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Low-amperage-direct Electric Current as an Alternative Technique to Sulphur Dioxide in the Stabilisation of Semi-sweet White Wines

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Abstract: An alternative technique to addition of SO_2 using low-amperage electric current was investigated for stabilisation of semi-sweet white wine. The influence of initial inoculation in the range of $2x10^4$ to $2x10^7$ cfu mL^{-1} on yeast inactivation by electrolysis (0.5 A-3 h) was studied in phosphate buffer in a preliminary study. Yeast inactivation was shown to depend significantly on initial yeast load, as revealed by a decrease in lethal efficacy for initial inoculation higher than $2x10^6$ cfu mL^{-1}. Wine stabilisation study was performed on white grape juice fermented at ambient temperature to 1,030 density, by applying 0.16 A for four hours. The treatment was shown to be effective in wine stabilisation as fermentation was successfully stopped. The influence of electrolysis on several physicochemical characteristics of the wine showed an increase in yellow colour intensity and in total and volatile acidity to an acceptable level. Provided that physicochemical characteristics of electrolysis –treated wine would be similar to SO_2 –treated wine, low-amperage electrolysis could be considered to be an effective method of wine stabilisation.

Key words: Low-amperage-direct, alternative, technique, sulphur, dioxide, stabilisation, semi-sweet, wines

Introduction

A new technique of food preservation using direct electric current was applied to stabilisation of semi-sweet white wines. Wine-making is a complex process where undesirable microorganisms can cause spoilage and have detrimental effects on the quality of the final product. With the increasing consumer demand for minimally processed, less heavily preserved, more natural and healthier food products, there is a growing interest in alternative preservation techniques which do not result in adverse changes to the flavour, taste and nutrient content of foods. In wine-making, sulphiting is an operation which consists of adding massive doses of sulphur dioxide (SO_2) to stop alcohol fermentation in order to maintain high residual levels of glucose in wine. However SO_2 may also have side effects such as headache in sensitive subjects. It has been demonstrated that several adverse reactions including asthma attacks, anaphylactic shock, urticaria and angioedema, nausea, abdominal pain and diarrhea, seizures and death may be related to ingestion of sulphite compounds (Yang and Purchase 1985). The World Health Organisation (WHO) has therefore strongly advised that sulphur dioxide levels in foods be lowered (Usseglio-Tomasset 1992). The antiseptic properties of SO_2 in protection against must oxidation and in the inactivation of contaminants have made this additive an indispensable part of wine-making. However the high levels of SO_2 authorised in wine might be shortly revised downwards by French legislation for wine technology. Moreover, consumers are increasingly concerned about health and safety aspects of food products and the use of chemical preservatives is not accepted by many consumers. Research is therefore being performed to find an alternative to SO_2 addition to stop alcohol fermentation.

The application of high electric field serial pulses to liquid foods has been shown to be a fairly efficient method of preservation. However the industrial plant required for such treatments represents a major investment for manufacturers. The method we propose uses low intensity electric current and is therefore potentially much simpler and cheaper to implement (El Murr 1990). We have previously demonstrated that electrolysis is an efficient method of yeast inactivation in phosphate buffer (Guillou and El Murr 2002). This new technique was applied to the stabilisation of semi-sweet wines as a substitute for addition of SO_2 and wine cooling. As far as we know there has been no report on the stabilisation of wine by using low-intensity direct electric current. The study reported here was divided into two parts. The influence of initial yeast inoculation on yeast inactivation by electrolysis was examined in a preliminary study and the effects of such treatment on wine stabilisation were investigated in the second phase.

Materials and Methods

Yeast: *Saccharomyces cerevisiae* yeast strain (FERMIVIN # 7013) provided by Gist-Brocadès was used for the study.

Electric Treatment

Influence of Initial Yeast Inoculation: Cells were grown at 28°C to late exponential growth phase in 100 mL of nutritive broth containing (g L^{-1}) : Yeast Extract (Merck), 10; pancreatic peptone (Difco), 10; D – (+) Glucose (Fluka), 20; and chloramphenicol (Merck), 0.25.

