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Controlling grape must fermentation in early winemaking phases: the role of electrochemical treatment

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ABSTRACT

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Aims: To contribute to an understanding of the phenomena related to the effect of low electric current (LEC) in grape must fermentation during laboratory and pilot plant scale winemaking, with selected co-culture yeasts (*Saccharomyces cerevisiae* strain 404 and *Hanseniaspora guilliermodii* strain 465).

Methods and Results: LEC (10, 30, 50 and 100 mA) was applied to fresh grape must as an alternative method to the usual addition of SO_2. Parameters such as polarity, treatment duration (24–96 h) and type of inoculum yeast were varied one at a time. LEC decreased the survival time and increased the death rate of *H. guilliermondii* strain 465 in co-cultures, whereas it did not affect the growth and survival of *S. cerevisiae* strain 40. A final comparison was made of the main physico-chemical parameters on wine obtained after the different tests.

Conclusions: The results have demonstrated that the low voltage treatment using a pair of graphite electrodes had a positive effect on grape juice fermentation (yeast microflora) during the early stages of winemaking, even with the potential of being an alternative method to the usual addition of SO_2.

Significance and Impact of the Study: These results could be of significant importance in developing new winemaking technologies for an innovative yeast fermentation control process for 'biological wine'.

Keywords: grape must, low electric current, sulphur dioxide, yeasts.

INTRODUCTION

Wine can be defined as the alcoholic product resulting from the fermentation of fresh grape juice (must) obtained from grapes with the genotypes of *Vitis vinifera* that have been propagated over the ages (Iocco *et al.* 2001).

In traditional winemaking the grape juice, after the grape pressing, is put into vats where fermentation takes place, spontaneously transforming the juice or must into wine, without any human intervention. The spontaneous fermentation of the juice involves, or can involve, many wild yeasts (*Candida*, *Pichia*, *Metschnikowia*, *Brettanomyces*, *Saccharomycodes*, *Zygosaccharomyces* and *Hansenula*) with the constant presence, and initial prevalence, of apiculate yeasts belonging to the *Kloeckera* and *Hanseniaspora* genera present on the grape or resulting from contamination of the equipment used (Usseglio-Tomasset and Ciolfi 1981; Romano and Suzzi 1993; Gil *et al.* 1996). Initially, in these conditions, there are few yeasts that have optimal enological characteristics, like those related to the genera *Saccharomyces*, but these become involved later to terminate the fermentation process (Martini *et al.* 1980; Rosini 1982). Initially, in such conditions, the enological characteristics of most of the yeasts are far from optimal and it is these yeasts that later become involved in terminating the fermentation process (Martini *et al.* 1980; Rosini 1982). Alcoholic

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fermentation is initiated, and almost exclusively sustained, by spontaneous microflora originating from: (i) incomplete sugar fermentation, because of the prevailing of alcohol intolerant yeasts and (ii) excessive production of undesirable compounds (acetic acid) by apiculate yeasts. Furthermore wines can be produced with compositional characteristics that differ from year to year, depending on the microbial species predominating at that time, and compositional lack of equilibrium and minor sensory properties (changes in characteristic aroma and flavours) can also affect the final wine product (Sponholz 1993; Romano *et al.* 1993; Romano *et al.* 1997; Ciani and Maccarelli 1998).

Although it has long been known that there are advantages to not leaving the must to its destiny, and not allowing the free growth of wild undesired yeasts, it is best to guide the fermentation and, in general, endeavour to favour the elliptical morphological yeasts of the species *S. cerevisiae* (Castelli 1954; Zambonelli *et al.* 1971a,b; Ciani and Rosini 1992; Giudici and Zambonelli 1992).

Traditional white winemaking presupposes, apart from skin and juice contact, several prefermentative treatments during which wild microflora are controlled through the addition of SO_2 (Fleet and Heard 1993; Romano and Suzzi 1993).

In enological practices it is quite usual to use SO_2, its different actions fulfilling different aims like that of acting as an antioxidant and antiseptic, and stabilizing the wine itself from the biochemical aspect: hindering oxidation, inhibiting the development of acetic bacteria and inhibiting the development of lactic bacteria, all of which have a selective action on the yeasts and, in the case of spontaneous fermentation, favour the development of the more suitable *S. cerevisiae* (Wedzicha 1984; Warth 1985; Henick-Kling *et al.* 1998).

From the microbiological point of view the antiseptic function of SO_2 is an important determinant for the yeast population, having been revealed to be selective especially towards lactic and acetic bacteria. Indeed apiculate yeasts show little resistance to SO_2, being almost totally inhibited at starting concentrations lower than 50 mg l^{-1}: instead the elliptical yeasts, particularly *S. cerevisiae*, have a resistance clearly higher than SO_2 1200 mg l^{-1}. The addition of SO_2 brings, together with its positive aspects, several risks. In fact SO_2 exerts a mutagen action, ascertained long ago in tests carried out on micro-organisms; such action is representative of deamination and transamination reactions, with damage to DNA free radicals, and, in other cases, with indirect effects involving RNA and the metabolism of proteins (Shapiro 1977).

Furthermore, the necessary sulphide amounts must be kept to the minimum, above all for health and organoleptic reasons (Cerutti 1993).

Although the initial addition of SO_2 into the must at the start of fermentation is such that only minimal quantities of it would be expected in the wine, even residual traces of SO_2 in the final product can, at the time of consumption, lead to a marked lack of appreciation, or even refusal, of a wine on the part of the consumer.

Among the solicitations the enological sector receives from the market is that of supplying a product that is as 'genuine' as possible (Chichester and Tanner 1972).

