated products, present a protein degradation probably more important than EHG 1 and 3 (lanes 3 and 5). They presented a discontinuous smear between 10,000 and more than 100,000, confirming that the procedure for deamidation is fundamentally a chemical hydrolysis. It is important to note that real MW are higher than MW after reduction treatment. The enzymatic hydrolyzates and the deamidated glutens contain some SS cross-linked peptides with MW larger than those indicated by electrophoresis.

The enological product called "egg albumin" (lane 11, Figure 1) consists of the whole hen egg proteins; the principal protein is the albumin with a molecular weight close to 45,000. Two distinctive bands were revealed at 15,000 and 90,000. Numerous minor proteins between 25,000 and 100,000 were also observed. Concerning the gelatins (gelatin 1 in lane 9 and gelatin 2 in lane 10), one can observe some smears confirming that these products were the result of an important hydrolysis of porcin collagens. The continuity in the molecular weight distribution seems relatively similar for gelatins 1 and 2. Although their analyses in SDS−PAGE did not present any noticeable differences, results will show variations in their efficiencies.

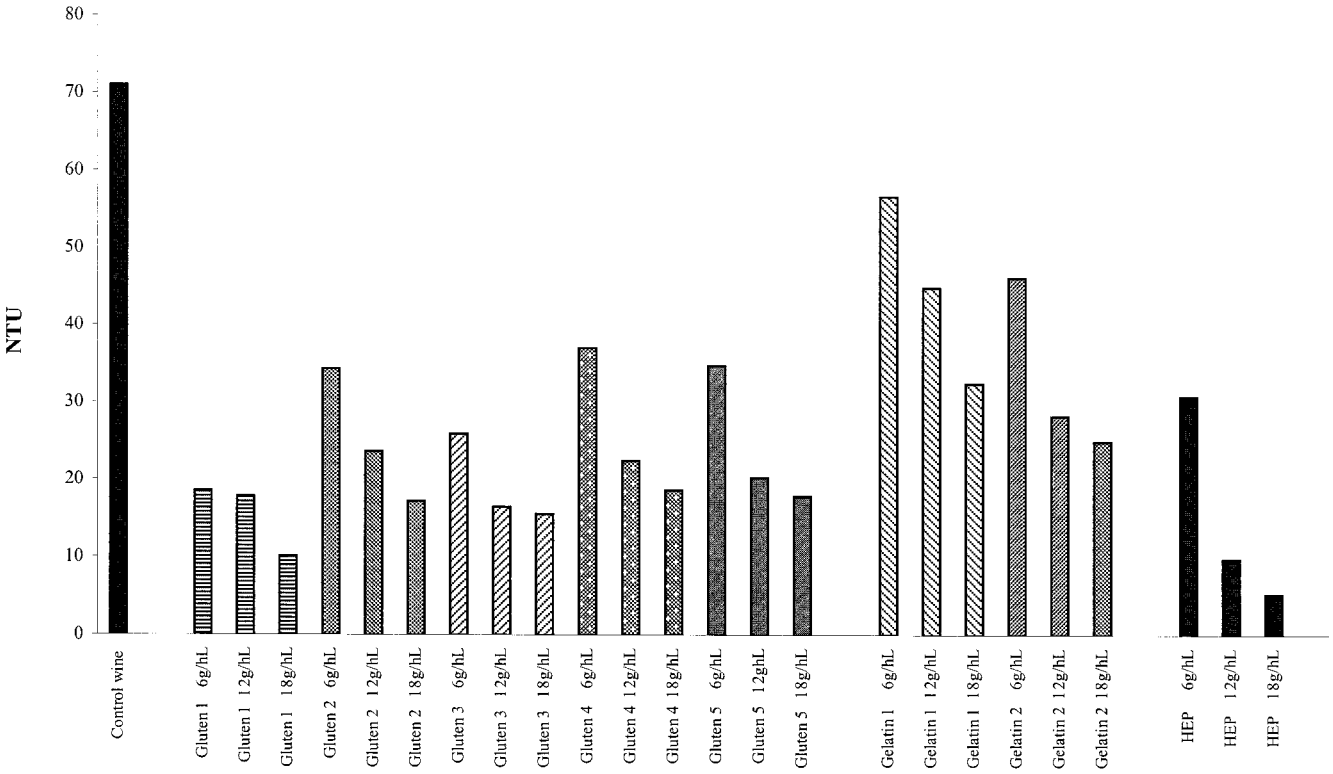


Figure 2. Effect of different finings on the turbidity of the Burgundy red wine. Measurements were made 156 h after treatments.

