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preliminary communication

Influence of the Production Yeast Strain on the Development of Malolactic Fermentation in White Wine

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Summary

The aim of the research was to study the influence of the hybrid strain of Saccharomyces 12233 × 6167, its parents – Sacch. bayanus 12233 and Sacch. cerevisiae 6167 – and the control strain Sacch. cerevisiae 220 on the growth of lactic bacteria in white wine. A number of winemaking cycles with three samples of must from white grape of typical Sicilian and Calabrian cultivars were carried out without the addition of SO_2. At the end of fermentation the wines were clarified and bottled, both with and without the addition of SO_2. The wines were stored at 15–20 °C for 90 days. The wines showed different levels of malic acid degradation as influenced by their ethanol content, the yeast strain used as a starter, and the levels of residual SO_2. The results demonstrate that the wines produced by the Sacch. cerevisiae strains were essentially unable to inhibit the start of malolactic fermentation, except when 80 mg/L of SO_2 were added to the wines. On the other hand, all the wines produced by the Sacch. bayanus 12233 effectively prevented the growth of lactic bacteria with just 40 mg/L of SO_2 and, for one cultivar, also without the addition of SO_2. The wines produced by the hybrid strain of Saccharomyces had an intermediary behaviour; therefore, with a low addition of SO_2, this strain stabilises white wines and prevents an excessive production of acids. This system of white wine microbiological stabilisation reduces SO_2 and offers considerable advantages for the health of the consumer.

Keywords: Sacch. bayanus, Sacch. cerevisiae, hybrid yeast, white wine, malolactic fermentation

Introduction

White wine is not produced by fermentation on the skins, and its tannin and extract content is rather low. This may make it more susceptible to the growth of lactic bacteria. The problem of the instability of white wine because of malolactic fermentation is more complex in wines from warm regions, where the malolactic fermentation is frequently undesirable as it results in a reduction of total acidity and fruity flavours *(1)*.

Malolactic fermentability of wines differs according to the strain of *Saccharomyces cerevisiae* used *(2)*. Thus it is possible to obtain microbiological stabilisation of wines by using *Sacch. cerevisiae* strains which guide winemaking and produce substances, such as succinic acid *(3)* and 2-phenylethanol *(4)*, that are capable of hindering bacterial growth.

Cryotolerant strains of *Sacch. bayanus, sensu (5)*, produce high amounts of these two substances. At present they are employed as starters in winemaking of acid deficient musts *(6)*. These yeasts, however, are not often used in winemaking, because they excessively increase titratable acidity.

An alternative could be offered by hybrid strains obtained via spore-conjugation of a cryotolerant strain of *Sacch. bayanus* and a non-cryotolerant strain of *Sacch. cerevisiae*. Different trials with these yeasts have shown that they exhibit an intermediary behaviour in the production of the secondary compounds of fermentation, including those responsible for the inhibition of malolactic bacteria *(7)*.

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The object of the research was to study the influence of these strains on the development of malolactic fermentation in white wine.

Experimental Part

Material and Methods

The hybrid strain of *Saccharomyces* 12233 × 6167, its parents – *Sacch. bayanus* 12233 and *Sacch. cerevisiae* 6167 – and the control strain of *Sacch. cerevisiae* 220 were employed. The first generation sterile hybrid strain 12233 × 6167 was obtained via spore-conjugation as described *(8)*. The identification of the *Sacch. bayanus* 12233 was authenticated by genomic comparisons *(9)*.

Three samples of must from white grape of typical Sicilian (*Catarratto* and *Grecanico*) and Calabrian (*White Malvasia*) cultivars of *Vitis vinifera* were utilised. The musts had the following composition: *Catarratto* – sugar 150 g/L, pH = 3.34, titratable acidity 6.60 g/L as tartaric acid, malic acid 2.87 g/L; *Grecanico* – sugar 180 g/L, pH = 3.23, titratable acidity 6.37 g/L, malic acid 1.40 g/L; *White Malvasia* – sugar 223 g/L, pH = 3.72, titratable acidity 4.30 g/L, malic acid 2.23 g/L.

