Biological Deacidification of Musts Induced by Yeasts or Malolactic Bacteria and the Effect on Wine Quality

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This study investigated the possible use of *Schizosaccharomyces pombe* and *Schizosaccharomyces malidevorans* for deacidification of red wines during alcoholic fermentation, in comparison with existing deacidification practices of induced or spontaneous malolactic fermentation (MLF) in South Africa. In red must, the effect of these yeasts on alcoholic fermentation rate, rate of L-malic acid removal, as well as the effect of mixed cultures with *Saccharomyces cerevisiae* at different inoculum concentrations and sizes of inocula on wine quality was investigated. In white must, the effect of pure culture inocula and temperature on wine quality and production of volatile bouquet substances was also studied. Both *Schizosaccharomyces spp.* were found to deacidify normal concentrations of L-malic acid in musts effectively at higher growth temperatures, but the ability of *Schiz. pombe* was inhibited by high concentrations of L-malic acid which was not pH dependent. Certain key volatile fermentation bouquet compounds were produced in very low quantities, which seems to be the reason for poor sensory quality wines produced by *Schizosaccharomyces*.

The need for deacidification of wine is not the same in all countries. Northern Hemisphere wine producing areas, including Eastern USA (Munyon & Nagel, 1977), more often have the need for deacidification because of too high acidity and too low pH due to cold weather and insufficient ripening of grapes. In Southern Hemisphere countries, the need is more often to acidify musts or wines to create a good acid balance with the correct amount of tartness. However, a survey of the incidence of malolactic fermentation (MLF) in South African red table wines has shown that spontaneous MLF occurred in 27,5% of the cases within a short period after completion of alcoholic fermentation and in most cases (66,2%), had been completed before bottling (Van Wyk, 1976). Most oenologists are of opinion that biological deacidification should be encouraged by adapting wine-making technology and by inducing bacteriological deacidification by starter cultures, in order to stabilize such wines biologically to prevent uncontrolled deacidification after bottling.

In contrast to MLF by bacteria, yeasts belonging to the genera *Schizosaccharomyces* convert grape sugar and malic acid to ethanol and carbon dioxide (Dittrich, 1963; Mayer, 1963). Although many studies have been undertaken dealing with wine deacidification by *Schizosaccharomyces spp.* as basis (Beelman & Gallander, 1979), most studies were conducted using *Schiz. pombe*. The reason *Schiz. malidevorans* was not studied to the same extent could be the formation of copious amounts of hydrogen sulphide during fermentation (Rankine & Fornachon, 1964; Rankine, 1966).

The amount of deacidification depends not only on the *Schizosaccharomyces* specie and strain (Benda & Schmitt, 1969; Beelman & Gallander, 1979), but also on temperature. The optimum deacidification temperature for *Schiz. pombe* was found to be 20°C–25°C (Spirov *et al.*, 1982). Dittrich (1963a) found a direct relationship between temperature and fermentation rate as well as deacidification rate. However, 35°C was found to be above the optimum temperature and fermentations were not completed. He states 30°C to be nearer the optimum temperature for *Schiz. pombe*. Contradictory to this, Benda and Schmitt (1966) found no difference in the degree of malic acid decomposition at 11°C and 20°C. This could have been due to both temperatures being far below the optimum for *Schiz. pombe*.

Beelman and Gallander (1979) concluded from work by Rankine (1966) and Peynaud *et al.* (1964) that malic acid utilization was pH dependent, but added that additional information is needed to verify that the amount of L-malic acid decomposed is inversely related to pH. It is possible that the lower limit of the pH range would be approx. 2,5, at which Yang (1973b) found only 70% utilization by *Schiz. pombe*. The high deacidification ability of *Schizosaccharomyces* of highly acid musts has been a great contributor to the betterment of the quality of these wines, as compared to the less deacidified wines fermented by *Saccharomyces* (Yang, 1973a; Ethiraj & Suresh, 1978). However, musts that were not excessively acidic were found to be over – deacidified, which could result in inferior wine quality (Yang, 1973a). The work by Rankine (1966) in Australia, indicated the possible use of selected yeasts for either maximum or minimum decomposition of L-malic acid.

Several researchers have found inferior quality of wines after fermentation with *Schizosaccharomyces* (Benda & Schmitt, 1966; Spirov *et al.*, 1982; Delfini *et al.*, 1983; Delfini & Pagliara, 1979). Nonomura *et al.*, 1968 (Beelman & Gallander, 1979) found wines from *Schizosaccharomyces* better tasting than wines fermented with true yeast, although these wines had inferior bouquets. However, many researchers have reported wines which did not differ in sensory quality from control treatments or *Saccharomyces* (Ethiraj & Suresh, 1978; Yang, 1973a; Ethiraj, Suresh & Onka-

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rayya, 1983; Svejcar, 1970; Benda & Schmitt, 1979). Beelman and Gallander (1979) also felt that most studies concerning wine deacidification by *Schizosaccharomyces* had incomplete sensory evaluation data with statistical analyses. Except for reports by Rankine and Fornachon (1964) as well as Rankine (1966), concerning H_2S production by *Schiz. malidevorans*, an aspect lacking in most research papers as to the lower sensory quality of *Schizosaccharomyces* wines, has been an accurate description or identification of the aroma or flavour deficiency of such wines. Carre *et al.* (1983) reported concentrations of groups of volatile fermentation bouquet substances, but did not correlate these with statistically analyzed sensory evaluation data.

Initially the use of mixed cultures was researched in order to attain the desired amount of deacidification (Dittrich, 1963a; Rankine, 1966), as well as to minimize unpleasant sensory characteristics produced by *Schizosaccharomyces* (Carre *et al.*, 1983; Ethiraj *et al.*, 1983). No report could be found in the available literature of comparative tests which included induced MLF by selected malo-lactic bacteria, in order to compare both deacidification abilities and rates as well as effect on sensory quality. To date, no research data on *Schizosaccharomyces* has been published by South African researchers. The aim of this work was to evaluate the possible use of *Schiz. pombe* and *Schiz. malidevorans* separately and in combination with *Saccharomyces cerevisiae* for biological stabilization of red wines, in comparison with existing biological deacidification practices in South Africa. Because of seemingly contradictory reports concerning the negative effects of *Schizosaccharomyces* fermentation on the production of bouquet substances, this was also investigated in white grape juice.