The strong reduction, or the elimination, of all these coadjutants, or of those processes that can affect the natural composition of wine, lies within a strategy aimed at an overall improvement of the quality of the final product.

In the light of the above-cited inconveniences, many organizations and international work groups like the International Office of Vine and Wine (OIV) and the Food Safety Panel of the Food Group have, for the last 30 years, been involved in endeavouring to reduce the use of SO_2 in wines and in other foodstuffs like beer, fruit and horticultural products, fruit juices and syrups/cordials. Therefore it is not surprising that the World Health Organisation (WHO) has recommended limiting, to as great a degree as possible, the use of SO_2 in the treatment of foodstuffs, even to the point of contemplating the possibility of its complete suppression.

On the basis of the numerous research studies on this argument, wine figures at the front line (Amati *et al.* 1978). It is known that the European Community (EC), in its efforts to harmonize the legislation of the member countries, set about reducing, starting from 1987, the maximum content of total SO_2 from 175 to 160 mg l^{-1} for red wines and from 225 to 210 mg l^{-1} for white and rosé wines. For a sugar content equal to or higher than 5 mg l^{-1} the tolerated maximum dose passes to 210 and 260 mg l^{-1}, respectively.

According to the actual indications of WHO the acceptable daily intake (ADI) of SO_2 in human foodstuffs has been cautiously established as 0·7 mg kg^{-1} of body weight, but even much lower doses can cause disturbances in subjects lacking specific enzyme alcohol dehydrogenase (Stevenson and Simon 1981).

Therefore it is evident as to how systems or techniques alternative to the addition of SO_2, which are able to selectively inhibit apiculate yeasts, are a sought-after goal.

Investigations have been carried out on the effect of low electric current (LEC) on the inactivation of micro-organisms (Stoner *et al.* 1982; Palaniappan *et al.* 1990, 1992; Liu *et al.* 1997); furthermore, the use of nonthermal methods in food processing has recently been under intense study (Raso *et al.* 1998; Ranalli *et al.* 2000, 2002; Knorr and Heinz 2001; Damar *et al.* 2002; Ulmer *et al.* 2002).

The objectives of the research discussed in the present paper were:

i to investigate the effects of LEC at different intensities in complex matrices (grape must), through the use of graphite electrodes;

ii to evaluate, in laboratory tests, the selectivity of the electrochemical effect on the microflora in grape must;
iii to verify, in a pilot plant, the applicability (efficiency) of LEC in grape must, allowing its proposal as an alternative to the addition of SO_2 to control the first fermentation phases of the fermentation process.

MATERIALS AND METHODS

Micro-organisms and media

Pure cultures of selected yeasts of the *S. cerevisiae* 404 (Di.PRO.VAL Strains Collection, University of Bologna, Italy) and *H. guilliermondii* 465 strains [Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands] were used for the micro-organism tests (Ranalli *et al.* 2002).

Preparation of fresh grape must

For the experiments on grape must, the juices were pressed from grapes taken from selected vines (Trebbiano) in the southern region of Italy (Molise); testing was performed in both the laboratory and a pilot plant.

The grape variety employed was the Trebbiano white berry grape found throughout Italy, known for its high productivity and with, on average, a sugar concentration of around 180 g l^{-1}.

The grapes were collected manually during the wine-grower's association harvest of 2001/2002.

The characteristics of the grape musts used, for both the laboratory and the pilot plant tests, were as follows: pH 3·5, sugars 182·30 g l^{-1}, total acidity 5·75 g l^{-1} and malic acid 3·8 g l^{-1}.

Laboratory test description

Samples of grape juice, pasteurized using low electrical treatment, were analysed. The laboratory tests (thus small scale) were carried out in four different 2·0 l vessels, three being treated and one as the control, and were performed at room temperature under stationary conditions for 24 h (considered a short period).

Pilot plant description

The cellars of a cooperative winegrower's association (Campobasso, Italy) were the site for the pilot plant experimentation, the fresh grape must being fractionated into 300 l containers (effective volume) made of stainless steel.

Three tests were set up: grape must (control), must with added SO_2, must subjected to electrochemical treatment.

After 12 days the samples were clarified at 4°C for 24 h, decanted and analysed immediately.

Electrolytic equipment

An operating electrolytic unit (R. DE Ponti Electronics, Treviglio, Italy) provided with two sections, each working independently, was utilized. Each section has a pair of electrodes that can be positioned as required and in which the electric power can be regulated, each electrode working independently of the other (Ranalli *et al.* 2002; Zanardini *et al.* 2002).

Microbial counts

The microbiological controls were performed by counting, at three different times, the yeast populations on YPD and Wallerstein (WL) nutrient agar media (Oxoid) in Petri dishes. WL nutrient medium was also used for the selection and counting of elliptical and apiculated yeasts (Ranalli *et al.* 2002). Ten colonies of these yeasts were then randomly isolated on each of the YPD plates, and characterized according to the criteria and methods described by Yarrow (1998).

ATP determination

ATP assay was performed using a specific enzymatic kit modified by Jago *et al.* 1989 (NRM/Lumit-Qm, code 9332-l; Lumac B.V., Landgraaf, The Netherlands) (Ranalli *et al.* 2002). A Biocounter 1500 P luminomete (Lumac B.V.) equipped with a photomultiplier tube set at 7200 RLU with 200 pg ATP in 100 μl of Lumit buffer and Lumit-QM reagent was used. (Ranalli *et al.* 1996, 1998).

