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professional paper

Selection of *Saccharomyces sensu stricto* for Mead Production

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Summary

The aim of this work was the selection of yeasts of oenological origin, able to carry out alcoholic fermentation of honey musts. We studied over one hundred strains of Saccharomyces sensu stricto. A preliminary screening with must prepared by mixing three parts of water and one of citrus honey was performed. The intensity of growth and the fermentation activity were evaluated. From the results of this screening, the strains with better characteristics were selected to test their fermentation performance using a must at higher sugar concentration. The majority of the yeasts did not show the ability to grow in the honey must during preliminary screening. Many of those strains that passed the preliminary screening manifested some defects when tested for fermentation performance. For some fermentation characteristics, such as fermentation vigour and ethanol production, the strains showed remarkable differences, which were particularly useful in the subsequent selection. Four strains exhibited good general performance. The research will go on to employ the best strains for the production of meads on an experimental scale, with or without the addition of nutrients, and using different varieties of honey; the meads thus produced will be examined for their chemical, physicochemical and sensory profile.

Key words: Saccharomyces sensu stricto, mead production, fermentation performance

Introduction

The interaction between honey and yeast determines the chemical, physicochemical, and sensory characters of mead.

Honey: A lot of variable composition factors affect honey fermentation: glucose content, complexity of sugar blend, *pH*, total acid, total nitrogen, mineral salts, enzymes, volatiles. If honey with defects and/or alterations is utilized, it will give a product of poor quality. The same will be observed if the honey used has been subjected to thermic treatments. Among the different methods proposed to avoid the thermic treatment of honey destined for mead production, ultrafiltration appears interesting as it eliminates not only bacteria and yeasts, but all colloidal materials and some proteins as well *(1)*. However, the finest honey for meading will be fresh honey; the storage may produce deterioration of colour, flavour, and enzyme content. Finally, it is not advisable to use honey with high moisture content. This is important because all unpasteurized honeys contain wild yeasts *(2)*. Due to the high sugar concentration, osmosis forces these yeasts into dormancy in low moisture honey. Conversely, in honey that has a higher content of water, the yeast may survive and produce undesirable fermentation.

Yeasts: Yeast requires nitrogen in the respiratory phase of growth. Since honey is poor in nitrogen, fermentations without the adequate addition of nutrients are notoriously slow *(3)*. The fermentation rate is dependent on the variety of honey, but it is possible to increase the fermentation rate by using the proper yeast strain, mixing during fermentation, adding nutrients, and adjusting the *pH (4)*. Spontaneous fermentation of the honey must is unadvisable because the wild yeasts result in off-flavours, which the honey flavour is not strong enough to mask. Different selected yeasts are commercially available for mead production. Yeast used

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for sparkling wines may be employed to produce a dry mead with a high alcoholic content. Yeast used for white wines may be employed to produce a standard mead with an intermediate alcoholic content. Flor or ale yeasts may be employed to obtain sweet meads; their best performance is with honey musts at low sugar content. There are also some strains specifically selected for mead production, with different characteristics, that work well but rather slowly. The employment of selected yeasts for oenological use does not always guarantee the completeness and the effectiveness of the fermentation process. The honey, in fact, according to the variety used, has different compounds that hinder the development of many microorganisms *(5)*. Consequently, fermentations are often difficult or incomplete; this may increase the volatile acidity of the mead, due to the activity of different microorganisms, and, therefore, it could produce a mead of low quality and stability. In general, if the honey must has a low sugar content and has the proper nutrients added, any selected strain is able to grow; on the contrary, if the honey must has a high sugar content and does not have nutrients added, the majority of the yeasts are unable to control the alcoholic fermentation. In this case, therefore, it is useful to employ strains selected for high fermentation vigour in must without added nutrients and for high production of ethanol, in addition to the normally required characteristics for the control of alcoholic fermentations.

The aim of this work was the selection of strains of *Saccharomyces sensu stricto* able to carry out alcoholic fermentation of honey musts. The selection concerned high fermentation vigour in must without added nutrients and high production of ethanol, in addition to the normally required characteristics for the control of alcoholic fermentations. The selection of yeasts was narrowed to those of oenological origin, because yeasts typical of honeys are not usually sufficiently ethanol-tolerant.

Material and Methods

Yeast strains

Only strains of *Saccharomyces sensu stricto* were chosen for testing, excluding those with flocculent growth, foaming, or those belonging to *Sacch. cerevisiae* p.r. *capensis* and *aceti*, usually considered unable to control the fermentation process. One hundred and twenty-two oenological strains with the following characteristics were studied:

