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Clarification of Muscat Musts Using Wheat Proteins and the Flotation Technique

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The bovine spongiform encephalopathy (BSE, mad cow) crisis has led some wine-makers to question gelatin as a fining agent and to reject the use of these animal proteins. The search for a substitute for gelatin was begun by comparing vegetable proteins (particularly gluten) with gelatin fining treatments. Clairette de Die (French-controlled appellation) is a sparkling sweet wine. The alcoholic fermentation begins in a tank, continues in a crown-cap bottle, and stops before the complete consumption of sugar. All particles present in the bottle have to be removed before corking, and it is important to have a must as clear as possible. This study concerned the clarifying of a Muscat must, treated with pectinases, using a very efficient flotation technique. The must is fined with bentonite/silica/protein (fish gelatin, wheat gluten, or lupin isolate) to induce flocculation and then pressurized (6 bar). After depressurization, microbubbles cling to flocculates and climb to the top of the flotation tank (this flotation foam is clarified using a rotary filter). At laboratory scale, gluten (20 g/hL) and gelatin (10 g/hL) (each combined with bentonite and silica gel) gave turbidities of 50 and 35 NTU, respectively (6.5 and 4.1% of that of the nontreated must). The Muscat must was also clarified by static settling. The turbidity decreased by 86% for the gluten/bentonite fining and by 60% for the gelatin/bentonite fining. Visually, gluten flocculation takes longer to occur and flocculates sedimentation is longer than with gelati, but the removal of insoluble particles is more complete and leads to lower turbidities. At an industrial scale, gluten (20 g/hL) and gelatin (10 g/hL) (each combined with bentonite and silica gel) gave turbidities of 60 and 48 NTU, respectively. Turbidities measured in the tanks 14 h after the flotation showed a better efficiency for the wheat gluten (24 NTU) compared to gelatin (28 NTU). This is explained by the static settling that completed the clarifying effect of the flotation. The last experiment showed comparable efficiencies for wheat gluten and lupin proteins. BSE caused a situation of crisis (in Europe particularly) leading the public and wine-makers to lose their confidence in the use of gelatin as fining agent and to reject animal proteins in general. It is proposed here that vegetable proteins could efficiently replace gelatin.

KEYWORDS: Flotation; vegetable proteins; wheat gluten; must; Muscat; bentonite; gelatin

INTRODUCTION

After pressing, a Muscat must is a colloidal solution and suspension. The particle density, which is close to that of the must, the electrical repulsion forces, and the diffusion phenomena lead to very slow and insufficient spontaneous clarification. Moreover, for the Clairette de Die elaboration (a sparkling wine with approximately 7.5% alcohol and 40 g/L residual sugar), pectinolytic enzymes are added to the Muscat grapes in the crusher to increase the extraction of aroma compounds. The consequence of this treatment is that the must presents a high turbidity (between 1000 and 1500 NTU). During alcoholic fermentation, the must is bottled when the density is ∼1040−1050 g/L; the fermentation continues in the bottle until the pressure reaches generally 6 bar and then stops spontaneously, and the sediment is removed after 6−8 months of bottle-aging. Riddling and disgorging are all the easier because the turbidity of the must is low. Centrifugation gives bad clarifications (G. Establet, personal communication); filtration is too long and expensive. For these reasons, organic and mineral fining agents are commonly used to clarify white musts using the flotation technique (*1*−*9*). Bentonite treament is also efficient for protein removal and reduces natural haze sometimes observed in the bottle (*10*, *11*). This treatment can also reduce browning (*12*),

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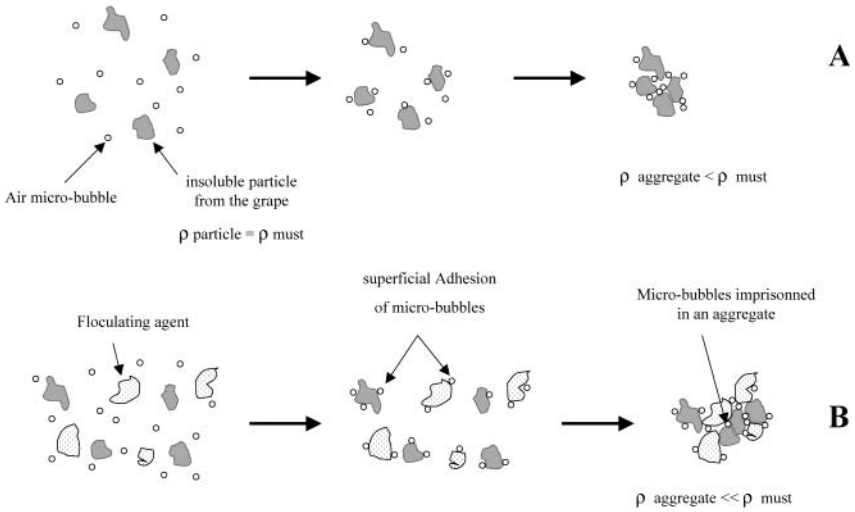

Figure 1. Principle of the flotation.

even if mineral fining agent is occasionally responsible for the loss of must and wine aroma (*13*, *14*).