MATERIALS AND METHODS

Organisms: *Saccharomyces cerevisiae* strain WE 14 was obtained as active dried wine yeast from the producer. *Schizosaccharomyces pombe* strain CBS 5682 and *Schiz. malidevorans* strain CBS 5557 were obtained from Prof. J. P. van der Walt, CSIR, Pretoria, South Africa. Both *Schizosaccharomyces spp.* are the type species. *Schizosaccharomyces malidevorans* was initially isolated from wine by Rankine and Fornachon (1964) and deposited at the Centraalbureau voor Schimmelcultures in Delft, Netherlands. *Leuconostoc oenos* (strain LO) was obtained from the VORI culture collection.

Inoculum preparation: *Saccharomyces cerevisiae* was rehydrated and inoculated according to the manufacturer's instructions. Both *Schizosaccharomyces spp.* were cultured in 11 Erlenmeyer flasks containing 500 ml sterile Colombar must on a shaker at 25°C for 18 hours. The *L. oenos* strain was grown in Tomato Juice Broth for 5 days at 25°C.

Vinification and must treatments: Cabernet Sauvignon grapes from the same vineyard (Welgevallen Experimental Farm, University of Stellenbosch) were used in 1984 (sugar 23,4°B; titratable acidity 5,6 g/*l*; pH 3,8) and in 1985 (sugar 21°B; titratable acidity 5,2; pH 3,9). The titratable acidity was adjusted to 7,0 and 7,5 g/*l* with L-Tartaric acid (Merck 804) in 1984 and 1985 respectively.

In the 1984 season, the effect of the following treatments at 25°C on the quality of Cabernet Sauvignon wine was studied: alcoholic fermentation by *Sacch. cerevisiae* and spontaneous MLF or MLF induced by inoculation with *L. oenos* after pressing the skins at half of the initial sugar concentration; alcoholic fermentation and malic acid deacidification by *Schiz. pombe* or *Schiz. malidevorans*.

In the 1985 season *Sacch. cerevisiae* was used for all treatments of Cabernet Sauvignon except one, where *Schiz. pombe* was used. In the control treatments MLF was allowed to occur spontaneously or was induced by *L. oenos*. Musts were treated in the following ways to study the effect of time of inoculation (11°B and 1°B) and size of inoculum (2%, 5%, 10% v/v) of *Schiz. pombe* on deacidification and wine quality. Grapes were crushed, destemmed and separated. The juice was divided on a mass basis before adding the skins to the juices. Samples were drawn regularly, treated with NaF, frozen and stored until analyses could commence.

In the second experiment in 1985, Chenin blanc grapes from Welgevallen, as well as a second harvest of Chenin blanc from the VORI vineyard were crushed and destemmed with addition of 60 mg/*l* SO_2, settled overnight, mixed and divided to obtain three lots with increasing malic acid concentrations. The pH was adjusted to 3,42 +/- 0,04 by reduction of the tartrate content with $CaCO_3$ (Munyon & Nagel, 1977). After mixing and adjustments, the lots had average L-malic acid contents of 7,93, 11,89 and 15,83 g/*l* respectively. These lots were subdivided into seven 6,5*l* duplicate sublots in 10*l* stainless steel canisters fitted with fermentation locks. After inoculation with the various yeasts, canisters were placed in a fermentation room maintained at 21°C. Samples were taken at regular intervals during fermentation and treated in the same manner as the first experiment.

For the third experiment the Chenin blanc treatment which contained 7,93 g/*l* L-malic acid was repeated in order to study the effect of yeast inoculum (*Sacch. cerevisiae*, *Schiz. pombe* and *Schiz. malidevorans*) and fermentation temperature (15°C and 21°C) on the quality of Chenin blanc wine.

Malic and lactic acid analyses: Malic acid was detected qualitatively by paper chromatography (Kunkee, 1968) for the 1984 Cabernet Sauvignon experiment. All other malic acid analyses, together with lactic acid were done by high performance liquid chromatography (HPLC). The method of Schwarzenbach (1982) was used with the following modifications: column temperature 40°C (constant); mobile phase, 0,013N H_3PO_4. A UV detector (KNAUER Spekralfotometer Model 87.00) was used for detecting eluting peaks, which were quantified by means of an APPLE IIe fitted with Chromatochart (Interactive Microware Inc. PA 16801) chromatography software (Van Rooyen & Van Wyk, 1986).

GC analysis: Quantitative determination of volatile fermentation bouquet substances was done by the method described by Marais (1986).

Sensory analysis: Wine quality was determined by a panel of 12 experienced judges, using the nine point score card (Tromp & Conradie 1979). In order to eliminate possible sensory differences in acidity due to deacidification and to compare *Schizosaccharomyces* and

Saccharomyces treatments for Cabernet Sauvignon and for Chenin blanc wines, a calculated amount of DL-malic acid was added 24 hours prior to judging. This amount was determined as the difference in total titratable acidity between the *Saccharomyces* and the other treatments.

Statistical analysis: Friedman's non – parametric two-way analysis of variance was used for wines tasted by the nine point score card. All experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Fermentation rates: It is evident from Table 1 that temperature had a profound effect on the alcoholic fermentation ability of both *Schizosaccharomyces spp.* in Cabernet Sauvignon and Chenin blanc musts. It is also clear that 25°C is closer to the optimum fermentation temperature for *Schizosaccharomyces spp.* than 15°C. It has been shown by various authors that the growth temperature for *Schizosaccharomyces spp.* is higher than for *Sacch. cerevisiae.* Peynaud and Sudraud, 1964 (Beelman & Gallander, 1979) found maximum growth at approx. 35°C. Yang (1973) reported faster alcoholic fermentation rates by *Sacch. cerevisiae* than *Schiz. pombe* at 12,8°C for white musts. Spirov *et al.* (1982) showed 25°C to be near optimum growth temperature for *Schiz. pombe* and Dittrich (1963) found the highest alcoholic fermentation rate at 25°C. Except for the *Sacch. cerevisiae* alcoholic fermentation in the 1984 Cabernet Sauvignon must, which took 43 days, all fermentations finished within an expectedly reasonable time. At this relatively high temperature of 25°C, both *Schizosaccharomyces spp.* had a fermentation rate approximately double that of *Sacch. cerevisiae.* In the Chenin blanc musts, *Sacch. cerevisiae* fermented at more than twice the rate of both *Schizosaccharomyces spp.* at both 15°C and 21°C. This illustrates the rate-enhancing effect of high temperature on fermentation by *Schizosaccharomyces* and parallels the results of Dittrich (1963a), who found that high fermentation temperature benefited the growth of *Schiz. pombe* such that after fermentation in a mixed culture with *Sacch. cerevisiae, Schiz. pombe* was the predominant yeast.