After culture cells were collected, washed and resuspended in 0.1 mol L^{-1} phosphate buffer (pH 7.1) at a final concentration of approximately 10^8 cfu mL^{-1}. One mL of this suspension appropriately diluted, was added to the electrolytic vessels containing 50 mL of the treatment medium to reach the desired microbial concentration. Microbial suspensions ranging from $2x10^4$ to $2x10^7$ cfu mL^{-1} in 0.1 mol L^{-1} phosphate buffer (pH 7.1) were submitted to the electrolysis treatment. Two thermostatically controlled electrolytic vessels were used for each experiment (Guillou and El Murr 2002). One of the vessels was fitted with two netting platinum electrodes (30 x 25 mm) parallel to each other at a distance of 20 mm apart. The two electrodes were connected to a DC power supply (Metrix AX322). The other vessel, containing the same microbial suspension, received no electric treatment and was hence used as a control of microbial death without electric current. Electrolysis was performed at 0.5 A (9 V) at 20 ± 1°C for three hours.

Wine Stabilisation Study: Six hundred millilitres of pasteurised white grape juice, chaptalised to 16.5°, were inoculated with *S. cerevisiae* cells. The gravimetric density of the microbial suspension was measured daily during fermentation at ambient temperature (20-24°C). When the density had reached 1,030 kg m^{-3} (arbitrarily chosen value) after about 8 to 10 days, the yeast suspension was equally divided between two 300 -mL flasks. One half of the suspension was chosen to study the influence of electrolysis on stabilisation of wine. The other half received no stabilising treatment and was hence used as a control. Electrolysis was performed at 20 ± 1°C in a 300 -mL thermostatically controlled electrolytic vessel fitted with two needle platinum electrodes (35 mm in length) connected to a DC power supply (Metrix AX322). A direct electric current of 0.16 A (28 V) was applied for four hours to the yeast suspension.

Influence of Electrolysis Treatment on Yeast Viability: Viability of *S. cerevisiae* was assayed by counting colony forming units (cfu). A 1-mL aliquot of the yeast suspensions was taken from the electrolytic vessels to assess yeast viability before and after electrolysis, and 0.1 mL of the serially diluted 1 -mL samples was surface-plated in duplicate on a Yeast extract Glucose Chloramphenicol Agar (YGC-A) medium (Merck). Several dilutions were used so that the number of colonies that appeared after 48 h incubation at 288°C would be large enough to be statistically meaningful (between 30 and 300).

In the study of the influence of yeast inoculation, viability of *S. cerevisiae* was assessed before and after electrolysis. Results are represented by the Log_{10}-cycle microbial reduction as calculated by Log_{10} (N_0 / N) where N_0 represents the initial viable yeast population and N the viable yeast population at the end of three hours' electrolysis. All treatments were performed in duplicate. The mean standard deviation is represented by an error bar.

In the wine stabilisation study, viability of *S. cerevisiae* was determined during and after electrolysis. Samples of treated and control wines were taken every 30 min during electrolysis and daily after electrolysis to estimate surviving fractions. Results are presented in the form of survival curves representing Log_{10} (N/N_0) as a function of time, where N_0 is the population of viable yeast before treatment and N, the population of viable yeast after electrolysis. The same mathematical equation that was used for thermal inactivation was used to represent the effect of microbial inactivation by electric current (survival curve) : Log_{10} (N/N_0) = - t / D where N_0 = initial viable population of microorganisms, N = viable population of microorganisms after electrolysis, t = electrolysis or post-electrolysis time and D = death rate constant which corresponds to one Log_{10}-cycle reduction of the cell population. D values were estimated from the slope of the regression line obtained from the linear portion of the survival curve. Each experiment was repeated twice in order to verify that all treatments gave the same trends.

Density Curves: In wine stabilisation study, daily measured density was plotted against post-electrolysis time. Each experiment was repeated twice in order to verify that all treatments gave the same trends.

Physicochemical Characteristics of Wine: The physicochemical properties of treated wine were compared to those of the control whose fermentation had continued spontaneously.

The alcohol content defined as the number of litres of ethanol contained in 100 litres of wine, both measured at

20°C, was determined by near infrared spectrophotometry as described by Dubernet and Dubernet (2000). Reducing sugars, total and volatile acidity, pH and chromatic properties were analysed using the Official European Community methods for the analysis of wines (Commission Regulation (EEC) No. 2676 / 90). Reducing sugars were determined by their reducing action on an alkaline solution of copper salt. The total acidity of the wine was defined as the sum of its titratable acidity levels when titrated to pH 7 against a standard alkaline solution. Volatile acidity resulted from the acids of the acetic series present in wine in a free state and combined as a salt. The chromatic properties of the wine were assessed by determining its absorbency at the wavelength of 420 nm.