Chemical–physical analyses

At the end of fermentative process, the wines underwent the following determinations: pH, titrable acidity, ethanol and sugar concentration were assessed using standard methods (Anon. 1990). The pH of the musts and wine was determined using a pH meter; the quantification of glycerol and of acetic, malic and succinic acids was performed by enzyme assay (TC glycerol no. 148270, TC acetic acid no. 148261, TC malic acid no. 139168, TC succinic acid no. 176281; Boehringer, Mannheim, Germany).

Microscope observations

Microbial growth and the dynamics of cell survival during the electrochemical treatment, and for the relative controls (no current), were determined using scanning electron microscopy (SEM) observation scanning. The samples for SEM observation were left to sit overnight in a solution of 2% glutaraldehyde (0·01 mol l^{-1} phosphate buffer), and then immersed in 1% osmium tetroxide. A microscope

operating at 10 kV was used (Zeiss DSM 940A; LEO Elektronenmikroskopie GMbH, Oberkochen, Germany).

Statistical analysis

All the data concerning microbial counts and biochemical responses underwent statistical analyses using the SAS statistical software package (SAS Institute Inc. 1989).

RESULTS

Experimental trials in the laboratory

The laboratory scale trials with graphite electrodes can be detailed thus: effective vessel volume under electrochemical treatment was 2·0 l; treatment tank height 18 cm; specific volume, 0·001 m^3/electrode pair; distance between electrodes 15 cm; electrode shape, cylindrical; electrode length, 30 cm; intensity of current, 10, 30, 50 mA; voltage, 2·5–3·0 V and dispersion area, 200 cm^2/electrode. Co-culture fermentation with *S. cerevisiae* strain 404, or *H. guilliermondii* 465 strain, was performed at 24 h in 2·0 l tanks containing 1·0 l pasteurized fresh grape must. The fresh must was inoculated with the yeast species under examination to an initial concentration of 2% v/v; each vessel had 70 ml of liquid paraffin added to it to minimize water evaporation and to avoid the surface coming into contact with oxygen. The fermentation was allowed to proceed without any agitation whatsoever.

Figures 1–5 show the results.

Figure 1 shows the effects on grape must containing the *S. cerevisiae* 404 and *H. guilliermondii* 465 strains: electrical currents of 10, 30, 50 and 100 mA were applied for a 24 h treatment time, using inversion polarity (60 s). It can be seen that greater variation was encountered in the *H. guilliermondii* 465 strain, the numbers being reduced in proportion to the intensity of the current (10–100 mA). Some effect on the cells of the *S. cerevisiae* 404 strain was noted, but it was less relevant.

Figure 2 shows the survival dynamics of the yeast strains *H. guilliermondii* 465 and *S. cerevisiae* 404 inoculated on the grape must for the different treatments (10–30–50 mA).

The inactivation of *H. guilliermondii* increased with increasing current intensity. In fact, treating the *H. guilliermondii* population with increasing electric current, from 10 to 50 mA, led to a linear decrease in the number of living cells (reduction 3 log cycles). Instead, treating the *S. cerevisiae* cells with LEC had a less relevant effect (reduction 1 log cycle).

Figures 3–5 show the SEM observations during the alcoholic fermentation of the complex grape must matrix inoculated with *S. cerevisiae* 404 and *H. guilliermondii* and submitted to 100 mA LEC-treatment for 8 h. It can be noted that LEC induced, in this complex organic media, a differential effect on the strains: progressive disorganization of the cytoplasm and its organelles; progressive separation of the cytoplasmic membrane, and leakage of the intracellular content, or cellular debris, involving membrane rupturing at the time of gemmation. In addition, LEC applied to the yeast cultures in the complex medium led to alterations in cell morphology and integrity, loss or even the disappearance of *H. guilliermondii* viability, and a marked prevalence of *S. cerevisiae* cells as surviving organisms.

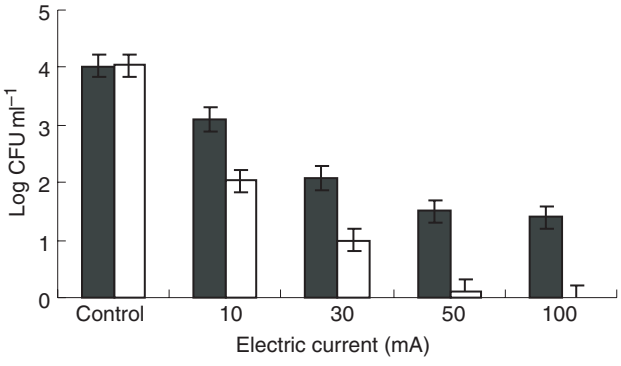

Fig. 1 Effect of different low electric current intensities (10, 30, 50 and 100 mA) on growth rate dynamics of co-culture yeasts *Saccharomyces cerevisiae* 404 strain and *Hanseniaspora guilliermondii* 465 strain on fresh grape must, determined after 24 h. *S. cerevisiae* (■), *H. guilliermondii* (□)

