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Influence of Lysozyme Treatments on Champagne Base Wine Foaming Properties

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The objective of this study was to estimate the effect of lysozyme on the foaming characteristics of Champagne base wine. Lysozyme additions were made to the musts and also to the wines before and after bentonite or charcoal treatments, which remove endogenous proteins. Treatments with bentonite diminished foamability and foam stability of wines, whatever the dose (30 or 80 g/hL) and variety [Chardonnay, −28%; Pinot noir, −20% (at 30 g/hL)]. An addition of lysozyme in must raised Pinot noir wine foamability by 21%, whereas the difference is hardly perceptible for Chardonnay wine (+3%). Pinot noir and Chardonnay wines, originating from lysozyme-treated musts, in addition to bentonite treatment on the wine, presented higher foamability than wines treated only with bentonite. Lysozyme was removed (91−100%) by the bentonite treatment. Then, it was not responsible for the increase in foamability but seemed to have a protective effect on the wine proteins. When wines were initially treated with bentonite (150 g/hL) and then enriched with 80 g/hL lysozyme, this enzyme was not able to restore foaming properties. Treatments with charcoal always diminished foamability. The average increase in foamability due to an addition of lysozyme after charcoal treatment (80 g/hL) was 23%. Results showed a real positive effect of lysozyme on foam stability when wines have to be treated with charcoal (+25% and +56% for the Pinot noir wine and the Chardonnay wine, respectively, at 30 g/hL).

KEYWORDS: Foam; lysozyme; Champagne wine; bentonite; charcoal; Chardonnay; Pinot noir; proteins; sparging procedure

INTRODUCTION

The ability of Champagne wine to form a persistent collar is an important feature in terms of product attractiveness to the consumer. Knowledge of enological practices that can modify the foaming properties of sparkling wine is then important for winemakers. Different studies have shown that proteins are largely involved in the stabilization of foam in Champagne wines, despite their low concentration that ranges from 4 to 20 mg/L (*1*). Indeed, Maujean et al. (*2*) found a correlation ($r = 0.845$) between foamability (using a gas sparging procedure) and the protein content of 31 wines, using the direct Bradford method (*3*). Foam was extracted from still wine foam (by constant artificial effervescence) and collapsed into a foam wine (*4*). This foam wine showed foamability and foam stability, respectively, 59 and 34% higher than in the base wine. In this foam wine, the protein enrichment rate was ∼20%. Another example of the relationship existing between protein content and Champagne wine foamability was given by mixing base wine with either ultrafiltrates or ultraconcentrates (molecular weight cutoff at 10 kDa) (*5*). These authors have demonstrated that the control of foam stability (once effervescence was stopped) was strictly correlated with the protein concentration.

Part of the wine proteins originate from the grape berry (*6*−*10*), whereas others come from yeasts (*10*−*13*). Evidence was also obtained, using an immunochemical technique, that proteins secreted by *Botrytis cinerea* are present in infected musts (*14*). In addition to endogenous proteins, wines can contain residual exogenous proteins originating from enological treatments such as gelatin plus tannins, the presence of which can modify Champagne wine foamability (*15*).

In the field of enology, hen egg lysozyme is used to prevent malolactic fermentation and to diminish the quantities of SO_2 added to the must or the wine. Lysozyme is a peptidoglycan *N*-acetylmuramylhydrolase (EC 3.2.1.17). Previous papers have shown that *Oenococcus oeni* species are sensitive to the action of lysozyme (*16*, *17*). This enzyme has also been investigated to reduce the lactic bacteria flora in musts and wines after completion of the malolactic fermentation. Quantities added to musts and wines ranged from 250 to 1000 mg/L (*16*−*18*), and all studied wines contained residual lysozyme.

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Table 1. Current Analyses of the Pinot Noir and Chardonnay Musts and Wines

process stage	musts		wines			
grape variety	Pinot noir	Chardonnay	Pinot noir		Chardonnay	
lysozyme	nontreated	nontreated	nontreated	treatment to the musts (50 g/hL)	nontreated	treatment to the musts (50 g/hL)
sugar (g/L)	159.3	155				
maturity index (sugar/total acidity)	15.3	10.9				
total acidity (g/L tartaric acid)	10.4	14.2	9.7	9.7	13.4	13.4
pH	3.24	2.96	3.33	3.33	2.97	2.97
alcohol (% v/v)			11.1	10.8	11.1	10.8
malic acid (g/L)	6.4	7.2	5.0	4.6	6.3	6.1
tartaric acid (g/L)	4.88	5.87	2.3	3.5	4.63	4.96
lactic acid (mg/L)			20	23	17	17

Now, lysozyme shows foaming properties depending on the structure of the protein and the concentration of the bulk protein solution (*19*). The formation of complexes between lysozyme and polysaccharides results in a substantial enhancement in foamability (*20*).

Wine is a very complex matrix containing numerous molecules of proteins, phenolics, and ionic and nonionic polysaccharides that can interact with the foam. The objective of this study is designed to estimate the effect of lysozyme on Champagne base wine foaming characteristics. To do this, we used Chardonnay and Pinot noir wines; lysozyme additions were made before or after enological treatments.

MATERIALS AND METHODS

Production of Musts and Wines. Grape berries of the Pinot noir and Chardonnay varieties were collected from the Champagne area (France). Grapes were hand-harvested in September 1998 and pressed with a Cocquart 4000 kg press (pressure between 1.5 and 2 bar). Musts were inoculated with a selected *Saccharomyces bayanus* at 10 g/hL (DV 10 strain, Martin Vialatte, Epernay, France). Musts underwent alcoholic fermentation in 12 hL tanks in the Lanson winery (Reims, France). SO_2 (60 mg/L) was added to the wine after alcoholic fermentation. Malolactic fermentation did not take place (only a trace of L-lactic acid) (**Table 1**). The decrease in malic acid content was explained by the metabolism of this acid by *S. bayanus* during alcoholic fermentation.