Statistical Analysis: Statistical analysis of the effect of initial yeast inoculation on microbial inactivation was performed by analysis of variance with differences determined by the method of least significant difference at the 5% ($P<0.05$) level.

Results

Influence of Initial Yeast Inoculation: Making of semi-sweet wines is based on arresting fermentation when the density of the wine has reached a certain value. This value depends on both the final residual sugar level desired and alcohol content. The amount of yeast cells in the fermented wine is presumed to depend on factors such as initial must contamination and climatic conditions influencing fermentation evolution. We therefore studied the influence of initial yeast load on yeast inactivation by electrolysis. Initial populations ranging from $2x10^4$ cfu mL^{-1} to $2x10^7$ cfu mL^{-1} in 0.1 mol L^{-1} phosphate buffer (pH 7.1) were submitted to electrolysis at 0.5 A at 20°C for three hours. Figure 1 summarises microbial inactivation induced by electrolysis according to initial microbial load. The effect of initial microbial inoculation on microbial inactivation induced by electrolysis was found to be significant ($P < 0.05$). Determination of the least significant difference showed that the experiment performed with an initial inoculation of $2x10^7$ cfu mL^{-1} significantly differed from the other experiments performed with smaller initial inoculations ($P < 0.05$). No difference was found between the experiments performed with an initial inoculation of $2x10^4$, $2x10^5$ or $2x10^6$ cfu mL^{-1} ($P > 0.05$).

Wine Stabilisation Study

Survival Curves: Fermented white grape juice was treated with a 0.16 A direct electric current for four hours at 20°C. After the first hour of treatment while there was no microbial inactivation, the population of *S. cerevisiae* treated with direct electric current decreased linearly with electrolysis time (Fig. 2). Slight yeast inactivation of approximately 0.15 Log_{10}-cycle was induced by the treatment. The D value determined from the regression line calculated from the last three hours of treatment was 21 h. Unlike yeasts treated in phosphate buffer (Guillou and El Murr 2002), yeasts in suspension in fermented grape juice were further inactivated once the electric current had been switched off (Fig. 3). The regression line calculated from the post-electrolysis survival curve made possible the determination of a D value of 66 h.

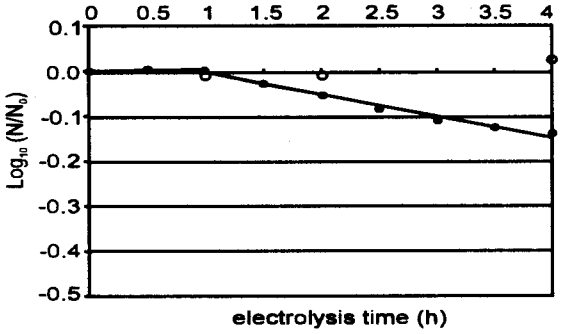

Fig. 1: Influence of initial inoculation on *S. cerevisiae* inactivation by electrolysis in 0.1 mol L^{-1} phosphate buffer (pH 7.1). Bars represent mean Log_{10}-cycle reduction [Log_{10} (N_0 / N)] induced by electrolysis at 0.5 A (9 V) at 20°C for three hours as a function of initial inoculation ranging from $2x10^4$ to $2x10^7$ cfu mL^{-1}. N_0 is the number of viable yeast cells before treatment and N, the number of viable yeast cells after treatment. Standard deviations are indicated by error bars

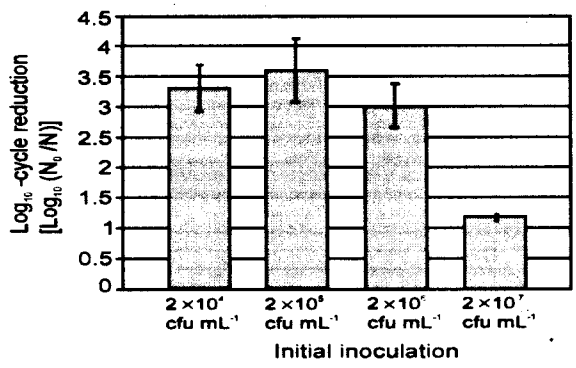

Fig.2: Survival curve of *S. cerevisiae* in suspension in fermented grape juice (density 1,030) treated with 0.16 A (28 V) at 20°C for four hours. Open circles : non-treated fermented grape juice (control), closed circles : electrolysis-treated fermented grape juice

Density: Fig. 4 shows that the density of treated fermented grape juice stabilised to a value of 1,025 in less than 48 h whereas the control density continued to fall throughout the 150 h of the experiment. We also demonstrated that longer electrolysis treatment provided a higher microbial inactivation rate after electrolysis, thereby leading to faster stabilisation of the density (data not shown).