Gelatin (combined to bentonite and silica gel) gives very good clarification, but some cases of bovine spongiform encephalopathy (BSE, mad cow) led to grave concerns, and wine-makers were encouraged to discontinue use of bovine gelatin. In Europe, the concern of transmitting this disease to humans led to a ban on the use of bovine plasma and blood cells, commonly but incorrectly called blood albumin and glue (Regulation CE 2087/97, Council of October 20, 1997).

Given the above, it was important to do research into treatments that could replace gelatin finings. We thus started our investigation with wheat prolamins, commonly called gluten, to clarify white musts. Different experimental procedures were established to compare gluten efficiency with that of gelatin.

The first experiments using wheat gluten to clarify musts were carried out in September 2000, with a laboratory flotation system. The results, very encouraging (data not published), allowed us to experiment in 2001 at an industrial scale (with authorization of the National Institute of Controlled Appellations). The aim of this work was also to estimate the efficiency of each fining agent (proteins, bentonite, and silica gel) during the flotation and to quantify possible synergy between them. The last point concerns the research of residual gluten proteins in the treated must. Gluten sometimes causes severe intolerance (coeliac disease), and it is then very important to know if it is completely eliminated after clarifying.

PRINCIPLES OF FLOTATION

Flotation is a solid−liquid separation process used when the density of the particles is lower than that of the liquid containing them. In a must, the greatest part of particles have a density close to that of the must. This density can be artificially reduced using gas bubbles that attach to insoluble particles from the grapeberry (**Figure 1**). When air bubbles are directly injected in the must to improve the separation of the particles, the process is called assisted flotation (**Figure 1A**). When air bubbles are injected in a must treated with a fining agent, the association between air bubbles and must flocculates gives insoluble complexes with very low density compared with that of the liquid. These complexes reach easily the surface of the tank; the process is called induced flotation (**Figure 1B**). When the diameter of the air bubbles is between 40 and 70 μm, the process is called dissolved air flotation. The speed with which the gas−particle complexes rise through the liquid to reach the liquid/air surface largely depends on the diameter of microbubbles, the mass of the particles, and the densities of the liquid, gas, and particles. The temperature and the hydrophobicity of the particles are also important parameters for must clarification using the flotation technique.

Table 1. Analytical Characteristics of the Muscat Musts before Fining and/or Flotation

	Muscat 1	Muscat 2	Muscat 3	Muscat 4
turbidity (NTU)	855	1446	1420	1265
density (g/L)	1080	1078	1076	1076
pH	3.30	3.25	3.37	3.29
total acidity (g/L H_2SO_4)	5.4	5.6	5.9	6.1
total SO_2 (mg/L)	13	30	22	
results presented in Figure	4, 5	6	7, 8	9

The density of must particles depends on the flocculation conditions following the pressing. At first, miniflocculates are formed. We observe after this an aggregation of these flocculates, leading to larger flocculates separable from the liquid. This second step is made easier when the must is treated with pectolytic enzymes, in the crusher or just after pressing. The vintage, the variety, and the maturity of the grapeberry also influence flocculation. In enology, it is possible to use flocculating agents to bind particles. These agents are mineral polymers (bentonite and silica gel) or natural polymers extracted from animal subtances (pork, bovine, and fish gelatins). In this study, proteins isolated from wheat (gluten) and lupin were investigated.

MATERIALS AND METHODS

Must. The Muscat variety musts (denoted Muscats 1−4) used in this study were from the Cave de Die Jaillance (Drôme, France). Grapes were hand-harvested in September 2001. Pectolytic enzymes (Inozyme, 1 g/hL; Institut Oenologique de Champagne, Epernay, France) and SO_2 (30 mg/L) were added in the crusher. After being crushed, the must pump sent the grapes to an industrial 8000 kg press (pressure between 1.5 and 2 bar). After pressing, the must was stored for 10 h in a tank (to allow the flocculation to occur) before being taken for a fining test or for clarification experiments. Must current analyses are reported in **Table 1**. Sugar content was determined using a Dujardin-Salleron (Paris, France) 1060-1090 Mustimeter, with a correction of the value at 20°C. The pH was determined by using an Orion 420A pH-meter (Thermo Electron Corp., Waltham, MA). Total acidities were determined by M/64 NaOH additions using blue bromothymol as a colorimetric indicator. One hour before flotation, compressed air was injected in the tank to put in suspension the particles/flocculates of the must.