- 60 Calabrian and Sicilian strains of autochthonous *Sacch. cerevisiae* p.r. *cerevisiae;*
- 29 Calabrian and Sicilian strains of autochthonous *Sacch. cerevisiae* p.r. *chevalieri;*
- 13 Calabrian and Sicilian strains of autochthonous *Sacch. cerevisiae* p.r. *bayanus;*
- 5 Calabrian and Sicilian strains of autochthonous *Sacch. cerevisiae* p.r. *coreanus;*
- 2 Calabrian and Sicilian strains of autochthonous *Sacch. cerevisiae* p.r. *italicus;*
- 2 Calabrian strains of autochthonous *Sacch. cerevisiae* p.r. *uvarum;*
- 2 strains of hybrid *Saccharomyces*, kindly provided by the Department of Agro-Food Protection and Valorization of Reggio Emilia (DI.PRO. V.A.L.);
- 1 strain of *Sacch. bayanus*, kindly provided by the DI.PRO.V.A.L.;
- 6 strains of *Sacch. cerevisiae*, kindly provided by the DI.PRO.V.A.L.;
- 2 commercial strains for oenological use (*Sacch. cerevisiae* VL1, *Sacch. bayanus* Fermichamp).

Honey

For all trials, one sample of unpasteurized orange honey, kindly provided by the producer Carlo De Blasio from Palizzi Marina (RC) – Italia, was used.

Screening

A preliminary screening with must prepared by mixing three parts of water and one of orange honey was performed. The must obtained was distributed in test-tubes in quantities of 10 mL and autoclaved at 121 °C for 15 min, without addition of nutrient. Each tube contained a small inverted insert tube to collect CO_2 that may be formed. The test-tubes were inoculated with each strain and incubated at 25 °C for 5 days. The intensity of growth and the fermentation activity of the different strains were evaluated by determining, respectively, the absorbance at 520 nm and the CO_2 production into the inverted tube.

Table 1. Principal fermentation characteristics of the four selected strains compared with two commercial strains for oenological use (*Sacch. cerevisiae* VL1, *Sacch. bayanus* Fermichamp)

Strain	Fermentation vigour after 2 days $\gamma(CO_2)$ / g/100 mL	Fermentation vigour after 7 days $\gamma(CO_2)$ / g/100 mL	*pH*	Titratable acidity γ / g/L	Fixed acidity γ / g/L	Volatile acidity γ / g/L	Ethanol volume fraction / %
Sc46	1.25	4.50	3.15	4.20	2.64	1.25	11.20
Sc251	0.90	3.55	3.20	4.31	2.88	1.15	11.05
Sc2535	0.40	3.05	3.19	3.34	2.09	1.00	13.50
220	0.85	4.65	3.18	3.34	2.21	0.90	11.25
VL1	0.40	2.90	3.14	4.13	2.25	1.50	11.50
Fermichamp	0.55	3.40	3.19	3.41	2.41	0.80	9.75

Selection

From the results of this screening, the better strains were selected to test their most common fermentation characteristics *(6)*, using a must at higher sugar concentration (two parts of water and one part of orange honey). The must was distributed in flasks in quantities of 100 mL, 10 mL of liquid paraffin was added to avoid the surface coming into contact with oxygen, autoclaved at 121 °C for 15 min, and inoculated in duplicate with 5 mL of 48 h precultures. The fermentations were performed at 25 °C, at the same time determining the weight loss caused by CO_2 production. The mass (in grams) of CO_2 produced was used to express strain fermentation vigour after two days and after seven days. When the CO_2 production ceased (25-30 days), the fermentation was considered completed and the samples were refrigerated for 48 h at 4 °C and analysed for *pH*, titratable acidity (expressed in mass concentration, γ, of tartaric acid), volatile acidity, and ethanol, using standard methods *(7)*. The fixed acidity, expressed in mass concentration of tartaric acid, was calculated by difference between titratable acidity and volatile acidity. The selection, for each tested characteristics, was effected by exclusion of the worst strains.

Results

Preliminary screening: Only 76 of the 122 tested strains (*i.e.* 62.3 %) showed, according to the utilized substrate, acceptable levels of growth and fermentation activity, because the absorbance at 520 nm increased by at least 20 % and CO_2 production occupied at least 2 of the inverted tubes. The other 46 strains were, therefore, excluded from the fermentation tests.

Fermentation vigour after two days: The ability of a strain to start the winemaking (fermentation vigour) is, perhaps, the most important oenological trait for selection of yeasts for mead making. Effectively, since honey is poor in nutrients, it is difficult for the yeasts to grow. In this trial, many differences were found among the 76 tested strains: the values of CO_2 mass concentration $\gamma(CO_2)$ varied between 0.21 and 1.25 g/100 mL of must. Thirty-four strains showed values of $\gamma(CO_2)$ above 0.67 g/100 mL of must (average value). The most numerous frequency class was that where values of $\gamma(CO_2)$ varied between 0.41 and 0.60 g/100 mL of must (Fig. 1). The 38 strains included in the 1st and 2nd frequency classes did not pass this test.