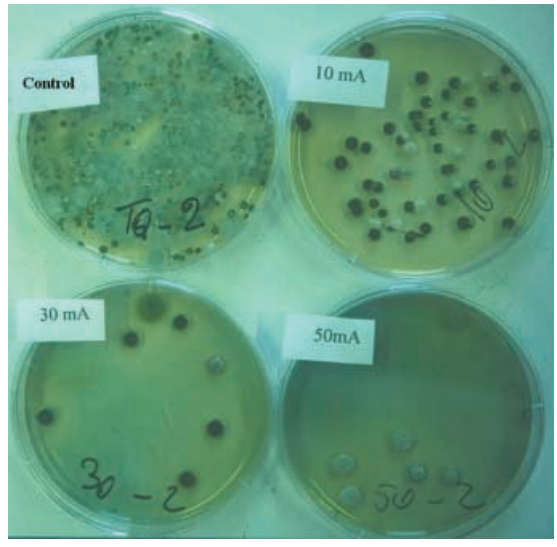

Fig. 2 Survival dynamics of co-culture yeasts *Hanseniaspora guilliermondii* 465 and *Saccharomyces cerevisiae* 404 strain on fresh grape must for different treatments (10–30–50 mA)

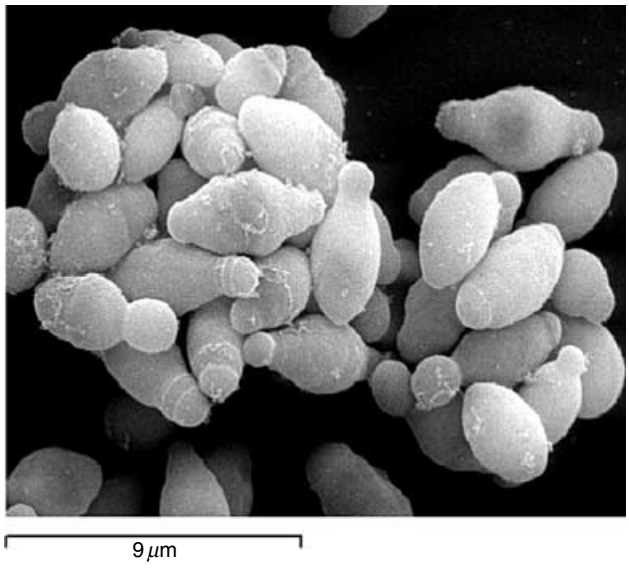

Fig. 3 Scanning electron microscopy of *Hanseniaspora guilliermondii* strain 465 cells on grape must (no treatment, control)

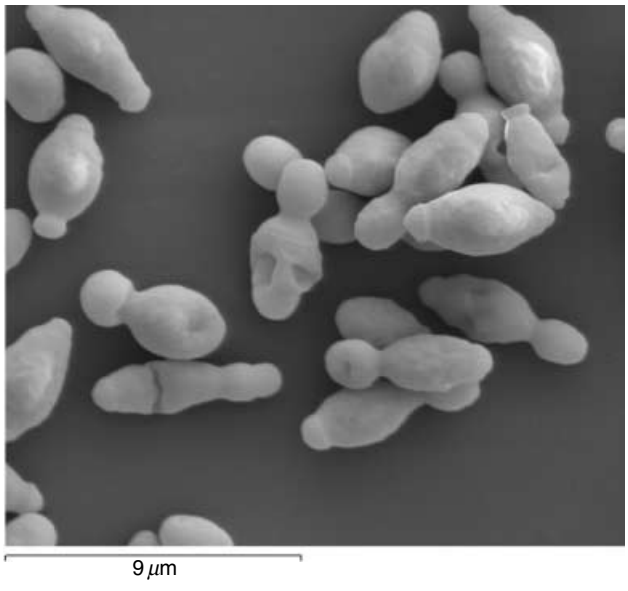

Fig. 4 Scanning electron microscopy of low electric current-treated (30 mA, 8 h) *Hanseniaspora guilliermondii* strain 465 cells on grape must

Experimental trials at the cellar pilot plant

The fermentation was performed by inoculating fresh grape must with pre-cultured cells of the *S. cereviseae* strain 404 (2% v/v), in three fermentors with a working volume of 150 l.

The first fermentor was equipped with two Gr-electrodes, and the current intensity applied was 100 mA, inversion polarity, 60 s; each electrode, 200 cm^2 in area; the inter-electrode distance 15 cm and treatment time, was 96 h. After the completion of 'the treatment' the fermentation was left to follow its natural course.

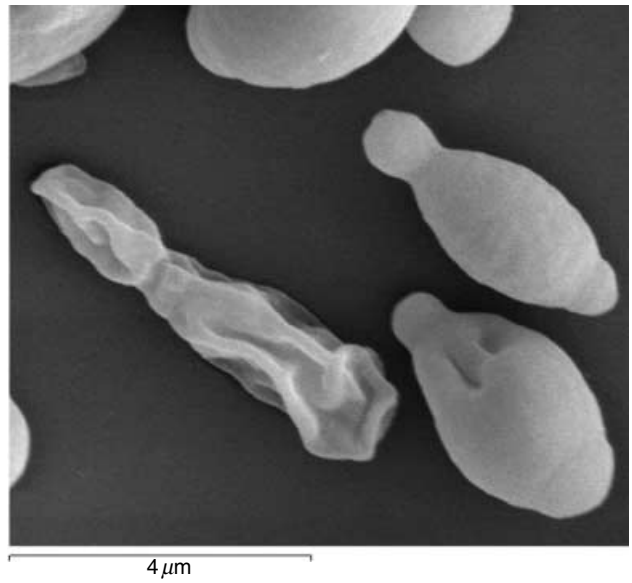

Fig. 5 Scanning electron microscopy of low electric current-treated (100 mA, 4 h) *Hanseniaspora guilliermondii* strain 465 cells on grape must

The second fermentor had 80 mg l^{-1} of SO_2 added to it, and no electric current was applied.

The third fermentor was used as the control, containing the inoculated grape must that underwent no further treatment whatever (neither LEC nor SO_2).