Small differences were noted in the fermentation rates with addition of *Schiz. pombe* to the fermenting 1985 Cabernet Sauvignon musts by *Sacch. cerevisiae.* These differences were probably not due to the addition itself, as the addition was done at a late stage and the pure *Schiz. pombe* alcoholic fermentation rate did not differ from that by *Sacch. cerevisiae.*

Malic and lactic acid concentrations:

Cabernet Sauvignon 1984: All treatments took 63 days for completion of deacidification, except for *Schiz. malidevorans* which took 42 days.

Cabernet Sauvignon 1985: Schiz. malidevorans was omitted after the 1984 experiment because of the poorer wine quality (Fig. 4). Figure 1 shows the concentrations of malic and lactic acids of the three deacidification treatments. It is evident that malic acid was metabolized soon after the second day (when skins were pressed at 11°B). The slowest rate of malic acid removal was in the case of spontaneous MLF, where no exogenous malic acid metabolizing organisms were added. In spite of this, total removal of malic acid took

TABLE 1

Fermentation rates of various must treatments by *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Schiz. malidevorans.*

Treatment	1984		1985	
	Time for completion of alcoholic fermentation (days)	Fermentation rate (days)	Time for completion of alcoholic fermentation (days)	Fermentation rate (days)
Cabernet Sauvignon 25°C (1984)				
Sacch. cerevisiae + spontaneous MLF	43	0,53		
Sacch. cerevisiae + L. oenos induced MLF	43	0,53		
Schiz. pombé	17	1,35		
Schiz. malidevorans	23	1,00		
Cabernet Sauvignon 21°C (1985)				
Schiz. pombe			31	0,69
Sacch. cerevisiae + spontaneous MLF			31	0,69
Sacch. cerevisiae + L. oenos induced MLF			27	0,79
Sacch. cerevisiae + 2% Schiz. pombe at 11°B			31	0,69
Sacch. cerevisiae + 5% Schiz. pombe at 11°B			31	0,69
Sacch. cerevisiae + 10% Schiz. pombe at 11°B			31	0,69
Sacch. cerevisiae + 2% Schiz. pombe at 1°B			31	0,69
Sacch. cerevisiae + 5% Schiz. pombe at 1°B			27	0,79
Sacch. cerevisiae + 10% Schiz. pombe at 1°B			24	0,89
Chenin Blanc 15°C (1985)				
Sacch. cerevisiae			12	1,87
Schiz. pombe			27	0,83
Schiz. malidevorans			31	0,72
Chenin Blanc 21°C (1985)				
Sacch. cerevisiae			7	3,01
Schiz. pombe			18	1,15
Schiz. malidevorans			17	1,21

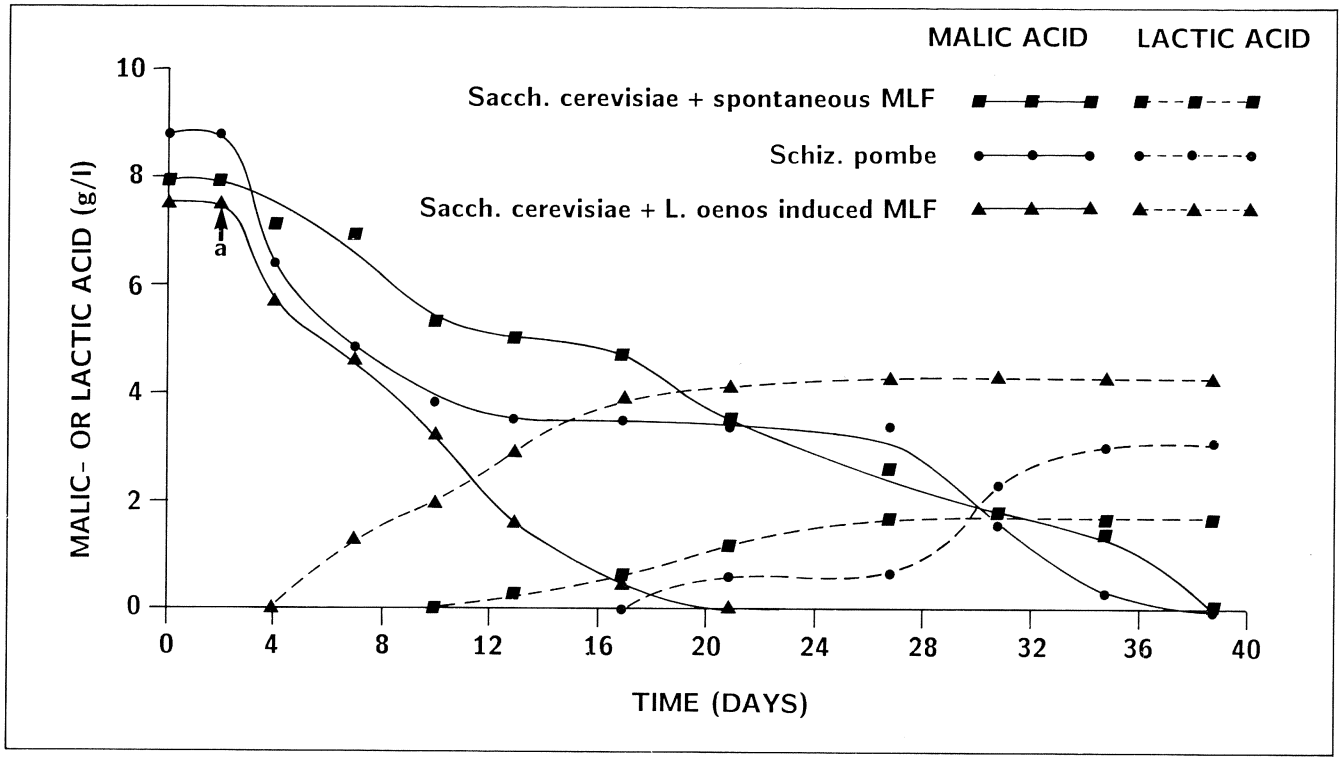

FIG. 1
Concentrations of malic and lactic acids in Cabernet Sauvignon musts deacidified by three different treatments.