These enological products show a very complex proteic composition with enormous differences between animal and vegetal proteins. It is also clear that, according to the industrial process, glutens present large differences in their proteic composition and biochemical characteristics such as isoelectric point (deamidation treatment increases the charge of proteins), molecular weight, and hydrophobicity (*15*, *16*).

Comparison among Gluten, Gelatin, and Egg-Protein Treatments after 156 h Settling. This test showed that the gluten clarifying efficiency largely depends on the quantity of fining agent used (Figure 2). For doses between 6 and 18 g/hL, glutens 3, 4, and 5 have relatively similar activities despite differences in their geographic origins (Australia and France) and industrial processes (thermal-treated gluten and deamidated glutens). Globally, gluten 2 gave turbidities a little better than those of glutens 3, 4 and 5, particularly if a low dose was used (6 g/hL). Gluten 1 gave the best results with a turbidity equal to 14% of that of the nontreated wine. We can yet observe that the effect of gluten 3 at 12 g/hL was similar to that of gluten 1 at 12 g/hL; also the effect of gluten 3 at 18 g/hL was similar to those of glutens 2, 4, and 5 at 18 g/hL It seems that a partial hydrolysis of prolamins induced a better flocculation than deamidated glutens.

On the basis of molecular composition (see above), it is not possible at the moment to understand why one gluten is better than another. Glutens are composed of numerous gliadins (*17*) and glutenins (*18*). Gliadins present differences in their amino acid compositions (*19*), the consequence being a difference of wheat protein hydrophobicity (*20*) and pH_i values (*21*). Now, these biochemical characteristics are responsible for proteins and wine phenolic compound interactions, leading to flocculation and clarification. Moreover, the must is a very complex biochemical medium containing colloidal particles and soluble colloids. To explain the differences in the clarifying efficiencies observed among glutens, it will be necessary to work with a model wine having a controlled composition. It will also be necessary to experiment with proteic fractions isolated from whole gluten, and the same fractions after enzymatic or deamidation treatments.

Results obtained with gelatin 1 were insufficient whatever the dose used (Figure 2). The clarifying effects are systematically better with gelatin 2 than with gelatin 1. Egg proteins gave the best results with very limpid red wines (Figure 2). For all products tested, one can note an increase in the fining efficiency when the doses increase.

In a Chardonnay white wine, the efficiencies obtained with the associations of hydrolyzed gluten plus tannins were higher than those obtained with the same gluten used alone (*22*). At the opposite, the affinity between deamidated gluten proteins and tannins (whatever the botanical origin of the tannins and the ratio of tannins/gluten used) was very bad. In a red wine containing endogenous phenolics, the higher clarifying effect of hydrolyzed glutens is then probably due to interactions between prolamins and phenolics.

Influence of Settling Duration on Clarifying Efficiency. Turbidities were also measured after 48, 72, and 96 h of settling. For all treatments (6, 12, and 18 g/hL), the turbidities obtained always decreased with time.

At first, treatments are compared for a dose of 6 g/hL (Figure 3). The best results were obtained for glutens 1 and 3, the two EHGs. Egg proteins also had a very good clarifying effect, even if turbidities were a few NTU higher than for EHG, whatever the time of clarifying. The two deamidated products (glutens 2 and 5) gave identical kinetics, as did the two EHGs. Peptides in deamidated glutens and EHGs differ widely in term of size, charge, and hydrophobicity. These differences in biochemical characteristics (induced by two different industrial processes) provide the explanation for different physicochemical properties. Gluten 4 (thermally treated) presented a slower clarifying start, probably because of the time needed for the flocculate formation.