The yeasts were cultured at 25 °C in 200 mL of pasteurised grape must. After 3 days, the precultures were inoculated into 4 L of each grape must, without the addition of SO_2. Spontaneous fermentation of the musts was also performed (*control*). Winemaking was carried out in duplicate at 20–25 °C and its progress was measured by determining residual sugar until the value of 1 g/L was reached. At the end of fermentation the wines were clarified by adding 300 mg/L of bentonite and 30 mg/L of gelatine and immediately analysed. Sugar, pH, titratable acidity, ethanol, total SO_2, and free SO_2 were determined by the usual methods. Malic acid, succinic acid, and acetic acid were tested, using specific Boehringer kits on diluted samples. Then the wines were poured into 750 mL bottles, both with and without the addition of 40 or 80 mg/L of SO_2. Each trial was carried out in duplicate. The bottles were topped up with sterilised liquid paraffin and stored at 15–20 °C for 90 days. At the end of that time residual malic acid and SO_2 were determined.

Results

The three grape musts produced fifteen wines, by regular fermentation that exhausted sugar within 12–25 days. Their analytical profile is reported in Table 1.

The cryotolerant 12233 and the hybrid 12233 × 6167 strains produced wine with higher levels of titratable acidity. This was due to their high contents of malic and succinic acid, while the quantities of acetic acid, compared with those produced by the normal strains or by spontaneous fermentation, were lower. Consequently, also the pH was lower. The cryotolerant and the hybrid strains produced less ethanol than both spontaneous fermentation and the normal strains. We conducted fermentation without the addition of SO_2, and so the level of this antiseptic was practically zero. Only the wines produced by the normal strains had medium-low quantities of SO_2, due to its production by yeasts during fermentation. We did not find measurable amounts of free SO_2 in any of the 15 wines examined. On the whole, the wines obtained through the hybrid strain 12233 × 6167 were more balanced and therefore were preferable for their analytical profile.

The wines in storage showed different levels of malic acid degradation as influenced by their ethanol content, the yeast strain used as a starter, and the levels of residual SO_2. Even if the decrease in malic acid concentration may not be entirely due to malolactic fermentation, this parameter is a useful reference point in establishing the microbiological stability of wines.

Fig. 1 highlights the malic acid degradation as influenced by the ethanol content in the wines, grouped by grape cultivar, after 90 days of storage without the addition of SO_2. In all the wines produced from the *Catarratto* grape must, malic acid was entirely degraded, probably because of their low ethanol content, which varied from 9.42% (volume fraction) of the cryotolerant strain to 9.92% (volume fraction) of the normal strain 220. Four wines produced from the *Grecanico* grape must completed malolactic fermentation; their ethanol content oscillated from 11.53% (volume fraction) of the hybrid strain to 11.89% (volume fraction) of the normal strain 220. On the other hand, the wine produced by the cryotolerant strain, despite its lower ethanol content (11.23% volume fraction), maintained almost 50% of residual malic acid. The wines produced from the *White Malvasia* grape must by the cryotolerant and the hybrid strains (respectively with 13.41 and 14.14% (volume fraction) of ethanol) showed excellent microbiological stability: they possessed over 90% of the original level of malic acid. However, malic acid was almost entirely degraded in the wines produced by the normal strains and by spontaneous fermentation, despite their higher ethanol content

Table 1. Analytical profile of the wines produced by different yeast strains from *Catarratto* (*Cat.*), *Grecanico* (*Grec.*), and *White Malvasia* (*W. Mal.*) grape musts.