as long as for the *Schiz. pombe* treatment. It is evident that *L. oenos* was most effective in reducing the malic acid content (21d), with the highest average daily rate of malic acid removal (Table 2), starting immediately after inoculation on the third day. From Fig. 1 it can be seen that production of lactic acid from malic acid started soon thereafter and of the three treatments, the most lactic acid was produced by *L. oenos*. It seems as if only half of the initial malic acid content was metabolized by *Schiz. pombe* after ten days, followed by a lag phase during which lactic acid started being produced. Of all treatments, *Schiz. pombe* had the highest rate of malic acid removal to half the initial concentration (Table 2), but was not capable of total metabolization of the malic acid present in the must. As *Schizosaccharomyces spp.* produce ethanol from malic acid and not lactic acid (Dittrich, 1963b), the natural malic acid bacteria must have grown to such an extent as to be able to metabolize the malic acid. This is apparent in the smaller final lactic acid concentration in comparison to the *L. oenos* treatment.

In contrast to this, the spontaneous MLF treatment (Fig. 1) produced even less lactic acid, which could be an indication of natural malic acid-metabolizing yeasts' activity in this must, of which the natural occurrence in newly fermented wines has been reported on (Van Zyl & Du Plessis, 1961). Delfini, Torreggiani and Ottina (1983) reported epidemic proportions of naturally occurring *Schizosaccharomyces* yeasts during the previous vintage which they ascribed to unusually high environmental temperatures. As South Africa is a Southern Hemisphere country, unusually high temperatures for Italy could well be within normal South African harvest time temperatures, where crushed grapes at 35°C are frequent.

Carre *et al.* (1983) advocated the possible use of *Schizosaccharomyces spp.* for deacidification of wine by addition towards the end of alcoholic fermentation to reduce adverse organoleptic consequences. They reported a considerable deacidification by this method. In our experiments this seems as effective as a pure culture addition of *Schiz. pombe* before alcoholic fermentation (Table 2). Addition of different sizes of *Schizosaccharomyces* inocula at different stages during alcoholic fermentation had no marked effect on the time for complete removal of malic acid. According to Beelman and Gallander (1979), true wine yeasts tend to outgrow the slow fermenting *Schizosaccharomyces* and this seems to have been the case in our experiment (Table 2).

Figure 2 shows a representative graph of malic and lactic acid concentrations for a 5% addition of *Schiz. pombe* at 11°B and 1°B during alcoholic fermentation. A similar phenomenon can be observed as in Fig. 1, viz. a reduction in the malic acid concentration up to approximately half of the initial concentration, followed by a lag phase and the final reduction to zero concentration, during which time production of lactic acid started and increased to a maximum level. Benda and Schmitt (1966), who measured total titratable acidity (TTA) in Riesling must during fermentation, found a sudden drop in TTA for treatments with *Schiz. pombe* at 11°C as well as 20°C, followed by a period of slow decrease in TTA. All combined treatments of *Saccharomyces* and *Schizosaccharomyces* exhibited the same tendency of a lower rate of malic acid utilization

TABLE 2

Time for, and rate of malic acid removal from Cabernet Sauvignon (1985) must and wine by spontaneous MLF, induction with *Leuconostoc oenos* or several treatments with *Schizosaccharomyces pombe.*

Treatments	Initial malic acid concentration (g/l)	Time for complete removal of malic acid (days)	Average daily rate of malic acid removal (g/l/d)	Rate of malic acid removal: initial to half concentration (g/l/d)	Rate of malic acid removal: half to zero concentration (g/l/d)
Schizosaccharomyces pombe	8,757	39	0,225	0,461	0,148
Sacch. cerevisiae + spontaneous MLF	7,887	39	0,202	0,219	0,188
Sacch. cerevisiae + L. oenos induced MLF 11°B	7,495	21	0,350	0,416	0,312
Sacch. cerevisiae + 2% Schiz. pombe at 11°B	7,870	35	0,225	0,179	0,303
Sacch. cerevisiae + 5% Schiz. pombe at 11°B	7,890	39	0,202	0,164	0,063
Sacch. cerevisiae + 10% Schiz. pombe at 11°B	7,878	35	0,225	0,164	0,358
Sacch. cerevisiae + 2% Schiz. pombe at 1°B	7,296	39	0,187	0,159	0,228
Sacch. cerevisiae + 5% Schiz. pombe at 1°B	7,128	39	0,183	0,119	0,396
Sacch. cerevisiae + 10% Schiz. pombe at 1°B	7,687	39	0,197	0,202	0,192

from initial to half concentration and an increase in the rate from half to zero concentration (Table 2). This increase coincided with lactic acid production, which indicates malolactic bacterial activity. Benda and Schmitt (1966) also found a similar phenomenon in their Rieslaner must, where approx. 2 g/l TTA reduction occurred by *Schizosaccharomyces* after 30 days. After two years, though, the reduction was 7,8 g/l, with a coinciding increase of 6 g/l of lactic acid.

The decrease in malic acid during fermentation can be ascribed partly to the ability of *Sacch. cerevisiae* strain WE 14, which was found capable of utilizing 20% of available L-malic acid (3 g/l) in a synthetic medium (Van Rooyen, 1987). This ability of *Saccharomyces* yeasts was also recognized by Rankine (1966), who found that these yeasts may metabolize up to 45% of L-malic acid in grape juice during normal alcoholic fermentation. Carre *et al.* (1983) reported 11,5% and 15,2% utilization of L-malic acid by *Sacch. cerevisiae.* None of the treatments in Table 2 were as effective as the *L. oenos* induced MLF for malic acid removal. When considering the role of the natural malolactic bacteria in the combined treatments, it can be assumed that they could have enhanced the rate of malic acid removal from half to zero concentration for the *L. oenos* induced MLF (Table 2).