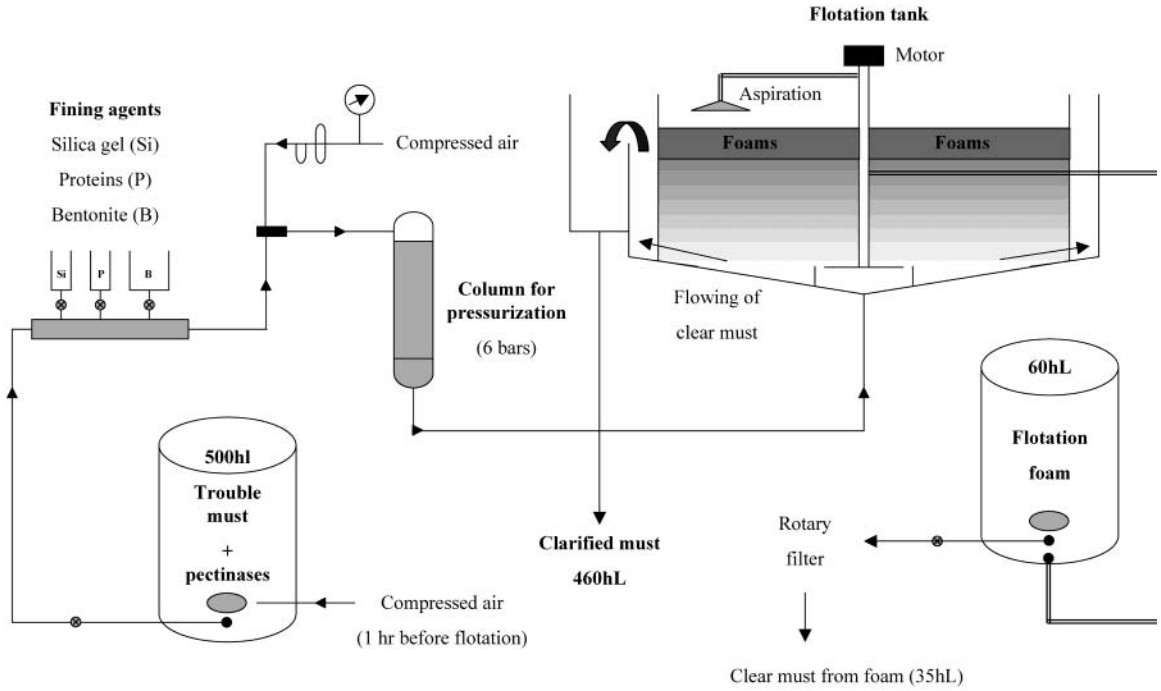

Figure 2. Industrial flotation system.

Table 2. Influence of the Initial Turbidity on the Efficiency of the Flotation Technique[a]

turbidity before flotation (NTU)	approximate ratio NTU/lower NTU	turbidity after flotation (NTU)	residual turbidity (%)
2852	12	128	4.48
1970	8	93	4.92
1420	6	79	5.56
916	4	68	7.42
522	2	53	9.6
256	1	37	14.45

[a] The must was fined with gluten (10 g/hL) + bentonite (20 g/hL) + silica gel (10 g/hL).

Musts with Controlled Turbidity. A Muscat must (20 L) was taken in a 500 hL tank (Muscat 3) and left in a plastic container during 2 h to allow the static sedimentation of flocculated particles. The supernatant (turbidity = 192 NTU) was racked and separated from the sediment (turbidity = 4360 NTU). Then, clear must and lees were mixed to obtain turbidities of between 250 and 2000 NTU (**Table 2**). Lees were agitated before each mixing to have exactly the same composition, particularly for size particles. The fining used for this last experiment was $bentonite_{20} + gluten_{10} + Si_{10}$. The dose of gluten was reduced with respect to previous laboratory and industrial trials (see **Figures 4**−**6**) because of its very good clarifying efficiency.

Enological Products. Gluten was supplied by a French company (Chamtor, Bazancourt, France). The producers guaranteed that the gluten did not come from genetically modified organisms. Gluten was obtained from vital gluten enzymatic hydrolysis. The isoelectric points of proteins were between 6 and 8. Lupin protein isolate was supplied by Groupe CANA (Ancenis, France). Gluten and lupin protein isolate gave complete suspensions without particle sedimentation; gluten was for this reason very easy to use at an industrial scale. Bentonite (denoted B) was a sodic activated bentonite (Volclay). The fish gelatin (denoted FG) was used at 45 °C for a complete solubilization. The silica gel (denoted Si) was a commercial solution (30% p/v SiO_2). Sulfur dioxide (SO_2) was added, at a concentration of 1 g/L, to all of the solutions of proteins (laboratory experiments only), which were kept at 4 °C for ideal preserving conditions. Bentonite was prepared in water to swell 12 h before must fining. All enological products were prepared as aqueous solutions/suspensions at 10 g/L for laboratory experiments. For industrial trials, concentrations were 100 g/L for the gluten, 50 g/L for the bentonite and fish gelatin, and 30 g/L for the silica gel.

Static Fining Experiments. Fining tests were carried out in plastic graduated cylinders (volume = 500 mL and internal diameter = 52 mm), filled to 260 mm with must. Finings were tested 10 h after pressing (Muscat 1). Enological products were introduced with automatic BioHit pipettors (Bonelles, France). Graduated cylinders were turned over twice to homogenize fining agents and must. Under Results, the type of agent used for fining and the quantity of fining agent added are noted as follows: for example, the abbreviation B_{20} means we have used bentonite at a dose of 20 g/hL; on the other hand, the abbreviation $B_{20} + Si_{10}$ means we have used bentonite at a dose of 20 g/hL combined with silica gel at a dose of 10 g/hL.

Clarification and Lees Volume after Static Fining. A 15 mL sample was taken from the graduated cylinder with a 25 mL plastic pipet. As liquid taken from the top is 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 into 200 mL of liquid and then unlocked to allow the sample to fill it. Experiments were carried out at pressing-room temperature (20 ± 2 °C). Turbidities and lees heights were measured 4 h after the addition of fining agents to allow the flocculates to slide down from the bulge onto the neck of the cylinder. Turbidities were measured with a turbidimeter Hach 2100 AN (Loveland, CO) calibrated with the Gelex secondary turbidity standards kit and expressed in nephelometric turbidity units (NTU). Lees height was measured with a ruler, converted to milliliters and finally to percentage lees/liquid (v/v).