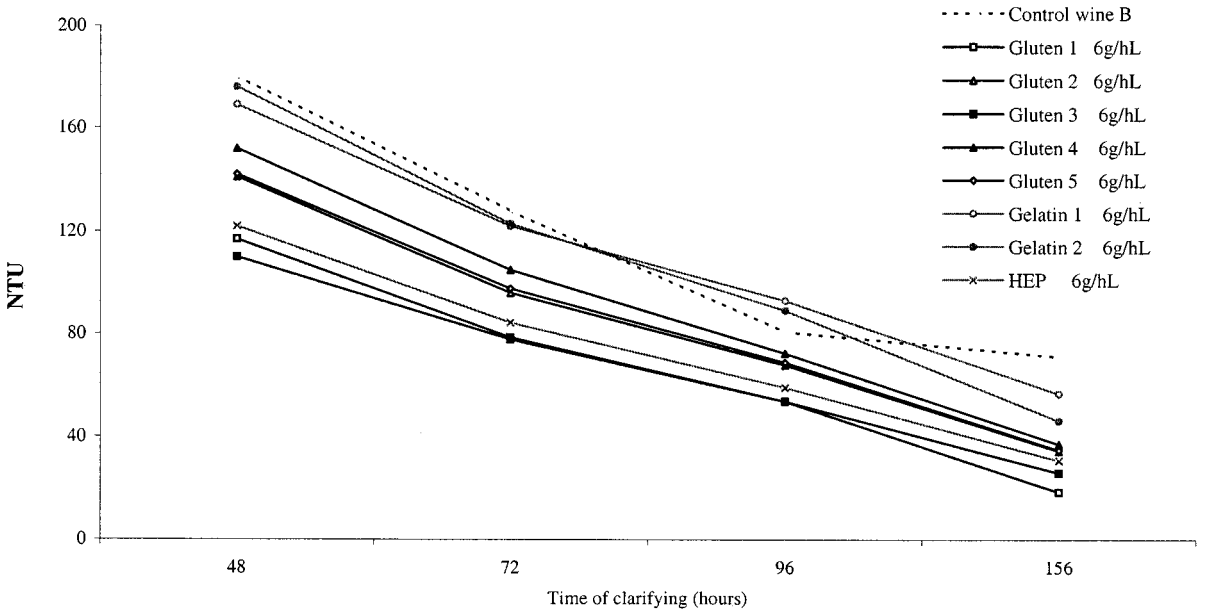


Figure 3. Kinetics of the Burgundy red wine clarifying. All treatments were done at a dose of 6 g/hL.

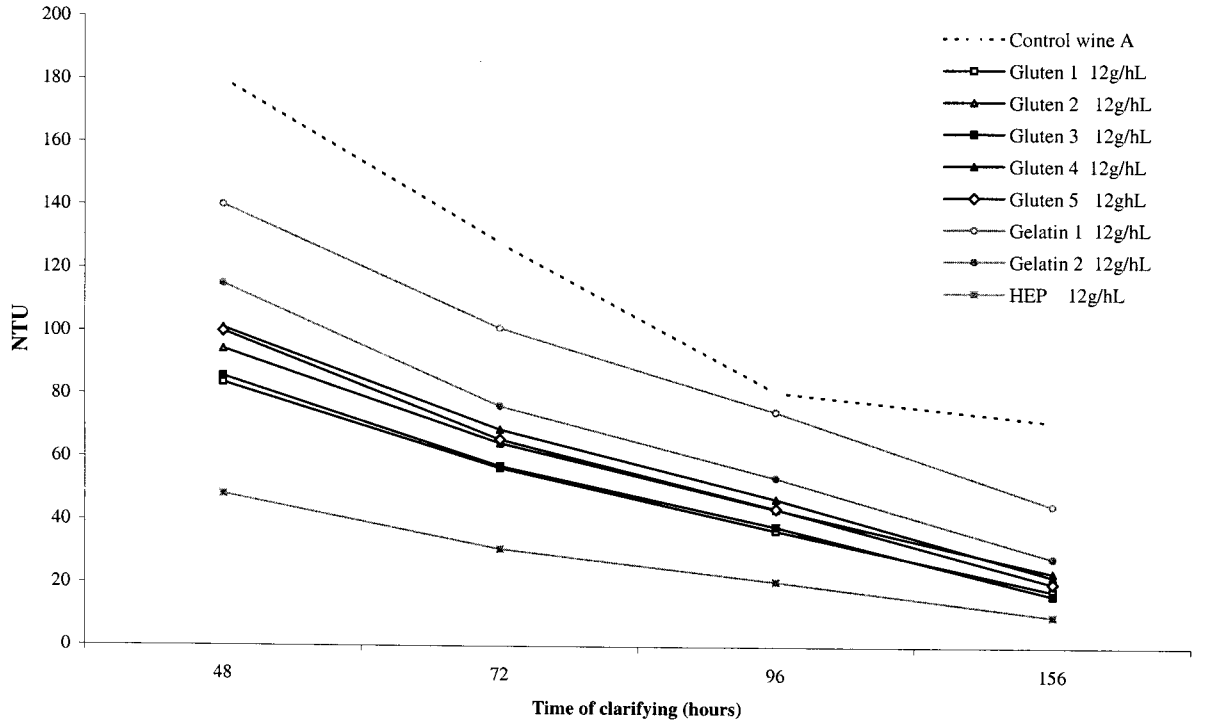


Figure 4. Kinetics of the Burgundy red wine clarifying. All treatments were done at a dose of 12 g/hL.