Physicochemical Characteristics: Several physicochemical characteristics reflecting certain organoleptic properties of wine were evaluated. Figure 5 illustrates the influence of the treatment on reducing sugar levels, alcohol content, volatile and total acidity, pH and yellow colour intensity. Alcohol fermentation arrested by electrolysis resulted in an increase in the residual amount of reducing sugars and a decrease in the alcohol content. Although no significant change in pH due to electrolysis was observed, total and volatile acidity and yellow colour intensity were shown to be higher in treated wine.

Discussion

Influence of Initial Yeast Inoculation: No significant effect of initial inoculation ($P>0.05$) was found for yeast populations less than $2x10^6$ cfu mL^{-1}. However an initial inoculation of $2x10^7$ cfu mL^{-1} resulted in significantly lower microbial inactivation by electrolysis ($P<0.05$). Zhang *et al.* (1994) showed that *S. cerevisiae* inactivation by pulsed electric fields depended on initial microbial load. Yeast inactivation was inversely correlated with initial yeast inoculation. However a slight difference (0.3 Log_{10}-cycle) in survival fractions was found between treatments performed with 10^5 and 10^6 cfu mL^{-1}. The lethal efficacy of pulsed electric fields was not tested on yeast populations of higher concentrations than 10^6 cfu mL^{-1}. They attributed the lower inactivation efficacy observed with the highest yeast inoculations to the presence of clusters of yeast cells and / or to their mutual dissimulation from electrical field effects. They reported they had also observed visible precipitation for 10^6 and higher initial yeast concentrations. In a study of the mathematical model of thermal destruction of bacterial spores, Cerf (1977) hypothesised that deviations from first order kinetics might arise from artefacts due to a 'protective effect' or a clumping phenomenon as the initial spore concentration was increased above 10^9 spores per mL. The lower efficacy of electrolysis for the $2x10^7$ cfu mL^{-1} yeast concentration could also be explained by cluster formation. Cluster-constitutive yeast cells might be inactivated only after cluster disintegration. Moreover we also observed that the $2x10^7$ cfu mL^{-1} yeast suspension was particularly viscous, which could be a determining factor in efficacy of electrolysis inactivation. Yeast concentrations in semi-sweet white wines just before sulphiting is generally in the range of $5x10^6$ to $2x10^7$ cfu mL^{-1}. In the present experiment, the concentration of the yeast suspension before electrolysis was $2x10^7$ cfu mL^{-1}. We have shown that electrolysis stopped fermentation for the highest yeast concentration tested in the preliminary study. As microbial inactivation increased with a decrease in yeast inoculation, fermentation would probably also be arrested by electric treatment for lower yeast concentrations.

Wine Stabilisation Study: Treatment of yeast suspensions in fermented grape juice with a 0.16 A direct electric current induced slight inactivation of approximately 0.15 Log_{10}-cycle. Electrolysis generated an antimicrobial effect that remained after electrolysis ceased. Yeast cells went on being inactivated after switching off the electric current. As there was no further inactivation after electrolysis in phosphate buffer, the phenomenon responsible

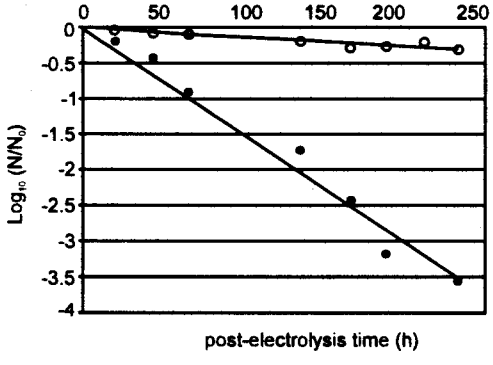

Fig. 3: Survival curve of *S. cerevisiae* in suspension in fermented grape juice following electrolysis treatment. Open circles : non-treated fermented grape juice (control), closed circles : electrolysis-treated fermented grape juice (0.16 A-4 h-20°C).