Lysozyme Treatments. For the first experiment (**Figure 1**), lysozyme was added after static settling (24 h at 15 °C) in 12 hL stainless steel tanks. Lysozyme theoretical concentrations (50 g/hL) decreased by 20% when lysozyme was added directly to the musts owing to chaptalization and topping up after alcoholic fermentation. For the second experiment (**Figure 2**), control wines (1.5 L) were enriched in lysozyme (20 or 80 g/hL) after alcoholic fermentation and bentonite treatment (150 g/hL).

Bentonite and Charcoal Treatments. The enological natrium bentonite (Volclay) was soaked in water (50 g/L, w/v) to swell 24 h before use. The vegetable charcoal resulted from plant calcination (Prolabo, France). Its medium screen was 10−74 μm. Its specific adsorption area was unknown. Treatments were carried out on Pinot noir and Chardonnay. The doses used were 30 and 80 g/hL for the bentonite and for the vegetable charcoal. All of the experiments were carried out with 1.5 L of wine, after or before the addition of lysozyme (**Figures 1** and **2**). Current analyses were made only for industrial musts and wines before and after lysozyme treatments (**Table 1**).

Standard Chemical Analyses of Musts and Wines. Enzymatic kits of Boehringer Mannheim were used to measure concentrations of L-lactic acid. The pH was determined by using an Orion 420A pH-meter. Tartaric acid was measured according to a modified Rebelein method, using ammonium monovanadate (*21*) and a SAFAS spectrophotometer. Total acidity was determined by 15.6 mM NaOH additions using blue bromothymol as a colorimetric indicator; results were expressed in grams per liter of tartaric acid. L-Malic acid was quantified using an automatic enzymatic method (Kone Progress apparatus). Sugar contents were determined using a Dujardin-Salleron 1060-1090 mustimeter, with a 20 °C correction of the value. Alcohol content was determined after distillation with a Dujardin-Salleron classe II alcohometer 9−16% volume (accuracy = 1/10% v/v).

Enological Lysozyme Purity. Enological lysozyme was provided by Fordras (Lugano, Switzerland). Its electrophoretic purity was compared by discontinuous SDS-PAGE with a highly purified lysozyme purchased from Boehringer Mannheim. The two electrophoretic profiles obtained present similar patterns, with only one band at 14.5 kDa. This very high electrophoretic purity revealed the absence of proteic contaminants. Enological lysozyme purity was also determined using a nitrogen chemiluminescence detector Antek 7000N (Houston, TX) with glycine as a standard and 5.4 as a nitrogen conversion factor. Chemiluminescence quantification also showed the absence of vehicles.

Protein Concentration in Wines. Wine protein contents were measured according to a modified Bradford method (*1*) to correct interferences essentially due to ethanol and phenolic compounds. In short, the wine protein reactivity with the Coomassie Blue Brilliant (A_{595}) is equal to the wine reactivity with the dye reagent minus the wine ultrafiltrate reactivity with the dye reagent (using a 3 kDa MWCO membrane) (Amicon, Beverly, MA). A_{595} results were after expressed in milligrams per liter equivalent bovine serum albumin (BSA) with regard to the standard curve. Each value was the average of three measures. The standard curve coefficient correlation is $R^2 = 0.9976$.

Lysozyme Quantification in Wine. Wine lysozyme concentrations were determined using a Waters (Milford, MA) HPLC system comprising a Waters 600 pump and a model W 490E UV detector, interfaced with a Millennium[32] chromatography manager (Waters). The lysozyme was analyzed by reversed phase chromatography on a TSK-gel 5PW-phenyl column (4.6 × 75 mm, Tosohaas). A linear gradient elution from 100% A and 0% B to 0% A and 100% B within 44 min was used. This was followed by a 18 min equilibrium period with 100% A at room temperature. Solvents used were as follows: A, 1% CH_3CN + 0.2% trifluoroacetic acid + 98.8% Milli-Q H_2O; B, 70% CH_3CN + 0.2% trifluoroacetic acid + 29.8% Milli-Q H_2O. The flow rate was 0.8 mL/min. The absorbance was measured at 225 nm. All standards were injected (20 μL) in triplicate. Concentrations of residual lysozyme in wines were calculated with regard to the standard curve ($y = 23239x$ and $R^2 = 0.9989$). Wine samples were injected only once because of the high R^2 for the standard curve. Lysozyme dissolved in a synthetic wine was eluted at 26 min, whereas no peak could be observed in the control wine profile for this retention time. Lysozyme concentrations in wines were determined before and after bentonite and charcoal treatment (not determined for 30 g/hL in the first experiment described **Figure 1**), 1 month after the enrichment of the wines and 4 months after being added into musts.