Strain	12233			12233 x 6167			6167			220			Control		
Cultivar	Cat.	Grec.	W.Mal.	Cat.	Grec.	W.Mal.	Cat.	Grec.	W.Mal.	Cat.	Grec.	W.Mal.	Cat.	Grec.	W.Mal.
titratable acidity/g L^{-1}	9.32	8.63	9.33	8.65	8.56	8.12	7.70	7.54	5.65	7.05	6.78	4.60	7.23	7.20	6.34
γ (malic acid)/g L^{-1}	2.96	1.87	3.60	2.95	1.79	2.61	2.88	1.37	1.56	2.60	1.23	1.29	2.55	1.02	1.06
γ (succinic acid)/g L^{-1}	0.79	1.30	2.45	0.58	1.23	2.25	0.40	0.52	1.42	0.35	0.66	1.16	0.39	0.73	1.35
γ (acetic acid)/g L^{-1}	0.30	0.25	0.26	0.35	0.26	0.32	0.32	0.25	0.52	0.35	0.26	0.50	0.43	0.38	0.68
pH	3.27	3.25	3.49	3.31	3.28	3.56	3.33	3.30	3.68	3.32	3.34	3.80	3.38	3.28	3.67
φ (ethanol)/% (vol. fract.)	9.42	11.23	13.41	9.85	11.53	14.14	9.90	11.87	14.66	9.92	11.89	14.70	9.83	11.54	14.55
γ (total SO_2)/mg L^{-1}	7	8	5	3	7	3	20	30	14	15	20	10	4	8	3
γ (free SO_2)/mg L^{-1}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

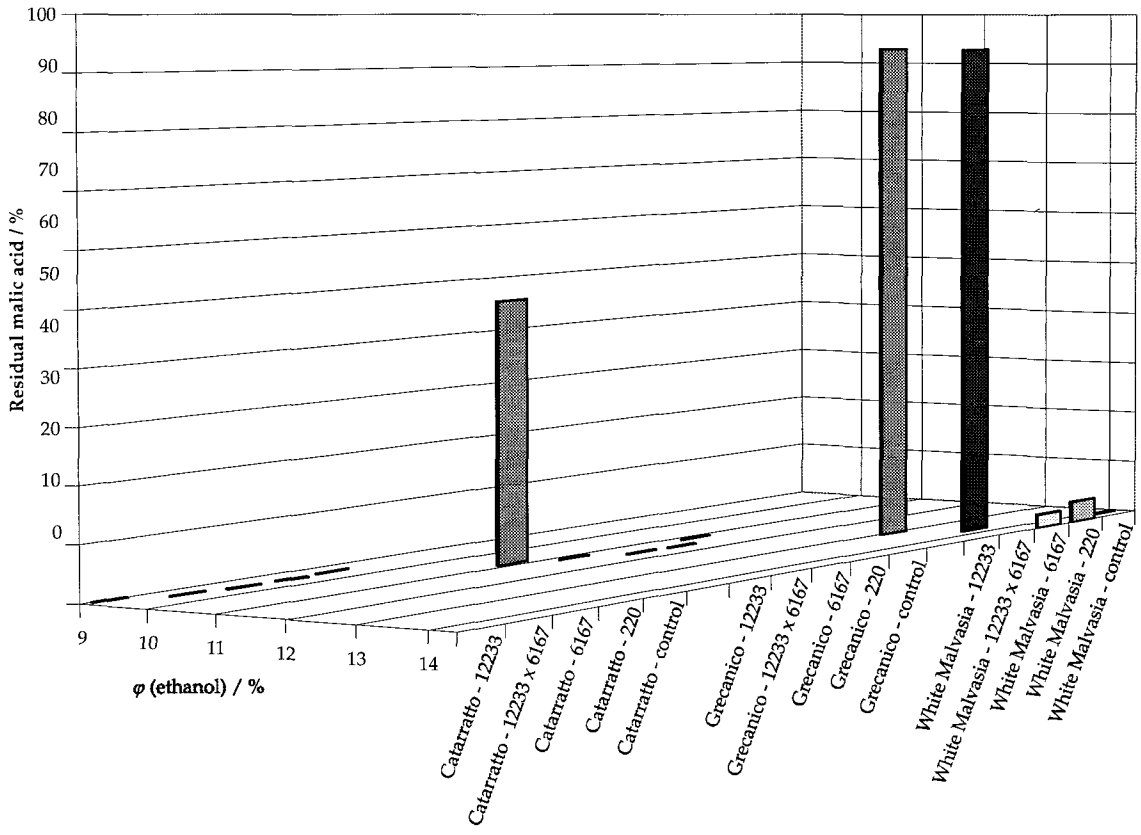

Fig. 1. Decrease of malic acid, after 90 days of wine storage without the addition of SO_2, as influenced by the ethanol content of each wine. (The wines are grouped by grape cultivar.)

which varied from 14.55% (volume fraction) of the spontaneous fermentation to 14.70% (volume fraction) of the normal strain 220.