Chenin blanc 1985: Figure 3 presents the effectivity of utilization of different concentrations of L-malic acid at a fixed pH of 3,42 by two *Schizosaccharomyces spp.* It would seem as if an increase in the malic acid concentration stimulated increased utilization, as well as increased rate of removal by both *Schizosaccharomyces*

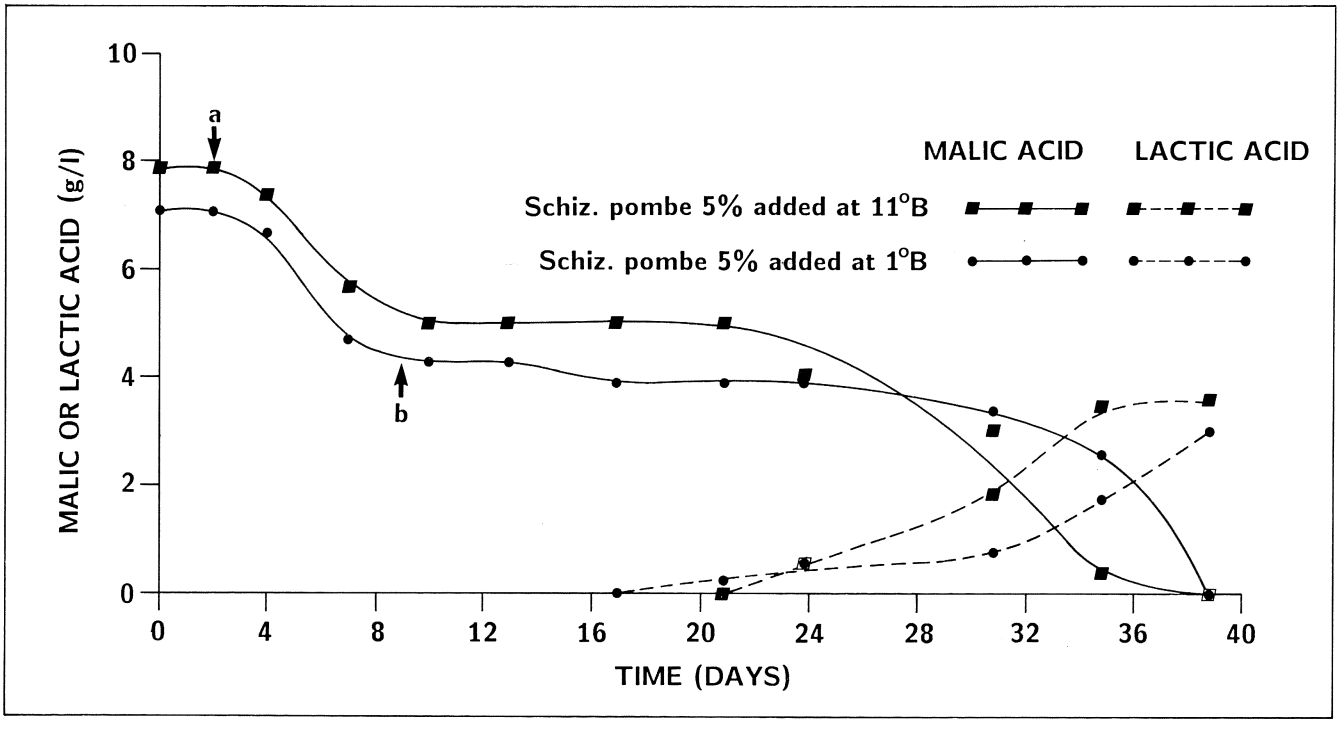

FIG. 2

Concentrations of malic and lactic acids in Cabernet Sauvignon musts fermented by *Saccharomyces cerevisiae* and inoculated with *Schizosaccharomyces pombe* at 11°B (a) or 1°B (b).

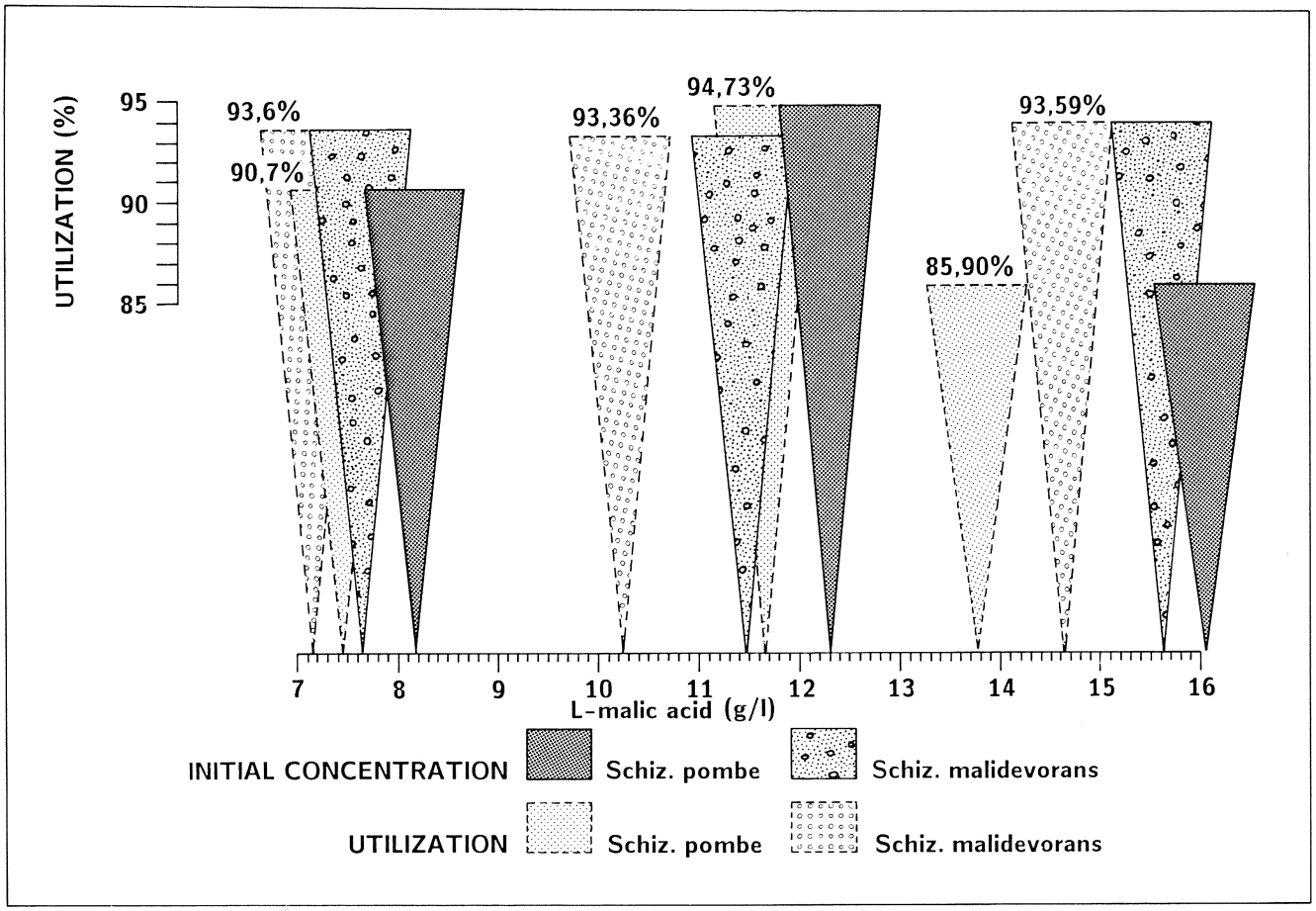

FIG. 3
Utilization of different initial concentrations of malic acid by two *Schizosaccharomyces spp.* in Chenin blanc must.