Foaming Properties. All foam measurements were carried out using the Mosalux apparatus (*2*). A glass cylinder (4 cm in diameter and 40 cm long) placed on a glass frit (pore size = 16−40 μm) was filled with the base wine to be analyzed (100 mL). All wines were filtered 0.45 μm before foam measurements. Carbon dioxide was injected into the glass cylinder through the glass-frit with a constant rate of gas flow (7 L/h) and under constant pressure (100 kPa). Foam height (measured in millimeters) was controlled by photoelectric cells (infrared beams). Each experiment was made in triplicate. The graphics were traced with one point for every 5 s. Each point was the average of three values. For each series, all measurements were made the same day to reduce the dispersion of the values. The foamability (millimeters) corresponded

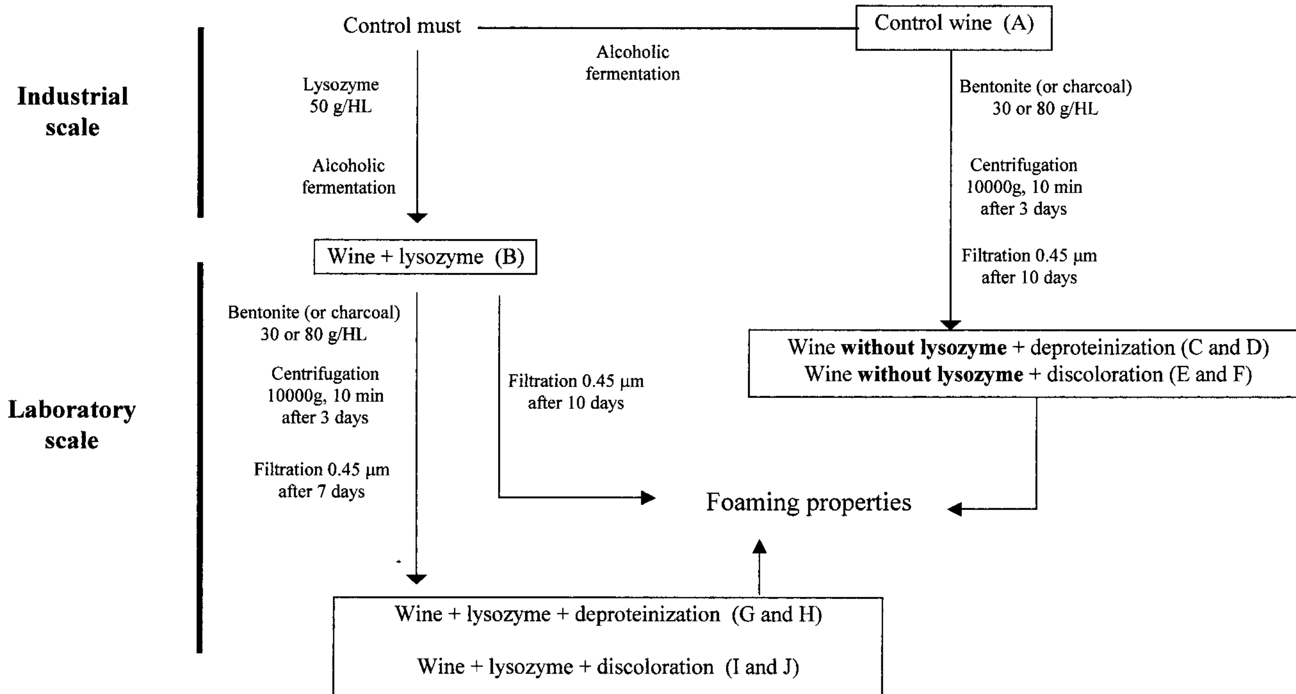

Figure 1. Description of the Chardonnay and Pinot noir wine production. First experiment: lysozyme is added to the must, and bentonite (or charcoal) treatment is carried out on the wine.

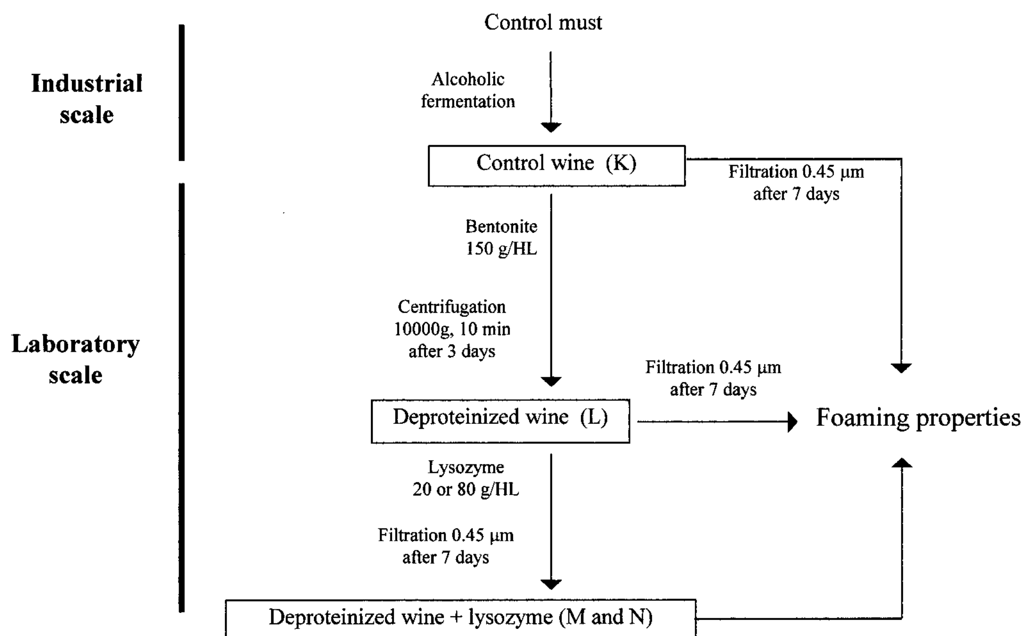

Figure 2. Description of the Chardonnay and Pinot noir wine production. Second experiment: bentonite or charcoal is added to the wine, and lysozyme treatment is applied on the centrifuged wine.

to the maximum foam height reached by the column of foam. Foam stability was the foam height (millimeters) after 8 min of sparging.