Fig. 2 highlights the malic acid degradation in the wines, as influenced by the yeast strain used as a starter; every wine was stored for 90 days either without the addition of SO_2, or after the addition of 40 or 80 mg/L SO_2. All the wines produced by the cryotolerant strain 12233 and stored after the addition of 40 or 80 mg/L SO_2, were well stabilised as regards the growth of lactic bacteria. For one cultivar (*White Malvasia*) the wine stored without the addition of SO_2 also remained stable; for another cultivar (*Grecanico*) the wine stored without the addition of SO_2 remained partially stable. The same behaviour was observed in wines produced by the hybrid strain 12233 × 6167, except for the wine produced from the *Grecanico* grape must stored without the addition of SO_2, which completed malolactic fermentation. The wines produced by the normal strains 6167 and 220 required the addition of 80 mg/L of SO_2 to prevent malolactic fermentation; stored in all other ways they showed different levels of malolactic activity. Finally, all the wines produced by spontaneous fermentation displayed a more or less intense malolactic activity and often complete malolactic fermentation.

Fig. 3 highlights the malic acid degradation as influenced by the levels of residual SO_2 after 90 days of storage, the wines being grouped according to the quantity of SO_2 added. The wines stored without the addition of SO_2 showed residual levels of the antiseptic, from 0 to 12 mg/L, due to its production during fermentation. The wines stored after the addition of 40 mg/L of SO_2 showed residual levels which varied from 20 to 30 mg/L. Finally, the wines stored after the addition of 80 mg/L of SO_2 showed levels from 40 to 70 mg/L. In general, the lower levels of residual SO_2 were observed in the wines produced by the cryotolerant strain and by spontaneous fermentation; the higher levels were observed in the wines produced by the normal strains. The hybrid strain always gave intermediate levels. No wine had measurable amounts of free SO_2.

The stability of the wines produced by the normal strains or by spontaneous fermentation depends strongly on the level of residual SO_2. However, in the wines produced by the cryotolerant and the hybrid strains the stability depends on other factors, that allow the wine to inhibit the start of malolactic fermentation, also at low or zero levels of residual SO_2.

Discussion

We noted that, in various winemaking cycles of musts inoculated with *Saccharomyces sensu stricto* strains, malolactic fermentation sometimes did not start *(10)*. This inhibition was produced by several cryotolerant strains of *Sacch. bayanus*, despite the inoculation of the wine with lactic bacteria and the addition of only 50 mg/L SO_2. The wines thus obtained were more stable with regards to chemical oxidation, and this contributed to a long-term maintenance of freshness and to a notable