But after 156 h, the efficiency of gluten 4 was comparable with those of deamidated glutens. The worst finings were observed for the two gelatin treatments. Gelatin 2 was a little better than gelatin 1; their electrophoretic profiles were, however, the same. This indicates that SDS−PAGE is not really a suitable method to compare enologic gelatins. For the control wine, the slope of the curve changed at the end of the kinetic. When the wine reached 80 NTU, the spontaneous clarifying became very slow, showing that fining was really necessary. To sum up, small doses of EHG (6 g/hL) can lead to a low turbidity and permit a direct filtration with a continuous alluviation filter (Kieselguhr). This is important for winemakers because it reduces the blocking of the filter.

At 12 g/hL (Figure 4), differences between glutens and animal proteins became more marked. Egg proteins gave better results than gelatin finings (this is currently observed in wineries) and also glutens. For HEP treatment, the difference of NTU between 6 and 12 g/hL was probably due to the difference of density between flocculates (appeared during fining) and the wine. We have already observed that there is not often a linear relation between the dose of fining protein used and the level if efficiency. Differences in the clarifying efficiencies were all the more marked that the kinetic was short. The five glutens constituted a more homogeneous group than the five same products at 6 g/hL (Figure 3). Nevertheless, comparison of the clarifying efficiencies shows that hydrolyzed glutens (glutens 1 and 3) gave results a little better than those for deamidated glutens (glutens 2 and 5). All gluten efficiencies were situated between the efficiency values for egg protein and gelatin. In wineries, egg-protein fining is a relatively limited practice