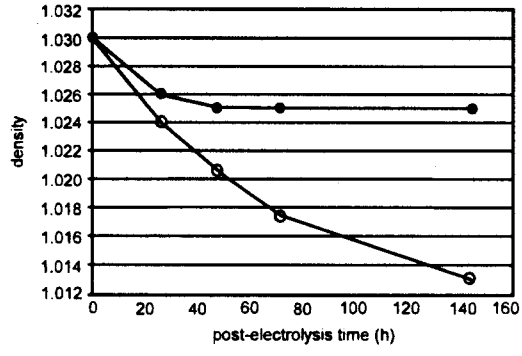

Fig.4: Fermented grape juice density *versus* post-electrolysis time. Open circles : non-treated fermented grape juice (control), closed circles: electrolysis-treated fermented grape juice (0.16 A- 4 h-20°C).

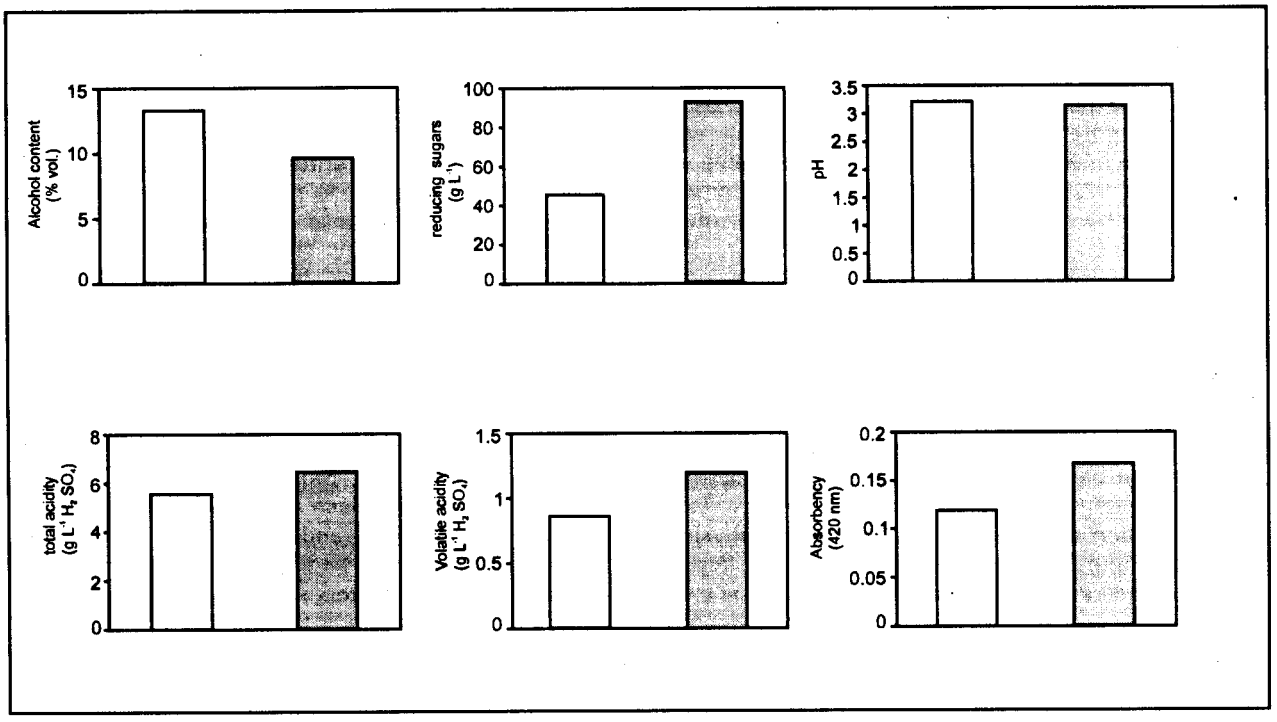

Fig. 5: Influence of electrolysis treatment on physicochemical characteristics of fermented grape juice. Bars represent physicochemical characteristics : alcohol content, reducing sugars, pH, total and volatile acidity, and absorbency at 420 nm.
( ) non-treated fermented grape juice (control), ( ) electrolysis-treated fermented grape juice (0.16 A - 4 h - 20°C).

for yeast inactivation by electrolysis in fermented grape juice might be different. Because of the complex nature of fermented grape juice, production of toxic substances could be assumed. Oxidation and reduction reactions occurring during electrolysis would presumably produce yeast growth inhibitors. The D value determined from the linear regression calculated from the post-electrolysis survival curve was approximately 66 h, which was three times the D value obtained during electrolysis. Microbial inactivation occurring during and after electrolysis would therefore have different inactivation rates, suggesting that factors inducing lethality during and after electrolysis would be of a different nature.