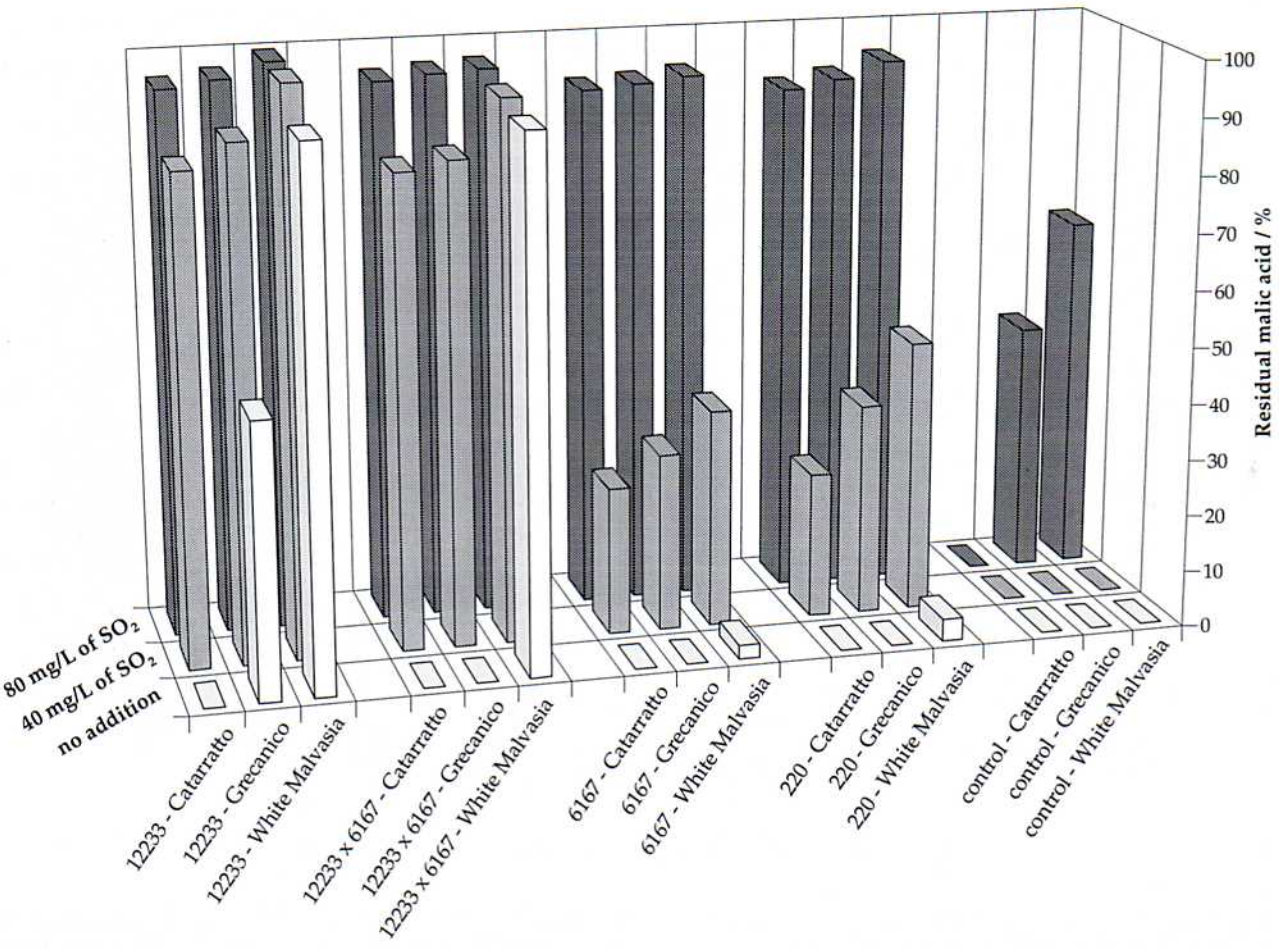

Fig. 2. Decrease of malic acid, after 90 days of wine storage either without the addition of SO_2, or after the addition of 40 or 80 mg/L SO_2, as influenced by the yeast strain used as a starter. (The wines are grouped by yeast strain.)

limitation of ageing. These are two extremely important properties for white wines. The enhanced stability of the wines obtained through cryotolerant strains is probably related to the high production of metabolites, such as succinic acid and 2-phenylethanol, which inhibit the growth of lactic bacteria *(11)*.

In these trials, the three grape musts tested, despite the absence of SO_2 during the winemaking, produced wines that display a different degree of stability, influenced by the ethanol level, the strain of yeast used as a starter, and the quantity of SO_2 added before storage. Wines produced from *Catarratto* and *Grecanico* musts can be stabilised with low levels of SO_2 by using cryotolerant yeast strains in the winemaking. Whereas, wines produced from *White Malvasia* musts can be stabilised by using the hybrid strain of *Saccharomyces* 12233 × 6167. This yeast is preferable since it gives an excellent stabilisation of the wine, even without the addition of SO_2 in winemaking and storage, and prevents an excessive production of acids.

Conclusion

The results demonstrate the influence of the production yeast strain on the development of malolactic fermentation in the conditions used. That is, white wines produced by the *Sacch. cerevisiae* 6167 and 220 were essentially unable to inhibit the start of malolactic fermentation, except when at least 80 mg/L of SO_2 were added to the wines. On the other hand, white wines produced by the *Sacch. bayanus* 12233 effectively prevented the growth of lactic bacteria with just 40 mg/L of SO_2 and, for one cultivar, also without the addition of SO_2. The wines produced by the hybrid strain of *Saccharomyces* 12233 × 6167 had an intermediate behaviour, therefore, with addition of low amounts of SO_2, this yeast too can stabilise white wines.