Industrial Flotator and Flotation. The indusrial flotator (**Figure 2**) was a Flottaflux 2-2A system (Padovan spa TMCI, Conegliano, Italy). After fining, the must was pressurized with air in the saturation column (6 bar) and sent (500 hL at 140 hL/h) in the flotation tank (80 hL). An aspiration system collected the flotation foam (60 hL) on the top of the flotation tank. Particles of the foam were eliminated with a rotary filter (Padovan spa TMCI), and a clear must (35 hL) was obtained. The fining treatment usually used by Cave de Die Jaillance winery is bentonite (20 g/hL) combined with silica gel (at a dose of 10 g/hL) and fish getatin (10 g/hL). In this study, this treatment was the industrial control (*13*). Two identical tanks, containing exactly the same must, were treated with $B_{20} + Si_{10} + gluten_{20}$ or with $B_{20} + Si_{10} + FG_{20}$. The industrial experiment was made twice, and the musts obtained with the same treatment were blended. Therefore, we obtained 1000

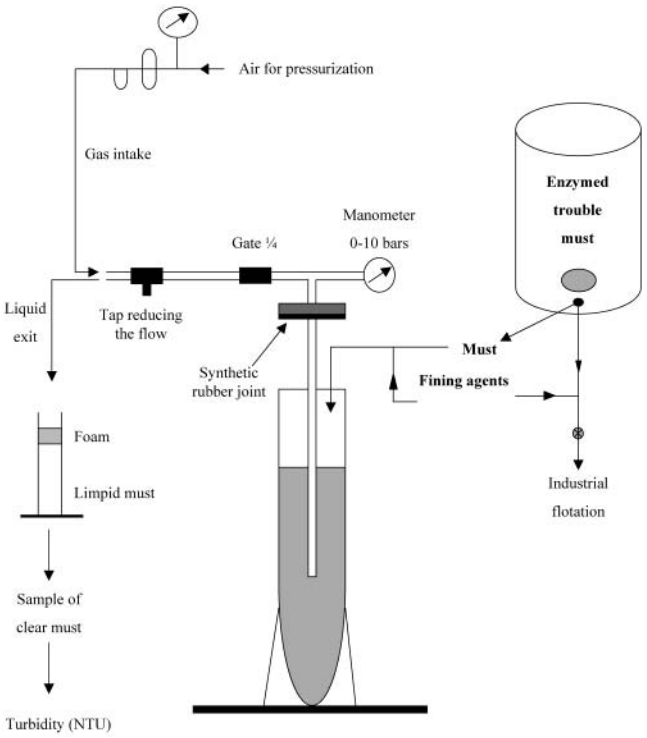

Figure 3. Laboratory flotation system.

hL of must clarified by flotation with gluten as protein fining agent and 1000 hL of must clarified by flotation using fish gelatin as protein fining agent.

Flotator and Flotation at Laboratory Scale. Laboratory experiments were carried out with a miniflotator (**Figure 3**) furnished by Padovan. The capacity of the tank (internal diameter = 80 cm, height = 44 cm) was 1.5 L. The whole system was in stainless steel, except for the manual one-quarter turn gate AISI 316. The enzymed must (1 L) is poured in the tank. Fining agents were added with automatic BioHit pipettors (Bonelles, France). The miniflotator was then closed and turned over twice to homogenize fining agents. After this, must was pressurized (6 bar) using the industrial pressurized air system. The flow rate of the air was 46 L/min (~7 L to reach 6 bar). The miniflotator was then turned over 20 times to dissolve and saturate the liquid with the air. The must was poured into a graduated cylinder using a stainless steel pipe (internal diameter = 12 mm, height = 44 cm) and a plastic pipe (internal diameter = 12 mm) owing to the pressure of the system. After 3 min, a sample was taken as for static fining and the turbidity was measured.

A preliminary study was conducted with different Muscat musts flotated using two different treatments: bentonite (20 g/hL) + gluten (20 g/hL) + silica gel (10 g/hL) and bentonite (20 g/hL) + gelatin (10 g/hL) + silica gel (10 g/hL). Standard deviations ($m \pm ts_m$, with $P = 0.95$ and $n = 3$) were situated between 3 and 5 NTU (with turbidities after flotation situated between 35 and 55 NTU).

Gluten Immunodetection in Muscat Must. The research of residual gluten proteins, in Muscat must and must obtained from foam filtration, was assayed using an ELISA method (Riedel de Haen, ELISA-Systems gliadin, ref 45230, Sigma-Aldrich Laborchemikalien GMBH, Seelze, Germany). Quantification was carried out at 450 nm with a Bio-Wittaker ELX 808 microtiter plate photometer (Biotek Instrument Inc., Winooski, VT), according to the manufacturer's procedure. Gluten content was calculated with regard to two standard curves (10−200 μg/L). Standard curves were obtained with gliadins extracted from the gluten used for the flotation experiments and also with a blend of gliadins extracted from 30 wheat glutens. The two curves were superimposable.