spp. However, it is evident that the utilization by *Schiz. pombe* might be inhibited by large amounts of malic acid, as is evident from the drop in percentage utilization from 94,7 to 85,9. Yang (1973b) indicated an increased percentage utilization by *Schiz. pombe* of a fixed malic acid content at increased pH values from 2,5 to 3,5. Above pH 3.0 malic acid utilization was complete. In musts with high TTA (9.3 and 16.2 g/*l*), he reported incomplete malic acid utilization by *Schiz. pombe*. Except for the pH dependency, our results indicate that high concentrations of L-malic acid may have an inhibiting effect on the deacidification ability of *Schiz. pombe*. This was not observed with *Schiz. malidevorans* (Fig. 3). Different abilities for malic acid utilization between *Schiz. pombe* and *Schiz. malidevorans* and also between strains of *Schiz. malidevorans*, were reported by Rankine (1966). He observed total decomposition of malic acid, independent of pH, by *Schiz. malidevorans* in musts up to the highest level tested, 116 meq./*l* (31,088 g/*l*), whereas *Schiz. pombe* did not show this ability.

Wine Quality:

Cabernet Sauvignon 1984: Except for the *Schiz. malidevorans* treatment, wines were of above average quality (Fig. 4). As these results proved the *Schiz. pombe* treatment was not significantly poorer than the *L. oenos* induced MLF treatment, it was decided to continue thorough experimentation in 1985 to ascertain whether *Schiz. pombe* might be used as a substitute for *L. oenos*, both for malic acid removal and for producing wines with comparable quality.

Cabernet Sauvignon 1985: The pure culture *Schiz. pombe* wines were significantly poorer than those in which spontaneous MLF or induced MLF by *L. oenos* took place. Figure 4 shows that malic acid removal by induction with different concentrations of *Schiz. pombe* at different stages during alcoholic fermentation (by *Sacch. cerevisiae*) had no significantly different effect on wine quality. This corresponds to the observations in Table 2, as these different sizes of *Schizosaccharomyces* inocula at different stages of fermentation had no marked effect on the time for complete removal of malic acid. A possible tendency could be found between the inocula at 11°B, that the lower inoculum size had less detrimental effect on wine quality (Fig. 4). This tendency is, however, not forthcoming from the inocula at 1°B. This could possibly be explained by the length of time during which *Schizosaccharomyces* could grow and metabolize in the must. The *Schizosaccharomyces* inocula at 11°B were added on the second day after fermentation had been initiated by *Sacch. cerevisiae*, while the inocula at 1°B were added after the fourteenth day.

Several references are made to the fact that *Schizosaccharomyces* tends to be overgrown by the faster fermenting *Saccharomyces* at lower temperature (Dit-