The environmental temperature, monitored throughout the trial period (15 days), showed an average value of 17°C, without any variation in the three trials compared during fermentation.

Figures 6 and 7, and Table 1 show the results of a multiple series of trials performed in the pilot plant at the cellar.

Figure 6 shows the variation, over time, of the yeast survival in the must, for the different treatments (electric current 100 mA, addition of SO_2, control).

There is a significant decrease in the yeast cell vitality compared with that of the control; at 18–24 h there appears a more marked effect for the LEC treatment than when SO_2 was added to the must (test 2).

Figure 7 shows the mean ATP content in the yeast culture of the *S. cerevisiae* 404 strain on grape must, for different times and different treatments.

As expected, there was a notable increase in the ATP content in the control after 17 h.

The application of a low intensity electric current led to the reduction of the total ATP content. This reduction was

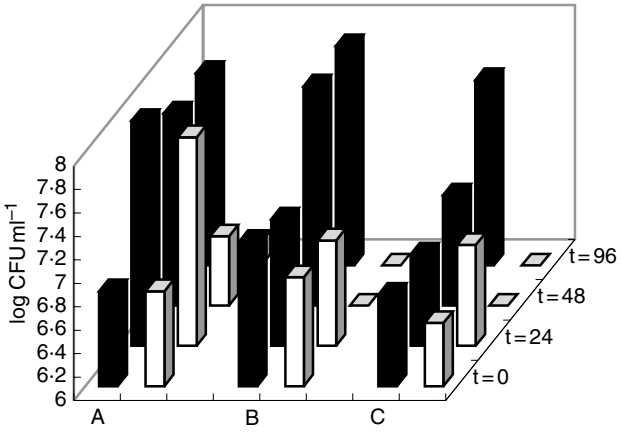

Fig. 6 Effect of different treatments on survival rate dynamics of co-culture yeasts *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* on fresh grape must, determined at different times. *S. cerevisiae* (■), *H. guilliermondii* (□). (a) Grape must: control; (b) grape must + SO_2; (c) grape must + LEC

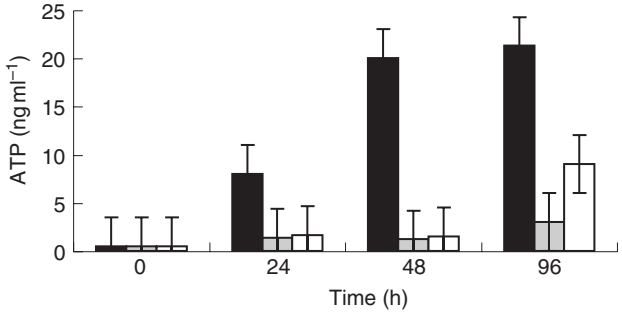

Fig. 7 Mean ATP contents, at different times and different treatments on grape must. Control (■), grape must + SO_2 (▒), grape must + low electric current at 100 mA (□)

already evident 17 h from the start of treatment and was still evident at 51 h, revealing an effect similar to that of adding SO_2 to grape must.

Table 1 shows the chemical–physical characteristics of the wines obtained by the different treatments discussed here (+SO_2, +LEC, fermentation with an inoculum); the data were recorded at the end of the alcoholic fermentation process of the musts (15 days).

The results for all the tests show that the sugar fermentation in the starting musts went to completion, the degree of alcohol being around 9·2°.

In the trials involving electric current the amount of volatile acidity, expressed as acetic acid, did not exceed 0·37 g l^{-1}, the resulting data being similar to the trials with SO_2 (control 0·80 g l^{-1}). Therefore enological yeast performance was not lowered by the electric current, confirming the results of the microbiological analyses.

Malic acid concentrations >5·2 g l^{-1}, that led to greater total acidity, occurred in the trials where electric current was applied or where SO_2 was added. The differences in the glycerol concentrations did not appear significant.

DISCUSSION

The present work investigated the effect LEC had on yeast in the early winemaking phase, both on the laboratory and the pilot plant scale. LEC treatment at different intensities was effective in controlling grape must micro-organisms like *H. guilliermondii* spp. and *S. cerevisiae* spp.; the inactivation mechanisms brought about by LEC were also investigated. The LEC inactivation of *H. guilliermondii* was shown to be proportional to the amplitude of the current (in accordance with Guillou and El Murr 2002). Such a relationship could be used to set current intensity and treatment duration in order to reach a definite reduction in a specific micro-organism population in a complex medium.

During LEC treatment, transmembrane potential differences lead to electrical breakdown and bring about local conformational changes in the bilayer, resulting in the destruction of transmembrane gradients and a loss of viability (Zimmermann 1996). These results indicate a linear relationship between intensity current, metabolic activity and membrane integrity, as was previously noted (Ranalli *et al.* 2002; Ulmer *et al.* 2002).

More research is needed to understand the mechanism by which yeast is inactivated during electrolysis. The dependence of such inactivation on different electrical treatments has already been described, both on a complex medium such as wastewater (Zanardini *et al.* 2002) and on a culture medium (Ranalli *et al.* 2002). Traditional microbiological analyses and biochemical enzymatic assays have confirmed

Table 1 Chemical–physical characteristics of wines harvested in 2002

	pH	Total acids (g l^{-1} tartaric acid)	Sugars (g l^{-1})	Alcohol (% Vol.)	Volatile acids (g l^{-1} acetic acid)	Malic acids (g l^{-1})	Succinic acid (g l^{-1})	Glycerol (g l^{-1})
Must	3·25	–	170	–	–	5·2	–	–
Trials + SO_2	3·28	9·3	<1	9·6	0·44	5·2	0·6	5·4
Trials + 100 mA	3·27	9	<1	9·3	0·37	5·3	0·6	5·2
Control + inocula	3·41	8	<1	9·2	0·80	4·0	0·4	4·8

the LEC effects on *S. cerevisiae* spp. and *H. guilliermondii* yeasts in the complex grape must matrix. When LEC treatments were applied to growing yeast microflora, the *H. guilliermondii* strain 465 was revealed to be more sensitive than *S. cerevisiae* (3 log cycles inactivation); this is probably because of its typical bipolar budding (Yarrow 1998) and the use of the specific electrodes (Shimada and Shimahara 1982; Matsunaga *et al.* 1984).