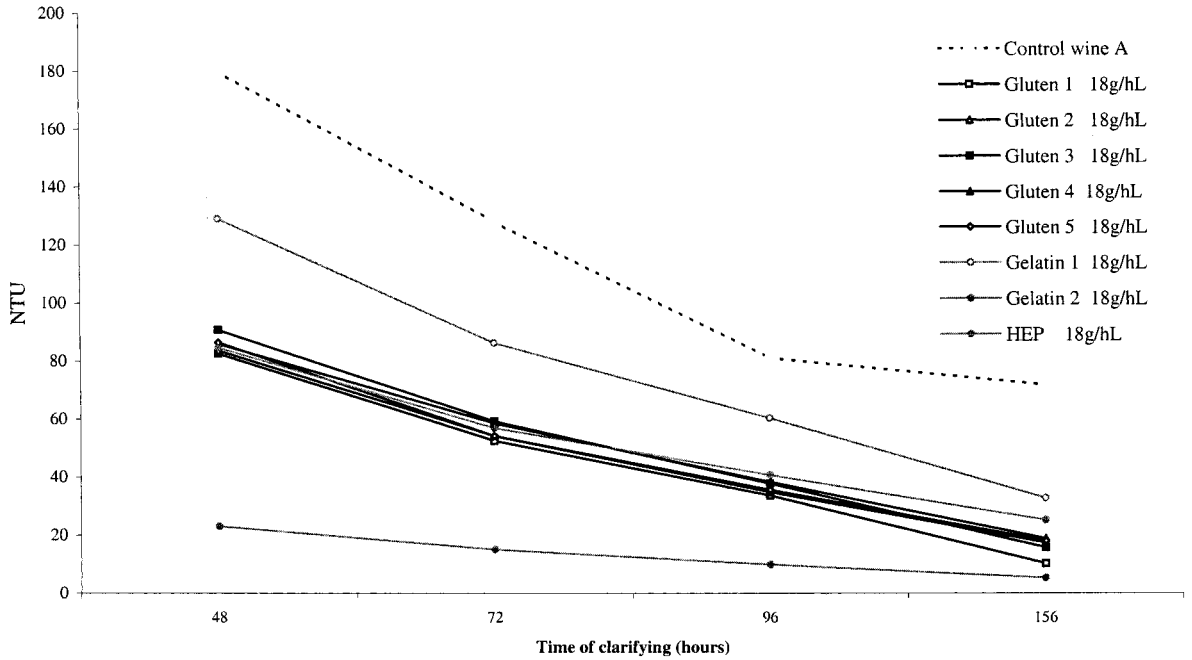


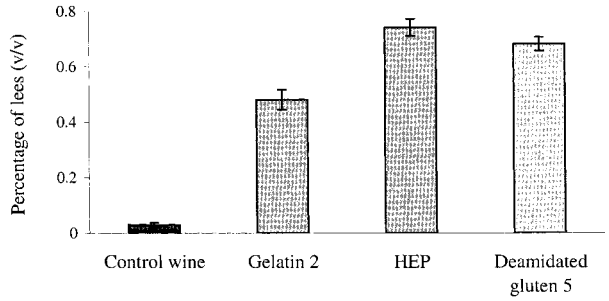
Figure 5. Kinetics of the Burgundy red wine clarifying. All treatments were done at a dose of 18 g/hL.

because this treatment is expensive. Moreover, aqueous suspensions of egg proteins are not easy to prepare. This was not the case here, for all tested glutens the utilization was very easy. The dispersion is easy to obtain, but it is nevertheless necessary to agitate the EHG suspension when taking the protein sample for fining. Otherwise, the sedimentation of a few particles occurs.

For treatments at 18 g/hL (Figure 5), the clarifying effects obtained with glutens 1 and 2 were really better with 18 g/hL (−86% and −76% of the turbidity compared to that of the control wine) than with 12 g/hL (−75% and −67% of the turbidity compared to that of the control wine). The same situation was observed for the two gelatins and for egg proteins. For glutens 3, 4, and 5, the increase in the clarifying efficiency was quite small when the dose of treatment increased (values are noted on Figure 2). A group was constituted by the five glutens and also the gelatin 2; it presented an intermediate position between egg proteins and gelatin 1. Distance with the control wine also increased when taking into account the slowing down of the natural sedimentation of particles. Egg-protein treatment gave a very low turbidity as early as 48 h. This means that egg proteins present a certain advantage for fining in cases in which a quick racking (of a few days) is required. Egg-protein fining also allowed a direct bottling, without any filtration. At the opposite, treatments with gluten (and particularly gelatins) need a longer settling time to give optimal results.

Influence of the Fining Type on Lees Volume. The untreated wine presented the lowest volume of lees (Figure 6). Gluten 5 generated a volume of lees equal to 0.68% (v/v). It is situated between the values observed for egg proteins and gelatin, which are the two animal fining agents actually used for red wine. The best result was observed for EHG fining. This value is yet very low. This parameter has to be considered when choosing the fining agent because it is related to the loss of wine.

Research of Residual Gluten Proteins in Wine. By using the dot-blot technique, a control experiment with preimmune antisera yielded no positive cross-reaction with deamidated gluten 5 proteins, for a scale of concentrations between 0.25



	lees % (v/v)	t. sm (n=3)
Control wine	0.03	0.0076
Gelatin 2	0.48	0.036
HEP	0.74	0.031
Deamidated gluten 5	0.68	0.0246

Figure 6. Volume of lees obtained for the different finings using animal and vegetal agents.

and 2.5 g/hL (Figure 7, strip A). Dot-blots also showed that the deamidated gluten protein-antibodies obtained were able to recognize their antigens (Figure 7, strip B), even for a concentration of 0.25 g/hL. A control experiment using preimmune antisera yielded no positive cross-reaction with the undiluted control wine and the undiluted wine treated with deamidated gluten 5 (Figure 7, strip C). Another control was assayed with gluten derivatives solubilized in a red wine (containing insoluble particles or clarified by centrifugation) to check the reactivity of the antibodies in the same conditions as those used for the analysis of the treated sample. It was not possible to obtain this control; the rapidity of the flocculate formation makes the experiment impossible. Finally, by using the same dot-blot technique, gluten-antibodies obtained were not able to recognize their antigens in the wine treated with 12 g/hL gluten (Figure 7, strip D). This result clearly indicates that if there were residual deamidated gluten proteins in wine, their concentration was lower than 0.25 g/hL or 2.5 mg/L. For the Codex Alimentarius, a food is gluten-free when it contains less than 10 mg/L gluten. At the opposite, it has been clearly shown (*23*) that animal fining proteins were not completely eliminated by settling and filtration of treated wines. In this assay, the red