RESULTS AND DISCUSSION

Current Analyses. Results are summarized in **Table 1**. The Pinot noir and Chardonnay musts had similar sugar concentrations. The Pinot noir must came from a blend of second pressings and presented a total acidity of 10.4 g/L expressed in tartaric acid. The Chardonnay must corresponded to a blend of top growths. Its total acidity (14.2 g/L) was higher than that of the Pinot noir must, mainly because of higher concentrations in malic and tartaric acids. The two musts presented a pH difference of 0.28 unit. All of these differences were also observed for the corresponding wines. However, the Chardonnay and Pinot noir wines originating from lysozyme-treated musts showed higher concentrations in tartaric acid (4.96 and 3.50, respectively) when compared with the corresponding control wines (4.63 and 2.30, respectively).

For the Pinot noir variety, malic and tartaric acid concentrations both decreased. The precipitation of potassium tartarate in wine always induces a decrease in pH. In contrast, the malolactic fermentation induces an increase in pH in this case. The resulting pH increased when compared with the pH of the must. It is not possible to give a scientific explanation because

Table 2. Foamability, Foam Stability, and Lysozyme and Protein Concentrations in Wines Produced According to the First Experiment (See **Figure 1**)[a]

		foamability			foam stability				
variety	wine (see treatments, **Figure 1**)	mm	diff (%) compared to control wine	diff (%) compared to bentonite-treated wine	mm	diff (%) compared to control wine	diff (%) compared to bentonite-treated wine	lysozyme content (mg/L)	protein (mg/L)
Pinot noir	control (A)	124			37			0	14.9
	50 g/hL lysozyme (addition to the must) (B)	150	+21		31	–16		261	
	30 g/hL bentonite (C)	99	–20		28	–24		0	6.6
	50 g/hL lysozyme + 0 g/hL bentonite (G)	113	-9	+14	37	0	+32		
	80 g/hL bentonite (D)	51	–59		24	–35		0	4.8
	50 g/hL lysozyme + 80 g/hL bentonite (H)	146	+18	+186	unstable			0 (–100%)	
Chardonnay	control (A)	144			32			0	11.8
	50 g/hL lysozyme (addition to the must) (B)	148	+3		32	0		263	
	30 g/hL bentonite (C)	104	–28		unstable			0	3.2
	50 g/hL lysozyme + 30 g/hL bentonite (G)	125	–13	+20	unstable				
	80 g/hL bentonite (D)	55	–62		26	–19		0	1.5
	50 g/hL lysozyme + 80 g/hL bentonite (H)	117	–19	+113	unstable			24 (–91%)	

[a] Lysozyme was added to the musts, and bentonite treatment was carried out on the wines.

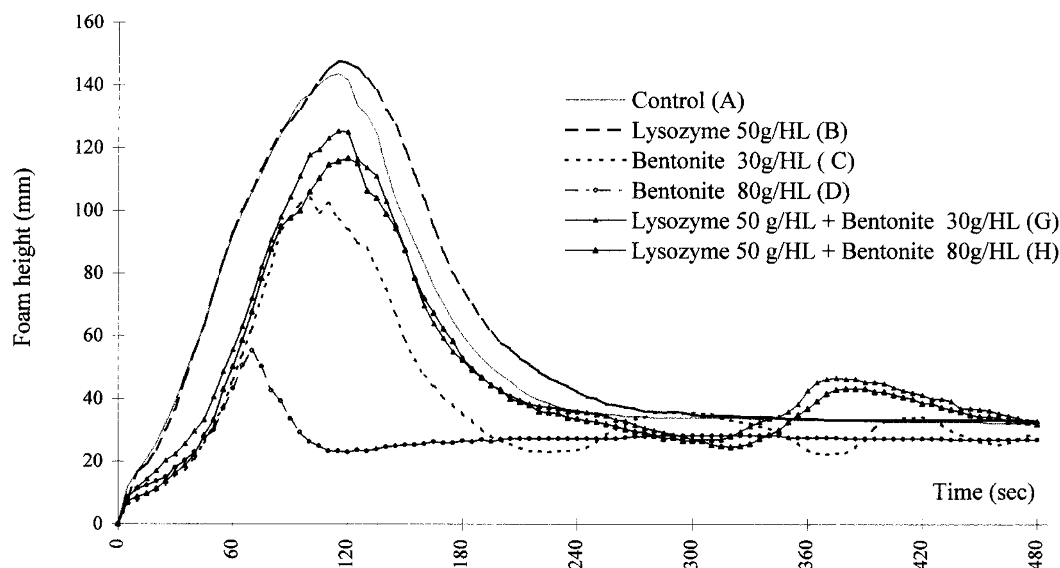

Figure 3. Foaming properties of the Chardonnay wine treated with bentonite at 30 and 80 g/hL and originating from a must enriched with lysozyme (see first experiment, **Figure 1**).

we do not know how each acid takes part in the acido-basic buffering capacity. If we consider the Chardonnay variety, we observe no variation of pH between the must and the wine. When lysozyme was added, tartaric stabilization was more difficult to obtain than for control wines. Lysozyme seemed to have a protective action in relation with tartaric stabilization, even if an enrichment with cream of tartar was made (J. P. Gandon, personal communication). Treated wines also showed low differences for alcohol and malic acid contents. We also observed lower values in malic acid and higher values for tartaric acid. pH and total acidities were nevertheless strictly identical.