Compared to the *H. guilliermondii* 465 strain there were significantly greater differences, indicated by the SEM technique, between the LEC-treated and untreated *S. cerevisiae* 404 strain. The SEM results revealed a relatively minor shrinkage of the cytoplasmic membrane, away from the outer membrane, in the treated yeast cells (Ranalli *et al.* 2002). At the same time, it was visually evident that in the *S. cerevisiae* 404 strains the LEC treatment led to higher membrane integrity and a less selective disintegration of the cell envelope. During the LEC treatment, potential differences in the transmembrane resulted in electrical breakdown and local conformational changes of the bilayer, resulting in the destruction of the transmembrane gradients and a loss of viability (Neumann 1989; Charpentier and Feiuillat 1992; Heinz *et al.* 2002).

The role of pH in micro-organism survival is related to the ability of the organisms to control cytoplasmic pH (Cimprich *et al.* 1995); with regard to yeasts, it has been reported that the effect of electrolysis inactivation gives pH values of between 4·0 and 5·0 (Nakanishi *et al.* 1998; Viljoen and Heard 2000). With respect to these authors our experimental results can be considered partially comparable: the pH of the grape must matrix was around 3·7. Nevertheless, it is known that the most sought after wines have a high acidic component, particularly malic acid, and, as a consequence, the pH is low, giving origin to well structured wines; thus such characteristics are looked for in musts destined for winemaking. Moreover the LEC effect in such musts is presumed to be further enhanced, giving the possibility of modulating, and reducing, LEC treatment times.

In the cellar pilot plant trials 100 mA current intensity was chosen in order to verify efficiency with respect to the defined treatment/volume ratio. The 100 mA LEC was applied in the first phase of the fermentation of the fresh grape must to alcohol, and its effectiveness in controlling the inoculated yeast microflora (*S. cerevisiae* 404 strain) was confirmed. Moreover, in the treatment studies combining different LEC intensities, employed as an alternative to the normal addition of SO_2, it was found that the effects of LEC were, to a large degree, comparable with the effect of adding SO_2, but differed greatly from the outcome in the controls.

In fact, the microbial counts and the different biochemical assessments, such as ATP content, during the monitoring of the fermentation phases revealed a certain comparability between LEC treatment and SO_2 addition, but there was no such comparability between these trials and the control.

The results of the fermentation test indicated a log-linear relationship between LEC treatment and SO_2-processing with reduced cellular activity in the must during the first fermentation stages.

In fact, compared with SO_2 addition inactivation, the LEC treatment increased inactivation by, on average, 1·6 log cycles. This was also confirmed by the data on ATP content, total ATP being a potential tool as a descriptive and predictive bioindicator of the general metabolic activities that occur during LEC treatment (Boron 1983).

The characteristics of the wines made it evident that the wines obtained in the trials where current was applied were similar in composition to those obtained adding SO_2 to the must.

The greater amounts of volatile acids in the trials conducted for comparison purposes demonstrated the equal efficiency of the current and SO_2 in inhibiting wild yeasts, like the apiculates, that are high producers of acetic acid (Lema *et al.* 1996). This is an aspect that appears particularly important as it highlights the current's role as an antiseptic when current is applied to must in the initial fermentation stage.

The analytical data were confirmed in the differentiated yeast counts taken during treatment. In fact apiculate yeasts were present in the control even after 4 days, while they were absent in must treated with either SO_2 or LEC.

Thus the very evident correlated advantages of such treatment raises the strong possibility of it providing a valid alternative to the widespread addition of SO_2 in the prefermentation stages of grape must.

In fact, in the light of what has been set out here, it appears evident that technological innovation in the fermentation process could lead to the total elimination of the practice of sulphation, offering LEC treatment as a substitute.

Although applying low intensity current can substitute the antiseptic activity of SO_2, it does not substitute the positive SO_2 action of stabilizing the colour of the wine from white grapes. Nevertheless an appropriate yeast could be used to overcome this limitation (Zambonelli *et al.* 1976).

From the viewpoint of practical application, the overall results appear of great interest for processes aimed at obtaining biological wines, offering a new point of reference to the production of such wines.

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REFERENCES

Amati, A., Guerzoni, M.E. and Galassi, S. (1978) Ricerche per limitare l'impiego dell'anidride solforosa in enologia. *L'Enotecnico* **14**, 6–17.

Anon. (1990) *Methods of Community Analyses of Wines. Regulation no. 2676/90 of the Commission September 17*, Brussels: European Economic Community.

Boron, W.F. (1983) Transport of H^+ and of ionic weak acids and bases. *Journal Membrane Biology* **72**, 11–21.

Castelli, T. (1954) Les agents de la fermentation vinarie. *Archiv für Mikrobiologie* **20**, 323–342.

Cerutti, G. (1993) *Il rischio alimentare*, 2nd edn. pp. 177–193. Milano: Tecniche Nuove.