RESULTS AND DISCUSSION

Influence of the Flotation Process (Laboratory Scale) and the Nature of the Fining Agents on Must Clarification (Muscat 1). Concerning the results presented in **Figure 4**, each value is the average of two experiments. We have decided to make only two trials to work in a time as short as possible (~5−6 h) and to keep a must as homogeneous as possible. During the depressurization of the must (previously at 6 bar), microbubbles are formed because the liquid becomes supersaturated with air. This assisted flotation (see **Figure 1A**) gave a turbidity equal to 31% of that of the untreated must (without fining agent), but the turbidity remained at 270 NTU, a too high value (**Figure 4**). When a mineral flocculating agent such as bentonite or silica gel was added, the turbidity of the must after flotation was nearly equal to 80% of that of the control must. These results showed that these two products, used alone, had poor efficiencies. Bentonite, which is a poor clarifying/fining agent for the flotation technique, is used instead to reduce the risk of proteic haze that is often observed for Muscat wines if musts are not fined. Bentonite was (and is still) used only for this main enological reason (which is a major and universal problem for white wines).

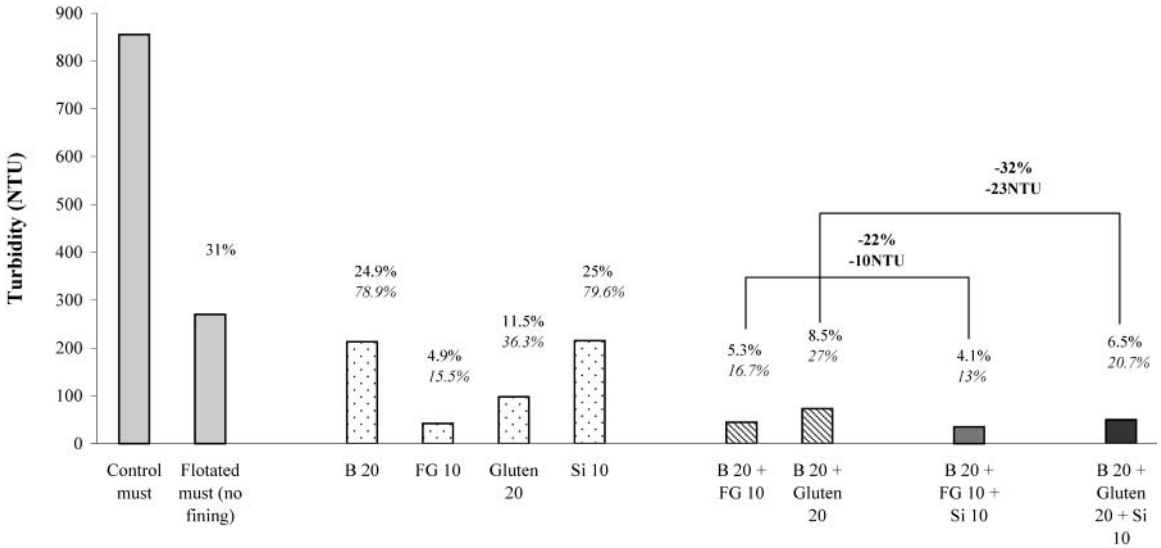

Figure 4. Clarification of a Muscat must using the flotation technique (laboratory experiments): influence of different fining agents (each value is the average of two experiments) [upper values (%), residual NTU compared to the control must; lower values (*% in italic*), residual NTU compared to the flotated must].

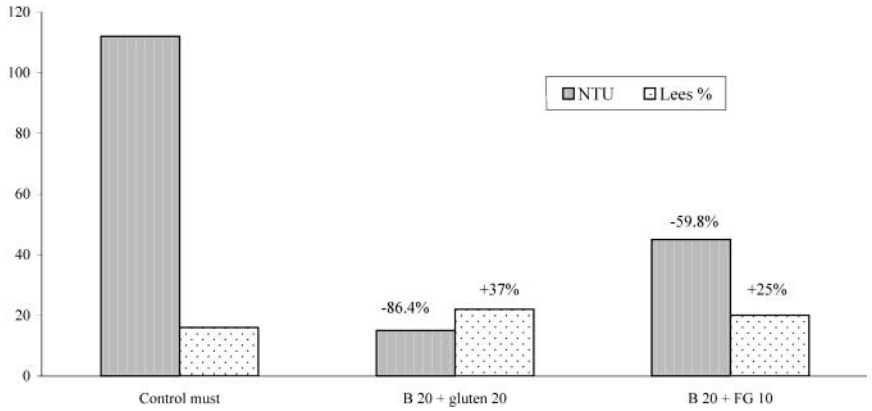

Figure 5. Static clarification of a Muscat must. Influence of bentonite–protein fining on the turbidity and lees height.