Foaming Properties. *Lysozyme and/or Bentonite Treatments (See **Figure 1**).* Treatments with bentonite alone diminished foamability (**Table 2**), whatever the dose used (30 or 80 g/hL) or the variety studied (Chardonnay and Pinot noir). For 30 g/hL, the decreases in foamability were nearly the same for the two wines (−28 and −20%, respectively). Foam stability also diminished for the Chardonnay and the Pinot noir. After an addition of 30 g/hL bentonite, the Chardonnay wine became unstable (**Figure 3**) and the foam stability could not be measured. This was also observed when the wines were treated with bentonite after an addition of lysozyme (**Figures 3** and **4**). This phenomenon had not been explained previously, although often observed after such treatment. An addition of lysozyme in must raised the Pinot noir foamability by 21%, whereas the difference was hardly perceptible for the Chardonnay wine (+3%). A large increase in this particular protein concentration can be the cause of small foamability change. Foam stability differences were also very small. Thus, the wine protein content seemed not to be correlated to foaming properties when an exogenous protein was added to wine. In contrast, previous experiments made with wines from the same region

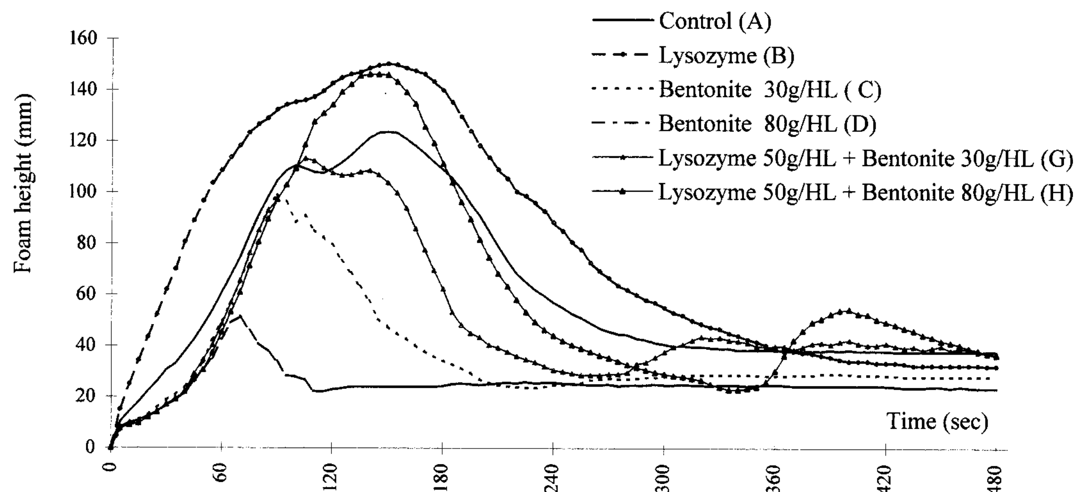

Figure 4. Foaming properties of the Pinot noir wine treated with bentonite at 30 and 80 g/hL and originating from a must enriched with lysozyme (see first experiment, **Figure 1**).

Table 3. Foamability, Foam Stability, and Lysozyme and Protein Concentrations in Wines Produced According to the First Experiment (See **Figure 1**)[a]

		foamability			foam stability				
variety	wine (see treatments, **Figure 1**)	mm	diff (%) compared to control wine	diff (%) compared to charcoal-treated wine	mm	diff (%) compared to control wine	diff (%) compared to charcoal-treated wine	lysozyme content (mg/L)	protein (mg/L)
Pinot noir	control (A)	124			37			0	14.9
	50 g/hL lysozyme (addition to the must) (B)	150	+21		31	−16		261	
	30 g/hL charcoal (E)	96	−23		36	−3		0	13.0
	50 g/hL lysozyme + 30 g/hL charcoal (I)	110	−11	+15	45	+8	+25		
	80 g/hL charcoal (F)	85	−31		34	−8		0	10.1
	50 g/hL lysozyme + 80 g/hL charcoal (J)	105	−8	+24	46	+24	+35	245 (−6%)	
Chardonnay	control (A)	144			32			0	11.8
	50 g/hL lysozyme (addition to the must) (B)	148	+3		32	0		263	
	30 g/hL charcoal (E)	88	−39		34	+6		0	11.4
	50 g/hL lysozyme + 30 g/hL charcoal (I)	89	−38%	+1	53	+65	+56		
	80 g/hL charcoal (F)	69	−52		33	+3		0	10.4
	50 g/hL lysozyme + 80 g/hL charcoal (J)	84	−42	+22	53	+65	+61	257 (−2%)	

[a] Lysozyme was added to the musts, and charcoal treatment was applied to the wines.