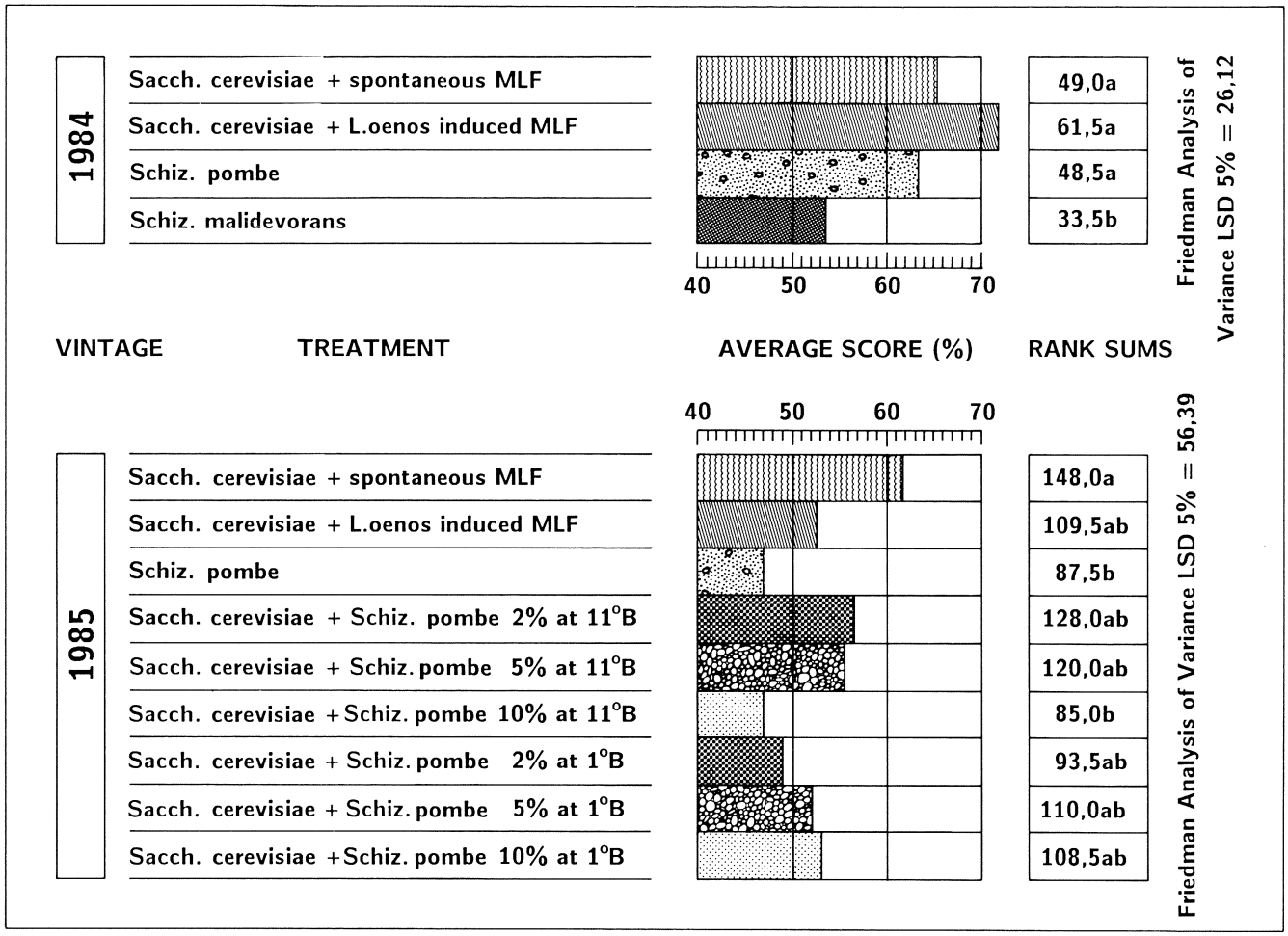

FIG. 4
Effect of different deacidification treatments on wine quality of Cabernet Sauvignon must of two vintages. Rank sums followed by different letters indicate significant differences.

trich, 1963a; Beelman & Gallander, 1979). One could therefore deduce that *Schiz. pombe* had not grown to the same extent as the initial inoculum prior to alcoholic fermentation.

It should also be borne in mind that Cabernet Sauvignon, in contrast to Chenin blanc wines, can mask a small amount of malodorous character. Carre *et al.* (1983) also referred to an attempt to reduce adverse organoleptic effects of *Schizosaccharomyces*, by addition towards the end of alcoholic fermentation.

Chenin blanc 1985: Considering our observations in the 1984 Cabernet Sauvignon experiment, where the *Schiz. pombe* treatment did not produce a wine significantly poorer to that of the *Schiz. malidevorans*, we compared the use of both *Schiz. pombe* and *Schiz. malidevorans* to *Sacch. cerevisiae* in Chenin blanc must. It is important to note that the wines were acidified 24 hours prior to judging (see Materials & Methods). This was done to ensure that any sensory differences would not be due to lower acidity or higher pH. For the wines fermented by *Schiz. pombe* and *Schiz. malidevorans*, the amount of DL-malic acid added was 2,6 g/*l* and 4,2 g/*l* respectively. This is also an added indication of the effectiveness of malic acid removal at the different temperatures. *Schiz. malidevorans* was included in the white wine quality experiment to determine possible differences *inter alia* with *Schiz. pombe.* As is evident from Fig. 5, there was no significant difference in wine quality at 15°C. The *Sacch. cerevisiae* wine was significantly better than the two *Schizosaccharomyces spp.* wines. At 21°C, however, *Schiz. malidevorans* produced a significantly poorer wine than *Schiz. pombe*, whereas the *Sacch. cerevisiae* wine was significantly better than the *Schiz. pombe* wine. The higher fermentation temperature (21°C) illustrates the detrimental effect of *Schiz. malidevorans'* metabolism versus that of *Schiz. pombe.* Although H_2S was not determined quantitatively, none of the judges commented on the presence of any H_2S or sulphury aroma in any of the wines. This contrasts with the findings of H_2S produced by *Schiz. malidevorans* previously reported (Rankine & Fornachon, 1964; Rankine, 1966).

Quantitative gas chromatographic analysis of volatile wine constituents in the Chenin blanc wines (Table 3) indicated that, at 15°C, (except for ethyl lactate values), *Saccharomyces cerevisiae* produced higher concentrations of ethyl and acetate esters as well as higher alcohols than both *Schiz. pombe* and *Schiz. malidevo-*

rans. The most marked difference was the higher iso-amyl acetate concentration produced by *Saccharomyces cerevisiae* at both temperatures.

Ethyl lactate values for both *Schiz. pombe* and *Schiz. malidevorans* at 15°C indicate bacterial metabolism of malic acid, but according to Zeeman, Snyman and Van Wyk (1982), it is doubtful whether this would have had a considerable effect on wine bouquet in view of the odour threshold in wine, as well as its rather neutral smell. Although a particular aroma property can only rarely be associated with a specific ester (Nykänen, 1986), an explanation for the higher sensory quality of *Saccharomyces cerevisiae* wine at 15°C (Fig. 5) could possibly be found in the overall higher values for the measured bouquet substances, as well as specifically the high iso-amyl acetate concentration (Table 3).

Van der Merwe and Van Wyk (1981), who investigated the contribution of fermentation products to the odour of dry white wines, found that addition of certain acetate esters, as a group, to de-aromatized wine caused a highly significant improvement to the odour. The single biggest difference between these Chenin blanc wines seems to have been the lower production of iso-amyl acetate and to a lesser extent hexyl acetate by the *Schizosaccharomyces* yeasts. The production of ethyl octanoate, which is believed to be a key fermentation volatile (Van der Merwe & Van Wyk, 1981), was also higher by *Sacch. cerevisiae* at 15°C than by the *Schizosaccharomyces* yeasts. The overall low production of volatile substances by *Saccharomyces* at 25°C may well have been the reason for the low sensory score.

Carre *et al.* (1983), reported on the analysis of groups of volatile bouquet substances and found that *Schiz. pombe* produced much lower concentrations of all these groups (including acetate esters) at 19°C than