Charpentier, C. and Feiuillat, M. (1992) Yeast autolysis. In *Wine Microbiology and Biotechnology* ed. Fleet, G.H. pp. 225–242. Switzerland: Harwood Academic Publishing.

Chichester, D.F. and Tanner, Jr F.W. (1972) Antimicrobial food additives. In *Handbook for Food Additives* ed. Da Furia, T.E. pp. 115–118. Cleveland, OH: C.R.C. Press.

Ciani, M. and Maccarelli, F. (1998) Oenological properties of non-*Saccharomyces cerevisiae* yeasts associated with wine-making. *World Journal of Microbiology & Biotechnology* **14**, 199–203.

Ciani, M. and Rosini, G. (1992) The use of pure coltures in wine-making: presence of the starter culture during industrial fermentation. *Industrie delle bevande* **22**, 202–206.

Cimprich, P., Slavik, J. and Kotyk, A. (1995) Distribution of individual pH values in a population of the yeast *Saccharomyces cerevisiae*. *FEMS Microbiology Letters* **130**, 245–252.

Damar, S., Bozoğlu, F., Hizal, M. and Bayindirli, A. (2002) Inactivation and injury of *Escherichia coli* 0157:H7 and *Staphylococcus aureus* by pulsed electric fields. *World Journal of Microbiology & Biotechnology* **18**, 1–6.

Fleet, G.H. and Heard, G.M. (1993) Yeasts – growth during fermentation. In *Wine Microbiology and Biotechnology* ed. Fleet, G.H. pp. 27–54. Sydney, Australia: Harwood Academic Publishers.

Gil, J.V., Mateo, J.J., Jiménez, M., Pastor, A. and Huerta, T. (1996) Aroma compounds in wine as influenced by apiculate yeasts. *Journal of Food Science* **61**, 1247–1249.

Giudici, P. and Zambonelli, C. (1992) Criteri di selezione dei lieviti in enologia. *Vignevini* **9**, 29–34.

Guillou, S. and El Murr, N. (2002) Inactivation of *Saccharomyces cerevisiae* in solution by low-amperage electric treatment. *Journal of Applied Microbiology* **92**, 860–865.

Heinz, V., Alvarez, I., Angersbach, A. and Knorr, D. (2002) Preservation of liquid foods by high intensity pulsed electric fields. *Trends in Food Science and Technology* **12**, 103–111.

Henick-Kling, T., Edinger, W., Daniel, P. and Monk, P. (1998) Selective effects of sulfur dioxide and yeast starter culture addition on indigenous yeast populations and sensory characteristics of wine. *Journal Applied Microbiology* **84**, 865–876.

Iocco, P., Franks, T. and Thomas, M.R. (2001) Genetic transformation of major wine grape cultivars of *Vitis vinifera* L. *Transgenic Research* **10**, 105–112.

Jago, P.H., Stanfield, G., Simpson, W.J. and Hammond, J.R.M. (1989) The effect of extractant and sample composition on the performance of a commercial firefly luciferase reagent. In *Rapid Methods in Microbiology* ed. Stanley, P.E., McCarthy, B.J. and Smiter, R. pp 53–61. Oxford, UK: Blackwell.

Knorr, D. and Heinz, V. (2001) Development of nonthermal methods for microbial control. In *Disinfection, Sterilization, and Preservation* ed. Block, S.S. pp. 853–877. Philadelphia, PA: Lippincott Williams & Wilkins.

Lema, C., Garcia-Jares, C., Orriols, I. and Angulo, L. (1996) Contribution of *Saccharomyces* and non-*Saccharomyces* populations to the production of some components of Albarino wine aroma. *American Journal of Enology and Viticulture* **47**, 206–216.

Liu, W.K., Brown, M.R.W. and Elliot, T.S.J. (1997) Mechanisms of the bactericidal activity of low amperage electric current. *Journal of Antimicrobial Chemotherapy* **39**, 687–695.

Martini, A., Federici, F. and Rosini G. (1980) A new approach to the study of yeast ecology of natural substrates. *Canadian Journal of Microbiology* **26**, 856–859.

Matsunaga, T., Namba, Y. and Nakajima, T. (1984) Electrochemical sterilization of microbial cells. *Bioelectrochemistry and Bioenergetics* **13**, 393–400.

Nakanishi, K., Tokuda, H., Soga, T., Yoshinaga, T. and Takeda M. (1998) Effect of electric current on growth and alcohol production by yeast cells. *Journal of Fermentation and Bioengineering* **85**, 250–253.

Neumann, E. (1989) The relaxation hysterisis of membrane electroporation. In *Electroporation and Electrofusion in Cell Biology* ed. Neumann, E., Sowers, A.E. and Jordan, C.A. pp 61–73. New York, NY: Plenum Press.

Palaniappan, S., Sastry, S.K. and Richter, E.R. (1990) Effects of electricity on microorganisms. A review. *Journal of Food Processing and Preservation* **14**, 393–414.

Palaniappan, S., Sastry, S.K. and Richter, E.R. (1992) Effects of electroconductive heat treatment and electrical pretreatment on thermal death kinetics of selected microorganisms. *Biotechnology & Bioengineering* **39**, 225–232.

Ranalli, G., Chiumenti, R., Donantoni, L. and Sorlini, C. (1996) Electrolytic treatment of swine liquid manure in a full scale experiment. *Journal Environment Science and Health* **A31**, 1705–1721.

Ranalli, G., Recchiuti, J.E. and Grazia, L. (1998) Bioluminescence and impedance monitoring to detect the activity of starter cultures during frozen storage. *Annali di Microbiologia ed Enzimologia* **48**, 169–180.