(*2*, *5*) have clearly demonstrated a correlation between wine protein contents and wine foaming properties. These discrepancies were probably due to protein characteristics. Very few studies on the kinetics of the adsorption of proteins at the air−water interface from bulk mixtures have been reported (*23*−*26*). The molecular factors that affect preferential adsorption of proteins at an air−water interface are not known, although it is intuitively understood that differences in surface hydrophobicity, hydrophilicity, and rate of adsorption might be involved. In the wine, the numerous yeast and grape berry proteins cover a large range of MW and pHi. Some of them are composed of only amino acids, whereas other are glycosylated (*7*, *10*). This complex composition has a buffering effect on wine foamability. It is not the case for the lysozyme because of its very particular biochemical characteristics: low molecular weight (14.3 kDa), high p*I* (10.4), and compact tridimensional structure (four disulfide bonds). It is a highly ordered, rigid, hydrophilic, and positively charged protein (*27*). Anand and Damodaran (*23*) studied the kinetics of adsorption of lysozyme and BSA in single-component and 1:1 mixture experiments. In the single-component system, a long induction period for lysozyme adsorption was observed, which indicates that there is an energy barrier for its adsorption at the air−water interface. This energy barrier during the initial stages of adsorption is attributable to its high electrochemical potential at the interface (*28*, *29*). Unlike lysozyme, BSA in the single-component system does not exhibit a lag time for adsorption. The presence of lysozyme in the bulk phase has neither the ability to displace BSA from the interface nor the ability to significantly influence the kinetics of adsorption of BSA. Moreover, the apparent diffusion coefficient of lysozyme decreases exponentially with an increase in BSA concentration in the bulk phase. This indicates that BSA successfully competes with lysozyme for adsorption, but it cannot displace lysozyme molecules already adsorbed at the interface. If we now consider the wine, lysozyme did not have the ability to significantly improve foam stability and foamability. This is probably related to competition between endogenous wine proteins and lysozyme. This was also due to

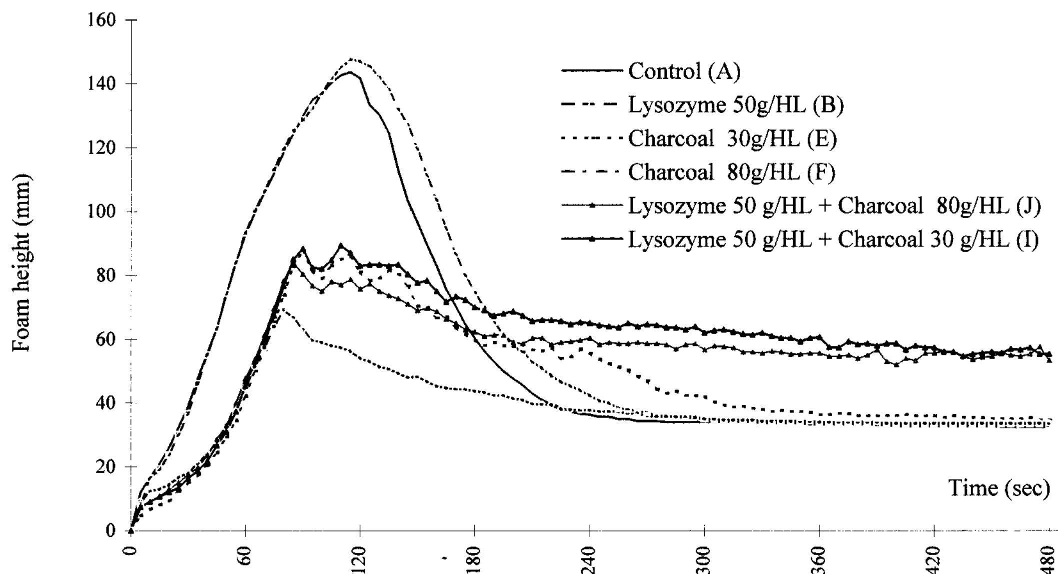

Figure 5. Foaming properties of the Chardonnay wine treated with charcoal at 30 and 80 g/hL and originating from a must enriched with lysozyme (see first experiment, **Figure 1**).

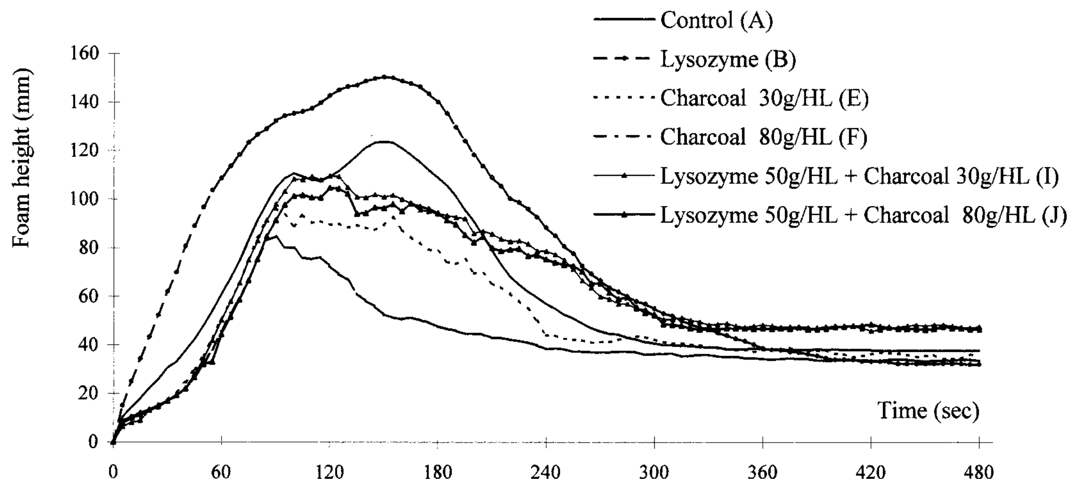

Figure 6. Foaming properties of the Pinot noir wine treated with charcoal at 30 and 80 g/hL and originating from a must enriched with lysozyme (see first experiment, **Figure 1**).

the energy barrier and the lag time required for lysozyme adsorption. Foam is a rapid and unstable phenomenon. This largely limited the presence of lysozyme at the air−wine interface.