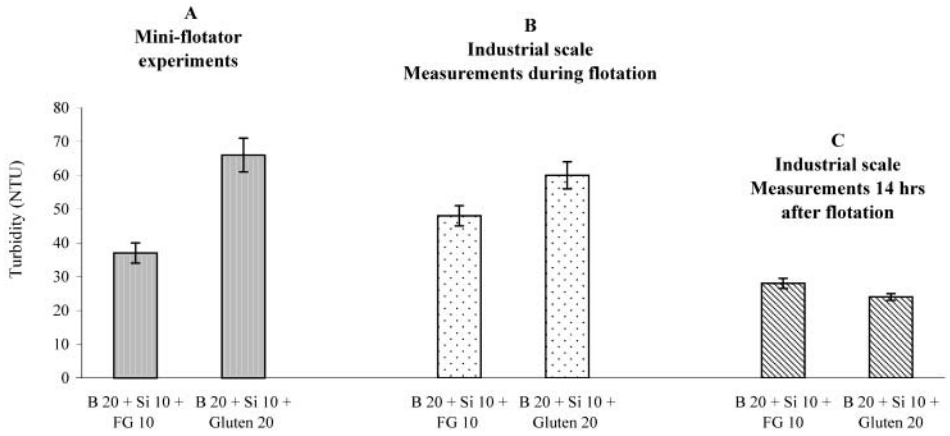

Figure 6. Clarification of a Muscat must using the flotation technique: comparison between industrial trials and laboratory experiments (three measurements), during and 14 h after flotation (average of three samplings). For industrial trials, measurements are the average of six measurements made during the process (15 min between each sampling).

On the contrary, wheat gluten and fish gelatin allowed good clarifications, the fish gelatin being a little better than gluten (−95.1 and −88.5% compared to the turbidity of the control must, respectively) (**Figure 4**). The combination B_{20} + FG_{10} gave an efficiency comparable with that of the must fined with FG_{10} alone. When gluten was combined with bentonite, the turbidity decreased from 36 to 27% compared to that of the flotated must without fining.

The clarification was all the more marked if silica gel was combined with gluten and bentonite. Comparison of B_{20} + $gluten_{20}$ vs B_{20} + $gluten_{20}$ + Si_{10} gave a difference in turbidity of ΔNTU = 23 (−32%); the turbidity decreased from 27% (−73%) to 20.7% (−79.3%) when compared to the flotated must (not fined), whereas it was −93.5% compared to the control must (no flotation). These differences were already noted in 2000 and 2001 with other Clairette and Muscat musts (not published). On the basis of these results, we can consider that the use of silica gel significantly improved the efficiency of the flotation technique when the protein used was the wheat gluten. The difference was less clear when the protein used was the fish glue, and it is not possible to state that B_{20} + FG_{10} + Si_{10} (NTU = 35) was more efficient than FG_{10} (NTU = 42), probably because bentonite + fish glue gave together a turbidity already very low.

Last, the combination $gluten_{20}$ + B_{20} + Si_{10} was less efficient than the treatment FG_{10} + B_{20} + Si_{10} (−87 and −95.9% in turbidity, compared to that of the flotated must and untreated must, respectively). Here again, results were confirmed with others experiments in 2000 and 2001 (Clairette and Muscat musts).

Fining Experiments with Static Clarification (Muscat 1). The must was clarified with the same treaments and doses as those used for clarification by flotation. Four hours after fining, the treatment $gluten_{20}$ + B_{20} reduced the turbidity of the must by ∼86% (**Figure 5**), whereas the decrease of the turbidity was only ∼60% with the combination FG_{10} + B_{20}. This higher clarifying efficiency also gave higher lees volume (**Figure 5**), but we absolutely have to keep in mind that the main problem is the decrease of the must turbidity. Visual observations were also made during the clarifying kinetics. It appeared that the sedimentation of the flocculates was faster with B_{20} + FG_{10} than for B_{20} + $gluten_{20}$ fining, but numerous small flocculates remained in suspension with the B_{20} + FG_{10} treatment; on the contrary, B_{20} + $gluten_{20}$ fining gave a much clearer must. This observation will allow a better understanding of the results of industrial flotation experiments.

Industrial Flotation Experiments (Muscat 2). Measurements were made just after flotation (between the flotation tank and the temporary storage tank). Turbidities decreased by 95.9 and 96.7% when the fining treatments were B_{20} + Si_{10} + $gluten_{20}$ and B_{20} + Si_{10} + FG_{10}, respectively (**Figure 6**), when compared with the control must without flotation (1446 NTU). The turbidity of the must flotated with the gluten as protein was 20% higher than that of the must flotated with FG as protein (**Figure 6B**). The same treatments were investigated with the laboratory flotator, with the same must. Results (**Figure 6A**) confirmed the lower efficiency of gluten (−95.4% of the initial NTU) compared to that of FG (−97.5% of the initial NTU). If we observe now the residual turbidities, the must flotated with FG_{10} has a turbidity 44% lower than that of the must flotated