This system of white wine microbiological stabilisation reduces SO_2 and offers considerable advantages for the health of the consumer. Indeed, the wines produced by the *Sacch. bayanus* 12233 and the hybrid *Saccharomyces* 12233 × 6167 strains have enhanced chemical and microbiological stability, and this contributes to a long-term maintenance of freshness and to a notable limitation of ageing.

Further investigations are needed to establish a correlation between the delayed malolactic fermentation, the production of specific compounds by the cryotolerant and the hybrid strains and their antibacterial action.

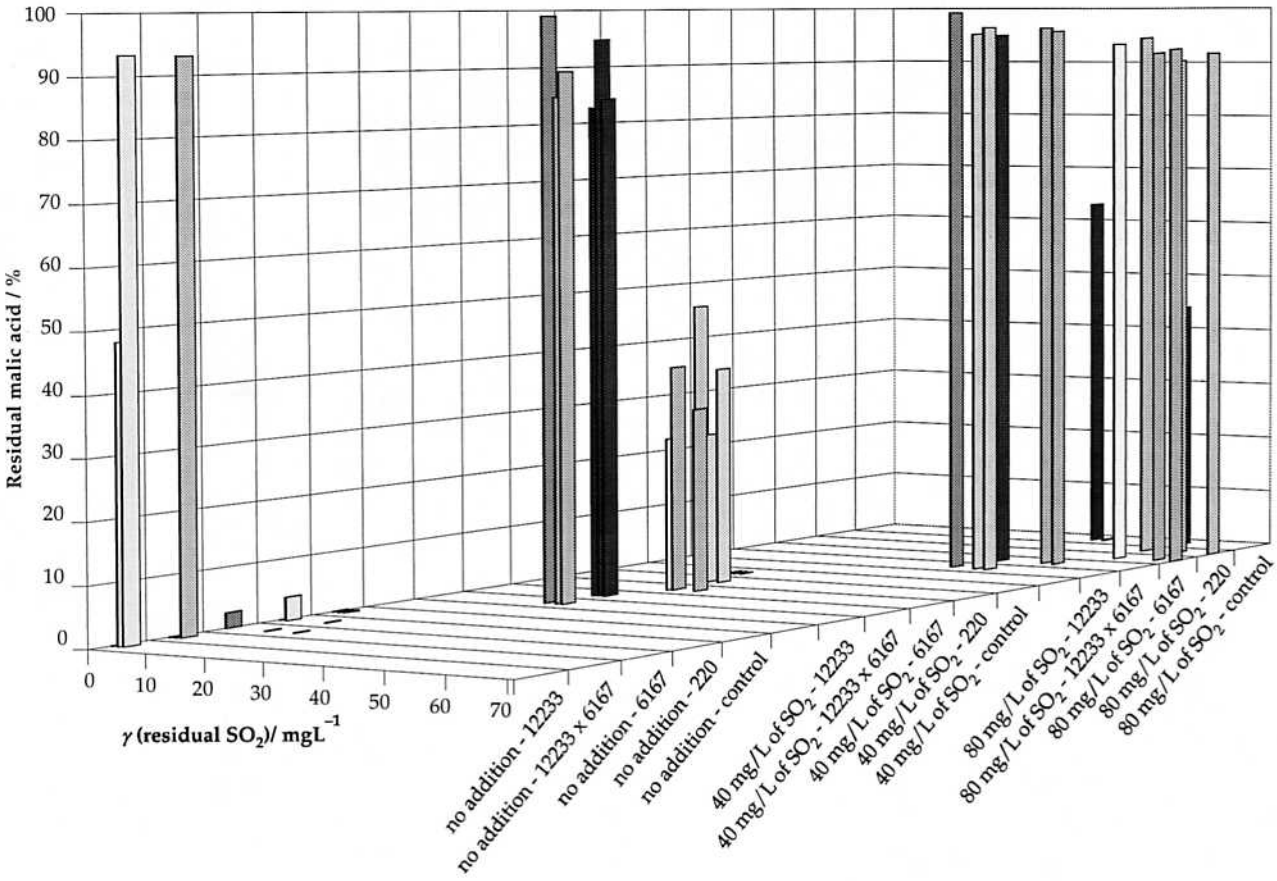

Fig. 3. Decrease of malic acid, after 90 days of wine storage either without the addition of SO_2, or after the addition of 40 or 80 mg/L SO_2, as influenced by the levels of residual SO_2. (The wines are grouped according to the quantity of SO_2 added before storage.)