Pinot noir and Chardonnay wines originating from lysozyme-treated musts plus wine bentonite treatment present a higher foamability than wines treated with only bentonite (**Figures 3** and **4**; **Table 2**). If wine protein was partially removed (30 g/hL bentonite), the increase in foamability due to the presence of lysozyme did not exceed 20% maximum. For bentonite treatment of 80 g/hL, protein content was 4.8 mg/L for the Pinot noir wine and only 1.5 mg/L for the Chardonnay wine. The increase in foamability when the lysozyme was added became very high for the Chardonnay wine (+113%) and for the Pinot noir wine (+186%). Lysozyme treatment seems to be of particular interest if severe bentonite treatment is necessary. Lysozyme content (as determined by HPLC) shows a very high elimination of this enzyme when bentonite treatment was applied (−91 and −100%). The adsorption of lysozyme by the bentonite in a model wine was already demonstrated in a previous work (*30*). Thus, lysozyme was not responsible for the increase in foamability. Lysozyme seems to have a protective effect on the endogenous wine proteins originating from the grape berry and yeasts. This point will also be discusssed within the second experiment. Lysozyme seems to be preferentially eliminated; this can be explained by its high isoelectric point equal to 10.4. At pH ∼3, it bears a positive net charge and is more easily adsorbed by the bentonite (which bears a negative net charge) than endogenous proteins having isoelectric points essentially ranging between 2.5 and 4.5 (*7*, *31*). Nevertheless, total protein content after bentonite treatment were not determined because values cannot be discussed; in fact, it is not possible to distinguish endogenous proteins (from grape berries and yeasts) and lysozyme contribution in a dye reaction. Surprisingly, if bentonite was added at 80 g/hL after lysozyme treatment, the foamability value became higher than the foamability of the same wine treated with lysozyme + 30 g/hL bentonite. This result has not been explained so far.

*Lysozyme and/or Charcoal Treatment (See **Figure 1**).* Treatments with charcoal alone always diminished foamability (**Table 3**; **Figures 5** and **6**) for the Chardonnay and Pinot noir wines. The decreases of foamability for concentrations varying from 30 to 80 g/hL were smaller (−23 and −31% for the Pinot noir wine, for example) than the difference observed between the two doses of bentonite (−20 and −59% for the same wine). The diminutions of foamability were fairly different for the two

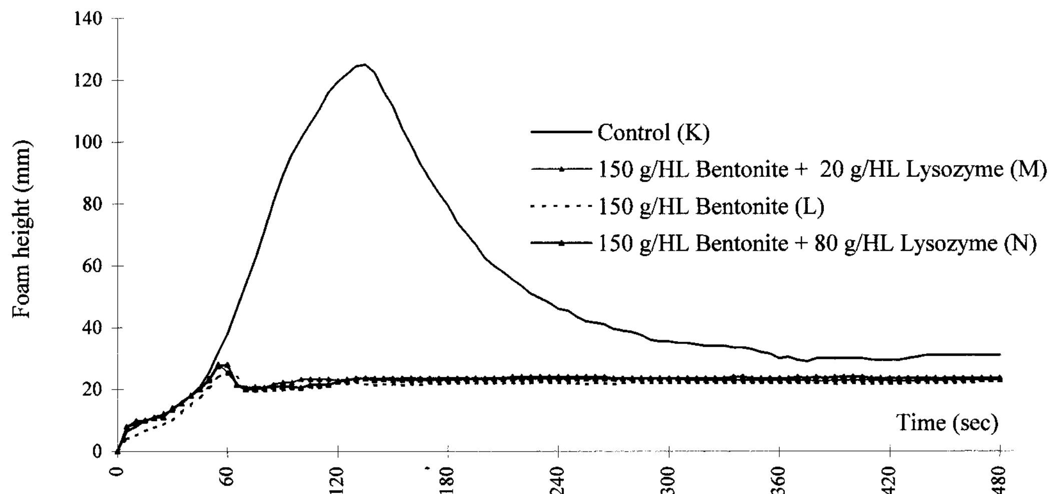

Figure 7. Foaming properties of the Chardonnay wine treated with bentonite and after addition of lysozyme (20 and 80 g/hL). The wine originated from a control must not treated with lysozyme (see second experiment, **Figure 2**).

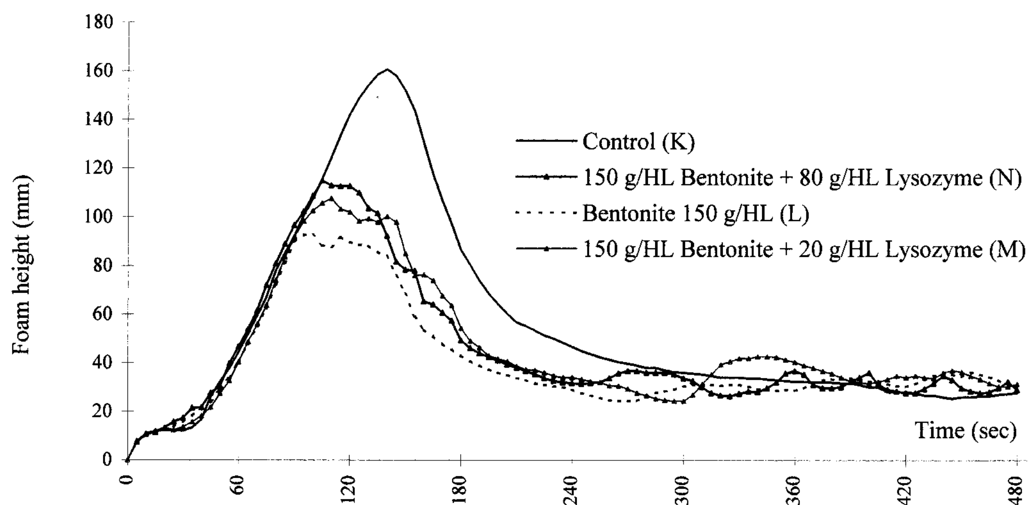

Figure 8. Foaming properties of the Pinot noir wine treated with bentonite and after addition of lysozyme (20 and 80 g/hL). The wine originated from a control must not treated with lysozyme (see second experiment, **Figure 2**).