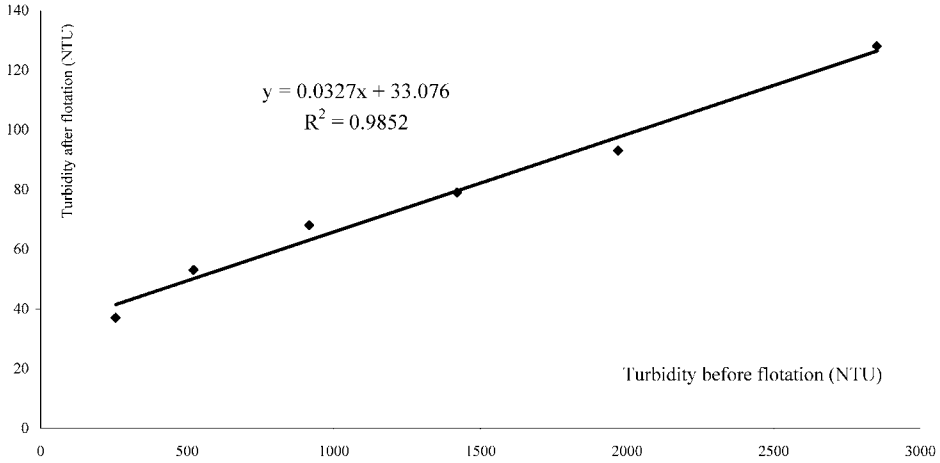

Figure 7. Influence of initial turbidity on the clarifying efficiency of the flotation. The fining treatment used was bentonite$_{20}$ + gluten$_{10}$ + Si$_{10}$.

with gluten. Differences in turbidity were twice higher at the laboratory scale than at the industrial scale. It will then be necessary to optimize the laboratory experimental procedure to obtain a better expectation of industrial trials. Turbidities were also measured in a temporary storage tank (500 hL), 14 h after the industrial clarification by flotation. The turbidity of the must flotated with gluten was 14% lower than that of the must flotated with FG$_{10}$ (**Figure 6C**). During these 14 h, a static settling occurred in the tank. Therefore, results of static settling (when the must was fined and clarified by sedimentation of flocculates) indicated a higher efficiency for fining with gluten (**Figure 5**), even if the kinetics was longer. We can hypothesize that small flocculates, present in the must after gluten fining, had higher density than flocculates formed with fish gelatin. According to this hypothesis (1) during the flotation, the higher mass will reduce the velocity of the flocculates rising through the liquid, and the flotation duration (~15 min at industrial scale) is not sufficient to allow a perfect clarification; and (2) during static settling, the sedimentation velocity will be higher for the same reason.

At the production scale, clarification occurred in two steps: during flotation, FG$_{10}$ was more efficient than gluten and gave lower turbidities; during the second step, the sedimentation of particles led to lower turbidities when the gluten was added to the must before flotation (always combined with bentonite and silica gel in this study).

Globally, these flotation experiments at an industrial scale gave nearly equal efficiencies, even if the gluten treatment was a little better. To improve the efficiency of the gluten fining, we suggest that the output of the pump that fills up the flotation tank be reduced; then flocculates will have more time to reach the surface. Theoretically, this should permit clearer musts to be obtained.

Influence of Initial Must Turbidity on Flotation Efficiency (Muscat 3). Turbidities were chosen to have approximately whole numbers for the ratios between each sample and the lowest turbidity (**Table 2**). The fining treatment used for this experiment was B$_{20}$ + gluten$_{10}$ + Si$_{10}$. The dose of gluten was reduced with respect to previous laboratory and industrial trials (**Figures 4−6**) because of its very good clarifying efficiency. Results showed a regular decrease of the must turbidity after flotation when the turbidity before flotation decreased (**Table 2**). Turbidities before and after clarification were correlated by a linear relationship with $R^2 = 0.985$ (**Figure 7**). The slope of the straight was relatively low. For this reason, when we clarified two musts that differed in turbidity by 1000 NTU before flotation (10 h after enzyme addition in the crusher and pressing, turbidities of Muscat musts are always situated between 1000 and 2000 NTU), the difference of turbidity after flotation was only 25 NTU (**Table 2**). This indicated that it is not essential to have turbidities as low as possible before flotation, because of the very high efficiency of the process. We also observed that the higher the turbidity before flotation, the higher the clarifying efficiency expressed in percent (**Table 2**). The percentage of particle removal by flotation decreases as the initial concentration in particles decreases. The relationship between these two parameters followed a power law (**Figure 8**), with $R^2 = 0.989$. The equation was

$$y_x = 224x^{-0.5}$$

$$y = (ab)^z = a^z b^z$$

$$y_{2x} = 224(2x)^{-0.5} = 224x^{-0.5}2^{-0.5}$$

$$y_{2x} = y_x(2^{-0.5}) \text{ with } 2^{-0.5} = 1/\sqrt{2} = 0.707$$

Practically, the residual turbidity of the must decreased by 29.3% (1−0.707) each time the initial turbidity was twice higher.

Now, if we consider absolute values (NTU), the turbidity after flotation was all the more lower than the turbidity before flotation.

Flotation with Lupin Proteins (Muscat 4). An experiment was also carried out at laboratory scale to compare the clarifying efficiency of lupin proteins with that of gluten, two protein isolates originating from vegetables. Turbidities of the musts fined with lupin or gluten were nearly equal after flotation and higher than the turbidity of the must clarified with FG (**Figure 9**). The experiment was done in triplicate for each fining treatment (it was possible because only three treatments were compared) and allows to calculate standard deviation and to do a variance analysis. The conclusions are as follows: (1) significant differences were observed between gluten and fish gelatin and between lupin and fish gelatin; (2) there was no significant difference between gluten proteins and lupin proteins.

However, industrial trials have shown that differences between vegetable and animal protein efficiencies were lower than those obtained using a laboratory flotator. Moreover, the sedimentation step following the flotation reduces the differences. For these reasons, a further study will concern the influence of must clarification with lupin proteins, at the production scale.