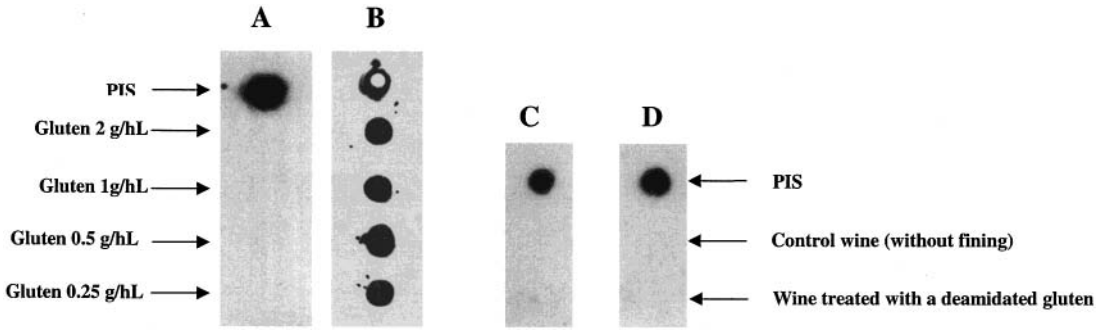


Figure 7. Research of residual gluten proteins in a Burgundy red wine after fining. Dot-blot on nitrocellulose strips with color developed using a chemiluminescent substrate. The first antibody was rabbit antigluten polyclonal antibodies. Strip A, deamidated gluten proteins colored with preimmune sera (PIS); strip B, deamidated gluten proteins colored with gluten antisera; strip C, control wine and gluten-treated wine colored with PIS; strip D, control wine and gluten-treated wine colored with gluten protein antisera.

Table 3. Effect of Wine Fining on the Sensorial Appraisal[a]

	correct responses	
triangular comparisons	olfactive test	gustative test
control vs gelatin	4	4
control vs deamidated gluten	5	4
deamidated gluten vs gelatin	5	6
deamidated gluten vs egg proteins	6	6

[a] Wines were compared using triangular tests. For 15 assessors, 9 correct responses are necessary to have a statistically perceivable difference.

wine clarified with wheat gluten may be considered as a gluten-free drink. In 2001, the same study was made with EHG antibodies and different red wines treated with EHG. The immunological detection was also negative (data not shown). These results seriously strengthen the interest that can be taken in the use of gluten to clarify red wines.

Effect of Gluten and Animal Finings on Sensorial Appraisal. For all comparisons, the number of correct responses (Table 3) was inferior to the critical value (equal to nine) given by the binomial law for a probability of 1/3 (*9*). The modification of composition due to finings was probably very small, so that it was not possible to differentiate the control Burgundy wine from the deamidated treated wine. For the dose used (12 g/hL), this also indicates the impossibility of softening the astringency of the wine. The dose necessary to reduce astringency (for example) was much higher than the dose able to correctly clarify this wine.

CONCLUSION

This first study shows that gluten proteins used at concentrations between 6 and 18 g/hL allow very good clarification of the Burgundy treated wine when compared with the untreated red wine. After wine fining, some glutens used at 12 and 18 g/hL gave turbidities situated between the minimal and maximal values obtained with gelatin and egg proteins. For low fining concentration (6 g/hL), deamidated glutens were less efficient than the two EHG tested (differences were less marked when the doses increased). A study made in 2001 with more than 10 red wines, from different French areas and different varieties, has shown that efficiencies were always better with EHG than with deamidated glutens (data not shown). Also, the volume of lees was higher with deamidated gluten than with EHG. Concerning celiac disease, deamidated gluten seems to give higher intolerance than EHG. For all these reasons, it is preferable to use EHG than deamidated gluten.

Wine clarifications are better with glutens than with gelatins, which are the more commonly used fining agents. Gluten treatments led to results a little less desirable than those obtained with egg proteins. Moreover, longer kinetics to reach low values of turbidity were observed with glutens, but only for high doses. Further experiments are needed, using red wines from different grape varieties and different wine-growing regions, to verify and generalize these first conclusions.

A further study will concern the influence of gluten hydrolysis level and deamidation level on the clarifying efficiency, two points of particular interest. Different proteic fractions isolated from whole gluten will also be tested.

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