Table 4. Foamability, Foam Stability, and Lysozyme Content in Wines Produced According to the Second Experiment (See **Figure 2**)[a]

		foamability			foam stability			
			diff (%) compared to			diff (%) compared to		
variety	wines (see treatments, **Figure 2**)	mm	control wine	bentonite-treated wine	mm	control wine	bentonite-treated wine	lysozyme content (mg/L)
Pinot noir	control (K)	161			31			0
	150 g/hL bentonite (L)	93	−42			unstable		0
	150 g/hL bentonite + 20 g/hL lysozyme (M)	108	−33	+16		unstable		176
	150 g/hL bentonite + 80 g/hL lysozyme (N)	115	−29	+24		unstable		819
Chardonnay	control (K)	125			37			0
	150 g/hL bentonite (L)	25	−80		22	−40		0
	150 g/hL bentonite + 20 g/hL lysozyme (M)	28	−78	+12	23	−38	+4	169
	150 g/hL bentonite + 80 g/hL lysozyme (N)	28	−78	+12	24	−35	+9	798

[a] Wines were treated with bentonite, and lysozyme was added after deproteinization.

wines (−23 and −39% for the Pinot noir wine and the Chardonnay wine using 30 g/hL charcoal). Foam stability was unchanged even if 80 g/hL charcoal was used (**Table 3**), but charcoal treatment never generated the foam instability currently observed with bentonite. When the charcoal treatment was 80 g/hL, protein contents were 10.1 mg/L for the Pinot noir wine and 10.4 mg/L for the Chardonnay wine. This indicates a poor affinity of charcoal with endogenous wine proteins. HPLC quantification also demonstrated that the lysozyme present in the treated wines was not adsorbed by charcoal. The Pinot noir

and Chardonnay wines treated with lysozyme (at the must stage) plus 30 g/hL charcoal (in wine) presented a foamability little higher than that of wines treated only with charcoal (**Figures 5** and **6**; **Table 3**). The increase in foamability due to the lysozyme was 22% for the Chardonnay wine and 24% for the Pinot noir wine. These increases were considerably lower than those observed in the case of comparison between bentonite treatment and lysozyme + bentonite treatment. Lysozyme had a bad protective effect on the wine foamability because charcoal added in a wine will adsorb endogenous compounds that participate in the formation of foam. If we consider foam stability, results showed a real positive effect of lysozyme when wines had to be treated with charcoal. The increase was high and variable depending on the wine variety (+25% and +56% for the Pinot noir wine and the Chardonnay wine, respectively, at 30 g/hL), but we also visually observed that bubbles were larger than the control wine bubbles. This characteristic is not appreciated by sparkling wine consumers.

*Bentonite Treatment after Lysozyme Treatment (See **Figure 2**).* In another experiment, wines were initially treated with bentonite and then enriched in lysozyme (**Figure 2**). Bentonite at a concentration of 150 g/hL completely destroyed the Chardonnay foamability (**Figure 7**) with a decrease of 80%. After an addition of 20 or 80 g/hL lysozyme, the wine foamability was still decreased by 78% compared to the control. Lysozyme was absolutely not able to restore the foaming properties. The same phenomenon was observed for the foam stability. For the Pinot noir wine (**Figure 8**), the decrease in foamability due to bentonite treatment was smaller than for the Chardonnay wine (−42 and −80%, respectively). Moreover, the addition of lysozyme partially restored the foamability (respectively, +16% for an addition of 20 g/hL and +24% for an addition of 80 g/hL). Foam stability remained poor in comparison with that of the control wine (**Table 4**).

Conclusion. Lysozyme had a protective effect on foaming properties when added before bentonite treatment. This enzyme could restore correct foamability (in particular for the Pinot noir wine studied), even if the deproteinization treatment was severe. Lysozyme treatment seems to be of particular interest if a bentonite treatment is needed. This increase in foamability was nevertheless poor or nil when the bentonite treatment was made in the wine before the addition of lysozyme, even in the case of high protein concentration. Finally, lysozyme could not suppress the instability induced by bentonite. This study also noted major differences in the foamability breakdown observed after a bentonite treatment. These differences were smaller with charcoal treatment, probably because of low protein adsorption. It seems that the effect of lysozyme was all the higher when wine foaming properties were sensitive to treatments with bentonite or charcoal.

In the future, it will be necessary to examine the interfacial properties of synthetic wines containing lysozyme and wine macromolecules with techniques such as bubble tensiometer and spectroscopic phase modulated ellipsometer. It will then be possible to characterize the layer of macromolecules and to explain the behavior of lysozyme in wine foaming properties.

Other aspects of our research to come are the relationships between the presence of lysozyme, tartaric stability, and proteic stability. The modification of the protein composition of a wine when lysozyme is added is of particular interest when one considers that the colloids present in wine have a protective effect on tartaric precipitation (*32*) and natural haze (*10*, *12*, *33*−*35*). However, they can also be responsible for protein insolubilization during aging or storage of wine under unsuitable conditions (*36*). The kinetics of clarifying and filtrability of lysozyme-treated wines are also of particular interest.

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