The treatment of fermented grape juice with 0.16 A resulted in stabilisation of density in less than 48 h, showing that fermentation had stopped. The aim of the study to stop fermentation in order to maintain a high residual sugar level was thus achieved. Because some physicochemical characteristics are responsible for organoleptic properties of wine, it was essential to study the influence of electric treatment on several physicochemical characteristics. As a first approach, alteration of wine physicochemical characteristics induced by electrolysis, were studied. The physicochemical properties of treated wine were compared to those of non-treated wine. Electrolysis induced a decrease in the alcohol content and an increase in residual sugar level, confirming that fermentation had been stopped. Wine colour strongly influences consumer judgement although it is not necessarily related to taste. White wines should be light yellow in colour with nuances of green. Semi-sweet white wines should have oxidised or amber nuances. The yellow colour intensity was shown to be slightly higher in treated wine, and could be considered a benefit of electrolysis. Acidity is a major physicochemical characteristic of wine as it results in non-desirable sensory perceptions such as acidity, astringency and bitterness. Volatile acidity is generally attributed to acetic acid. *S. cerevisiae* metabolism generally produces 0.2 to 0.4 g L^{-1} acetic acid in wine (Ribereau-Gayon 1961). Volatile acidity was measured at 0.9 and 1.2 g L^{-1} H_2SO_4 (corresponding to 0.5 and 0.7 g L^{-1} acetic acid) in control and treated wines respectively. Total and volatile acidity were found to be higher in treated wine. However semi-sweet wines tend to have higher volatile acidity than dry wines. A volatile acidity of 1.1 - 1.2 can be tolerated in semi-sweet wines.

Several authors have proposed alternative methods to addition of SO_2 such as natural antimicrobial compounds from plants, animals and microorganisms. Lysozyme, which is a bacteriolytic enzyme, has been studied as an alternative to sulphur dioxide in Italy and in France to contain undesired malolactic fermentation of white wines

(Amati *et al.*, 1992 and Gerbaux *et al.*, 1999). Gerbaux *et al.* (1999) showed that SO_2 could not be totally replaced because of its essential antioxidant action. Nevertheless malolactic fermentation could be stopped with a combination of lysozyme and lower doses of SO_2 without detrimental consequences on wine quality (Gerland *et al.*, 1999). The possible use of nisin, a lactic acid bacteria bacteriocin, has been evaluated in wine-making (Radler 1990; Strasser de Saad *et al.*, 1995) and was shown to be effective in the inhibition of most lactic acid bacteria of importance in wine-making but not in the inhibition of wine yeast. Whereas the enforcement of easy rules can normally lead to reasonable sulphite levels in dry wines, an alternative method to sulphiting is still necessary to stop the alcohol fermentation of semi-sweet white wines. Some sauternes wines have been treated by high hydrostatic pressure (Federighi *et al.*, 1995). Crapisi and Lante (1998) observed no changes in the organoleptic properties of wine induced by high hydrostatic pressure. However this expensive treatment is unlikely to be implemented in wine technology. Selecting specific yeast species which could spontaneously stop fermenting when the alcohol content reached 13° has been studied (Poulard 1991, personal communication). This has not yet led to any successful industrial application. Fermentation can be stopped by the removal of the yeasts from the wine using tangential filtration (Chancel, 1990). However accumulation of microbial cells and formation of biofilms on membranes after several operating cycles would require a suitable method of membrane cleaning and disinfection. The method proposed in this paper was shown to stop efficiently alcohol fermentation even at a yeast concentration of $2x10^7$ cfu mL^{-1} at which a microbial load effect was observed. Moreover electrolysis did not induce detrimental consequences on wine quality. To show if electrolysis could replace addition of sulphur dioxide for stabilisation of semi-sweet white wines, physicochemical properties of SO_2- and electrolysis- treated wine should be compared. Further work is currently in progress to extend the present findings. More research is also needed to understand the lethal mechanism of the electric treatment. Growth inhibitors should be identified in order to verify their lack of untoward effects. Experiments should be performed to study the influence of amperage, duration of electrolysis, and size and shape of electrodes on physicochemical characteristics of treated wine in order to process wines on an industrial scale.

Acknowledgements

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