Ranalli, G., Iorizzo, M. and Grazia, L. (2000) Effects of low electric current on media and on grape juice. In *The Rising Power of Yeasts in Science and Industry* ed. van Dijken, J.P. and Sheffers, A. pp. 369–370. Delft, The Netherlands: Delft University Press.

Ranalli, G., Iorizzo, M., Lustrato, G., and Grazia, L. (2002) Effects of low electric treatment on yeast microflora. *Journal of Applied Microbiology* **93**, 877–883.

Raso, J., Calderón, M.L., Barbosa-Cánovas, G.V. and Swanson, B.G. (1998) Inactivation of Zygosaccharomyces bailii in fruit juices by heat, high hydrostatic pressure and pulsed electric fields. *Journal of Food Science* **63**, 1042–1044.

Romano, P. and Suzzi, G. (1993) Sulfur dioxide and wine microorganisms. In *Wine Microbiology and Biotechnology* ed. Fleet, G.H. pp. 373–393. Chur: Harwood Academic Publishers.

Romano, P., Suzzi, G., Zironi, R. and Comi G. (1993) Biometric study of acetoin production in *Hanseniaspora guilliermondii* and Kloekera apiculata. *Applied Environmental Microbiology* **59**, 1838–1841.

Romano, P., Suzzi, G., Comi, G., Zironi, R. and Maifreni, M. (1997) Glycerol and other fermentation products of apiculate wine yeasts. *Journal of Applied Microbiology* **82**, 615–618.

Rosini, G. (1982) Influenza della microflora saccaromicetica della cantina sulla fermentazione del mosto d'uva. *Vignevini* **9**, 43–46.

SAS Institute Inc. (1989) *SAS/STAT Users' Guide*. Version 6, 4th edn, Cary, NC: SAS Institute Inc.

Shapiro, R. (1977) Genetic effects of bisulfite (sulfur dioxide). *Mutation Researches* **39**, 149–175.

Shimada, K. and Shimahara, K. (1982) Responsibility of hydrogen peroxide for the lethality of resting *Escherichia coli* B exposed to alternating current in phosphate buffer solution. *Agricultural and Biological Chemistry* **46**, 1329–1337.

Sponholz, W. (1993) Wine spoilage by microorganisms. In *Wine Microbiology and Biotechnology* ed. Fleet, G.H. pp. 395–420. Sydney, Australia: Harwood Academic Publishers.

Stevenson, D.D. and Simon, R.A. (1981) Sensivity to ingested metabisulfites in asthmatic subjects. *Journal Allergy Clinical Immunology* **68**, 26–32.

Stoner, G.E., Cahen, G.L. Jr, Sachyani, M. and Gileadi, E. (1982) The mechanism of low frequency alternating current electrochemical disinfection. *Bioelectrochemistry and Bioenergetics* **9**, 229–243.

Ulmer, H.M., Heinz, V., Ganzle, M.G., Knorr, D. and Vogel R.F. (2002) Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. *Journal of Applied Microbiology* **93**, 326–335.

Usseglio-Tomasset, L. and Ciolfi, G. (1981) Osservazioni sulla flora lievitoforme presente sulle uve al momento della vendemmia. *Vignevini* **8**, 51–53.

Viljoen, B.C. and Heard, G.M. (2000) *Saccharomyces cerevisie*. In *Encyclopedia of Food Microbiology* Vol. **3**, ed. Robinson, R.K., Batt, C.A. and Patel, P.D. pp. 1918–1928. New York, NY: Academic Press.

Warth, A. (1985) Resistance of yeast species of benzoic and sorbic acids and to sulphur dioxide. *Journal of Food Protection* **48**, 564–569.

Wedzicha, B. (1984) *Chemistry of Sulphur Dioxide in Foods*. London, UK: Elsevier Applied Science.

Yarrow, D. (1998) Methods for the isolation, maintenance and identifications of yeasts. In *The Yeasts, a Taxonomic Study* ed. Kurtzman, C.P. and Fell, J.W. pp. 77–100. Amsterdam, The Netherlands: Elsevier Science B.V.

Zambonelli, C., Guerzoni, M.E., Nanni, M. and Gianstefani, G. (1971a) Selezione genetica nei lieviti della fermentazione vinaria. Studio dei caratteri: 1) l' energia di fermentazione. *Rivista di Viticoltura ed Enologia* **24**, 373–378.

Zambonelli, C., Guerzoni, M.E., Nanni, M. and Gianstefani, G. (1971b) Selezione genetica nei lieviti della fermentazione vinaria. Descrizione dei metodi e dei criteri seguiti. *Rivista di Viticoltura ed Enologia* **24**, 287–293.

Zambonelli, C., Soli, M.G., Romano, P. and Grazia, L. (1976) Influenza del ceppo di lievito sulla stabilità del colore dei vini bianchi. II. Analisi del ceppo 633. *Annali di Microbiologia* **26**, 109–118.

Zanardini, E., Valle, A., Gigliotti, C., Papagno, G., Ranalli, G. and Sorlini C. (2002) Laboratory-scale trials of electrolytic treatment on industrial wastewaters: microbiological aspects. *Journal of Environmental Science and Health* **A37**, 1463–1481.

Zimmermann, U. (1996) The effect of high intensity electric field pulses on eucaryotic cell membranes: fundamentals and applications. In *Electromanipulation of Cells* ed. Zimmermann, U. and Neil, G.A. Boca Raton, FL: CRC Press.