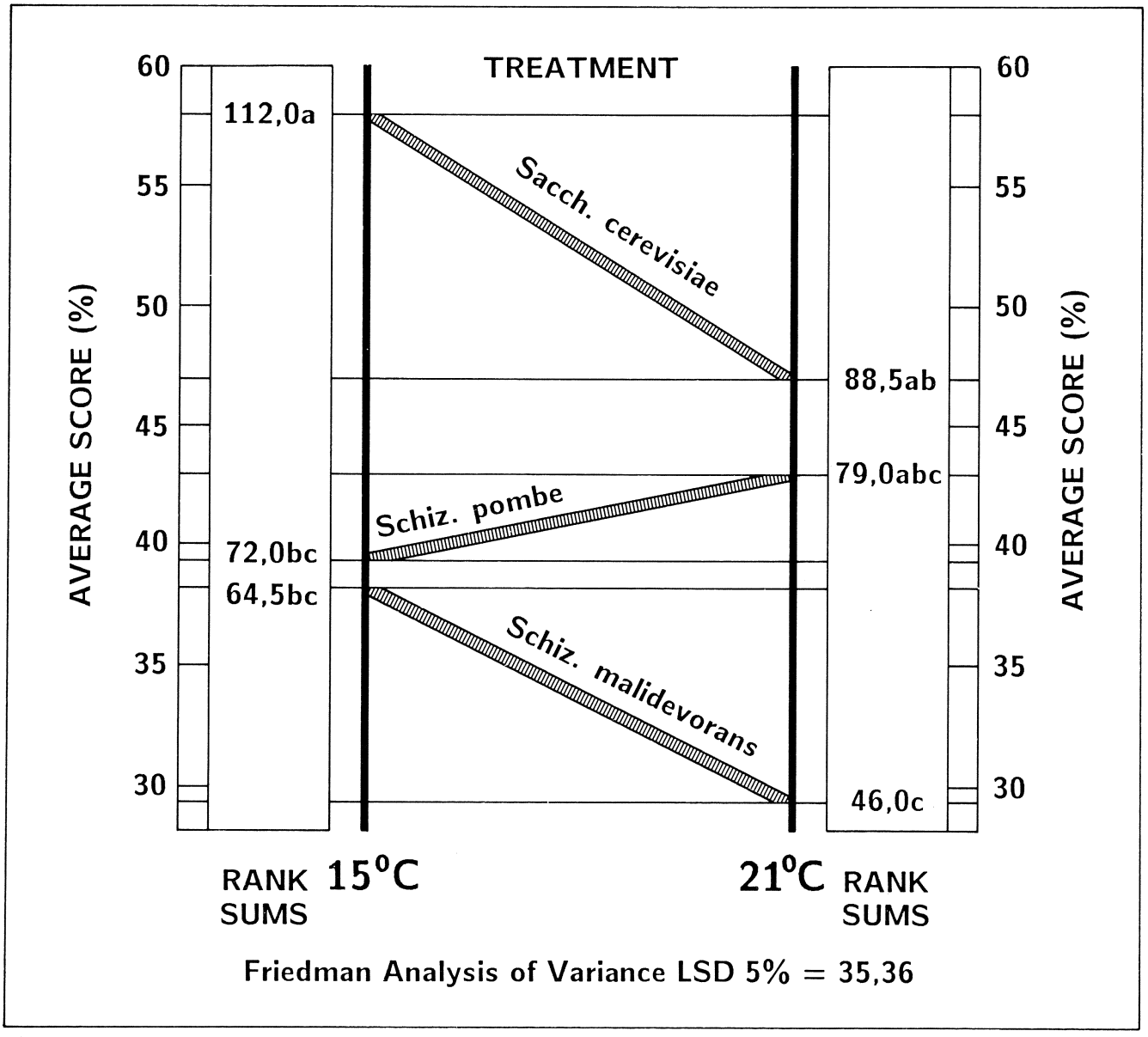

FIG. 5
Effect of yeasts and fermentation temperature on the quality of Chenin blanc wine. Rank sums followed by different letters indicate significant differences.

TABLE 3

Effect of yeast and temperature on the production of volatile constituents (μg/*l*) during fermentation in Chenin blanc must.

Volatile constituents	Sacch. cerevisiae 15°C	Schiz. pombe 15°C	Schiz. malidevorans 15°C	Sacch. cerevisiae 21°C	Schiz. pombe 21°C	Schiz. malidevorans 21°C
Ethyl hexanoate	710.4	758.9	672.1	505.9	687.4	606.7
Ethyl lactate	2.7	2931.9	3123.0	665.1	163.8	526.0
Ethyl octanoate	1302.4	1217.7	1128.2	692.0	997.9	940.7
Ethyl decanoate	208.8	170.6	287.9	143.6	260.6	580.7
i-Amyl acetate	1417.7	202.8	232.1	1087.7	185.2	483.3
Hexyl acetate	153.5	113.2	11.7	91.3	10.5	29.0
Phenethyl acetate	654.8	1093.3	690.1	475.5	904.0	1262.7
i-Butyl alcohol	946.9	773.1	385.0	1196.8	686.1	1620.5
n-Butyl alcohol	22.2	20.1	13.0	31.1	19.0	48.1
i-Amyl alcohol	39290.6	24770.4	25908.8	36181.6	28098.0	31555.3
Hexanol	1348.6	1751.8	1810.8	713.0	1926.5	1744.5
Phynethyl alcohol	11837.1	13786.9	7878.8	7352.7	11959.0	11400.4
Butyric acid	509.6	341.9	237.2	268.6	220.4	309.2
Hexanoic acid	3864.3	4351.5	3846.0	2596.1	4319.2	3439.1
i-Valeric acid	399.8	397.0	310.3	219.6	318.3	286.5
Octanoic acid	6841.8	7031.4	5416.9	3838.3	6212.1	5455.0
Decanoic acid	2332.7	2188.3	1860.5	1261.8	1973.2	1664.2
Dodecanoic acid	74.8	69.6	114.6	44.2	100.0	62.6

Sacch. cerevisiae, but did not present any sensory evaluation data. Results of this study confirm these observations.

CONCLUSIONS

The retarding effect of low temperature on the alcoholic fermentation ability of *Schizosaccharomyces pombe* and *Schiz. malidevorans* was such, that growth of naturally occurring malolactic bacteria could commence. Because of the natural microflora occurring on red grapes which are harvested and fermented at relatively high temperatures, bacteria are always potentially active unless specific preventative measures are taken. In such mixed cultures both types of organisms metabolize the malic acid present in the must. It would therefore depend on growth limiting factors such as temperature, acidity and initial inoculum size to what extent one organism would be able to dominate the other.

Although *Schiz. pombe* seemed to be inhibited by very high concentrations of L-malic acid, even at a fixed pH of 3,42, its effectivity would depend on growth conditions. *Schiz. malidevorans* was most effective in deacidifying low and high L-malic acid concentrations in Chenin blanc musts.

It would seem as if the inability of the *Schizosaccharomyces* yeasts to produce greater quantities of key fermentation bouquet compounds, could be the major contributing factor to the low sensory quality of such wines.

If fermentation and deacidification is planned with the use of *Schizosaccharomyces* yeasts, it can be concluded that at the high fermentation temperatures required for complete deacidification, the wine quality will be adversely affected in comparison to the use of *Sacch. cerevisiae* together with induced MLF by a selected strain of *L. oenos*.

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