References

1. M. A. Amerine, H. W. Berg, R. E. Kunkee, C. S. Ough, V. L. Singleton, A. D. Webb: *The Technology of Winemaking*, AVI Publishing Co., Westport (1980).
2. S. Lafon-Lafourcade, A. Lonvaud-Funel, E. Carre, *Antonie Van Leeuwenhoek, 49* (1983) 349–352.
3. L. C. Basso, D. M. G. Alves, H. V. Amorim, *11th Sinaferm. Anais Society Brazilian of Microbiology*, São Carlos (1996) pp. 1–6.
4. M. E. Guerzoni, F. Gardini, *Ind. Bev. 17* (1988) 239–245.
5. A. Vaughan-Martini, A. Martini, *Syst. Appl. Microbiol. 16* (1993) 113–119.
6. L. Bertolini, C. Zambonelli, P. Giudici, L. Castellari, *Am. J. Enol. Vitic. 47* (1996) 343–345.
7. L. Castellari, S. Franzoni, L. Bertolini, C. Zambonelli, *Ind. Bev. 25* (1996) 414–419.
8. O. Winge, O. Laustsen, *Compt. Rend. Trav. Lab. Carlsberg Sér. Physiol. 22* (1938) 235–244.
9. P. Giudici, C. Caggia, A. Pulvirenti, S. Rainieri, *J. Appl. Microbiol.* (1998) in press.
10. A. Caridi, V. Corte, R. Genova, *8th International Congress of Bacteriology, Applied Microbiology and Mycology Divisions*, Jerusalem (1996) p. 174.
11. A. Caridi, V. Corte, *Biotechnol. Lett. 19* (1997) 723–726.

Utjecaj proizvodnog soja kvasca na razvoj jabučno-mliječne fermentacije u bijelom vinu

Sadržaj

Svrha je rada proučavanje utjecaja hibridnog soja Saccharomyces 1233 × 6167 (čiji su roditelji Sacch. bayanus 12233 i Sacch. cerevisiae 6167) i kontrolnog soja Sacch. cerevisiae 220 na rast mliječnih bakterija u bijelom vinu. Proveden je određen broj ciklusa proizvodnje vina s tri uzorka mošta od bijelog grožđa tipičnih sicilijanskih i kalabrijskih sorti bez dodatka SO_2. Na kraju fermentacije vina su bistrena i punjena u boce, s dodatkom SO_2

i bez njega. Vina su čuvana pri 15–20 °C tijekom 90 dana. U vinima je došlo do različitog stupnja degradacije jabučne kiseline pod utjecajem alkohola u vinu, kvaščevog soja koji je upotrijebljen kao starter i razine preostalog SO_2. Rezultati pokazuju da vina proizvedena sa sojevima Sacch. cerevisiae ne mogu inhibirati početak jabučno--mliječnog vrenja osim ako je u vino bilo dodano 80 mg/L SO_2. S druge strane, sva vina proizvedena sa Sacch. bayanus 12233 uspješno su sprječavala rast mliječno-kiselih bakterija uz dodatak 40 mg/L SO_2, a u jednoj sorti grožđa čak i bez dodatka SO_2. Vina proizvedena s hibridnim sojem Saccharomyces omogućila su djelomičnu inhibiciju, a uz mali dodatak SO_2 stabilizirana su bijela vina i spriječena je prekomjerna proizvodnja kiselina. Taj sustav mikrobiološke stabilizacije bijelih vina snizuje količinu SO_2 i ima znatne prednosti što se tiče zdravlja potrošača.