On the basis of the molecular composition of wheat gluten (*15−25*) and lupin proteins (*26−32*), it is not possible at the

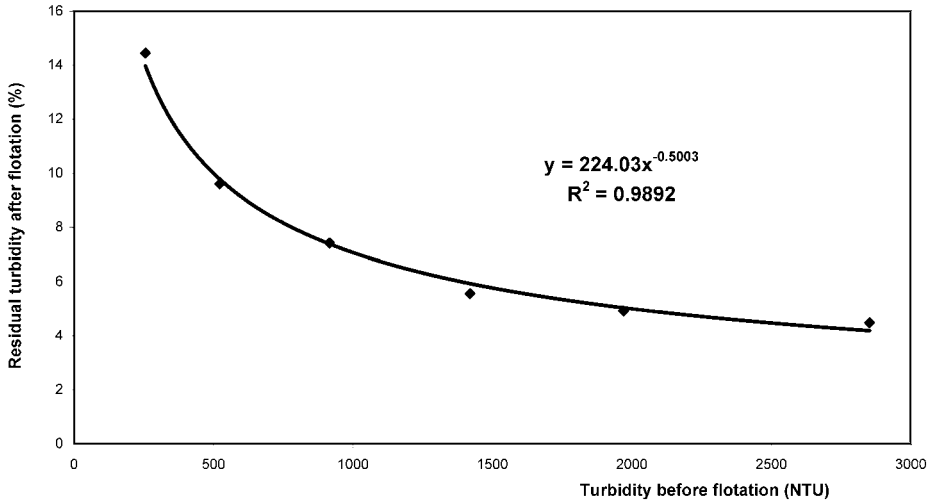

Figure 8. Relationship between the turbidity of the Muscat must before flotation and the residual turbidity (percent) after flotation. The fining treatment used was bentonite$_{20}$ + gluten$_{10}$ + Si$_{10}$.

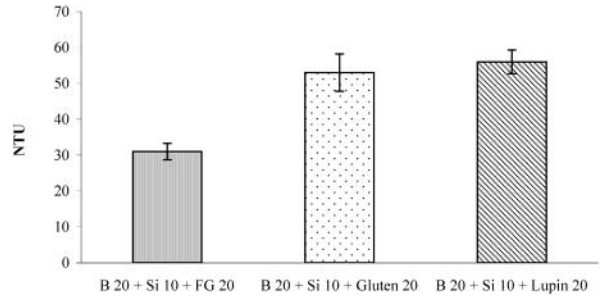

Figure 9. Clarification by flotation: comparison between animal (fish gelatin) and vegetable proteins (wheat gluten and lupin). Experiments (three for each treatment) were done at laboratory scale.

moment to understand why the gluten is a little better than lupin protein isolate. Glutens are composed of numerous gliadins (*16*) and glutenins (*18*). Gliadins present differences in their amino acid composition, the consequence being a difference of wheat protein hydrophobicity (*23*) and pH_i values (*20*). These biochemical characteristics are also induced by the industrial processes (partial enzymatic hydrolysis for the gluten, no hydrolysis for lupin proteins). Now, these biochemical characteristics are responsible for protein 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 mechanisms observed with gluten and lupin protein isolate, it will be necessary to work with a model must having a controlled composition. It will also be necessary to experiment with proteic fractions isolated from lupin and gluten and the same fractions after enzymatic treatments.

Research of Residual Gluten Proteins in Muscat Must (Muscat 2). We particularly insisted on the detection of possible residual gluten, for it has been clearly shown that animal fining proteins are not completely removed by the settling and filtration of treated wines (*33*). Coeliac disease (nontropical sprue) is an intolerance to food, and susceptible individuals show lesions of the intestinal mucosa after consumption of the gliadins of wheat. The drastic decrease in absorptive surface is the cause for the malabsorption accompanied by weight loss and disturbance of growth (*34*, *35*). By using an ELISA immunotechnique, gluten antibodies were not able to recognize their antigens in the Muscat must treated with 20 g/hL gluten (values were the detection limits of the ELISA method used). This result clearly indicates the absence of residual deamidated gluten proteins in the must (<1 mg/L, which is the limit of the detection kit). For the *Codex Alimentarius*, a food is gluten-free when it contains <10 mg/L gluten. In this assay, the white must clarified with wheat gluten derivatives may be considered to be gluten-free. These results seriously strengthen the interest one can take in the use of gluten to clarify musts.

Conclusion. This study shows that gluten proteins (combined with bentonite and silica gel) allowed very efficient clarification of the treated must when compared with the control must. Moreover, the industrial scale trials showed that grape juice clarification using the flotation technique was a little better with bentonite−gluten than with the bentonite−fish gelatin combination, which are the more commonly used fining agents. This confirms the possibility for vegetable proteins to replace animal proeins, as already observed for red and white wines (*36*, *37*).

A further study will focus on the influence of must fining on the resulting wine composition and sensory perception. Clarification represents one step in the wine-making process that can modify major wine characteristics such as foaming properties (*38*, *39*) proteic stability (*40*, *41*), and tartaric stability (*42*, *43*), phenomena in which colloids are largely involved.

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