Fermentation vigour after seven days: This parameter measures the strain's ability to complete fermentation and to consume sugars. In this trial, only 32 of the 76 tested strains showed fermentation vigour above the average value of $\gamma(CO_2)$ = 3.32 g/100 mL of must. Many differences were again observed, with values of $\gamma(CO_2)$ that varied between 1.90 and 4.65 g/100 mL of must. The most numerous frequency class was that where values of $\gamma(CO_2)$ varied between 3.01 and 3.50 g/100 mL of must (Fig. 2). The 50 strains included in the 1st, 2nd, 3rd, and 4th frequency classes did not pass this test.

pH and titratable acidity: In this trial, wines with *pH* values that varied between 3.01 and 3.39 (average value 3.18) were obtained. More than half of the strains (44) produced a *pH* that varied between 3.16 and 3.25 (Fig. 3). The 33 strains included in the last four frequency classes, with higher *pH* values corresponding to lower levels of titratable acidity, did not pass this test.

Fixed acidity: Since the fixed acid content of the honey must is very low, and it is useful to add tartaric, malic or citric acid, it is obviously preferable to use the strains that give a mead with medium-high fixed acidity, and to exclude those that produce lower values. The fixed acidity expressed as mass concentration of tartaric acid varied between 1.29 and 3.06 g/L; 34 strains exhibited a value above average (2.17 g/L). The most numerous frequency class was that where values varied between 2.01 and 2.25 g/L (Fig. 4). The 22 strains included in the 1st, 2nd, and 3rd frequency classes did not pass this test.

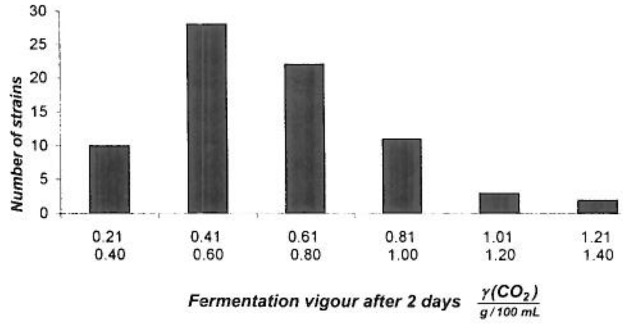

Fig. 1. Distribution of fermentation vigour after two days in tested strains

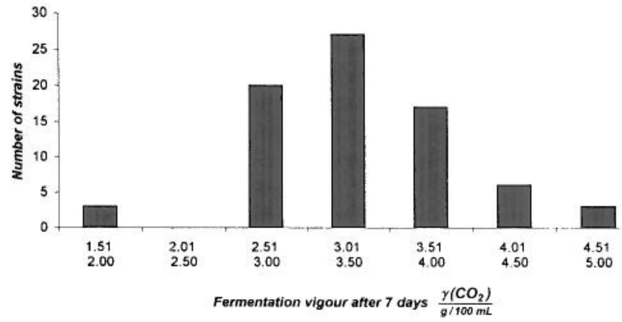

Fig. 2. Distribution of fermentation vigour after seven days in tested strains

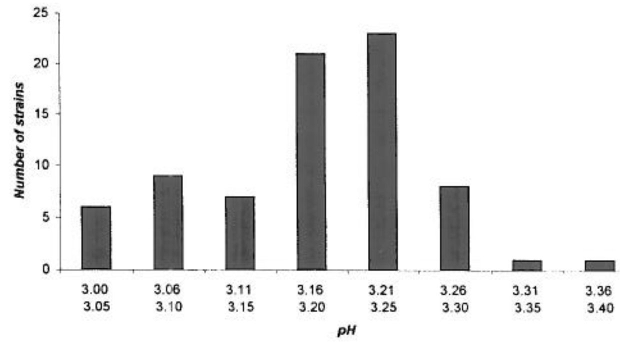

Fig. 3. Distribution of *pH* in meads fermented by tested strains

Volatile acidity: The production of acetic acid is strain specific, but depends on the composition of the substrate. The strains that produce high levels of acetic acid have to be excluded. In this trial the strains showed many differences: they produced from 0.80 to 2.40 g/L of acetic acid, with an average value of 1.37 g/L. The most numerous frequency class was the 3rd, with 26 strains (Fig. 5). On the whole, the rather high production of acetic acid probably depends on the choice of not adding nutrients to the honey must. In fact, it has been demonstrated in winemaking that drastic treatments to clarify the grape musts increase the production of acetic acid, probably because of the impoverishment of the substrate *(8)*. The 46 strains included from the 3rd to the 7th frequency classes did not pass this test.

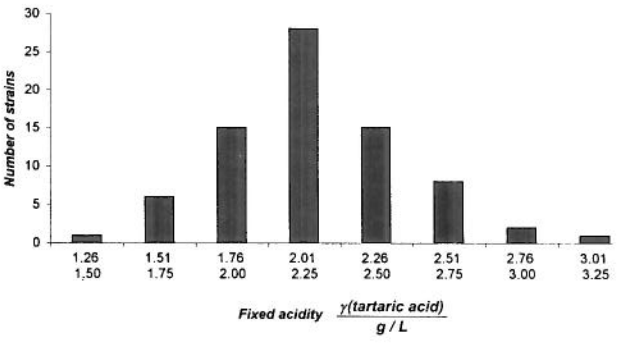

Fig. 4. Distribution of fixed acidity in meads fermented by tested strains

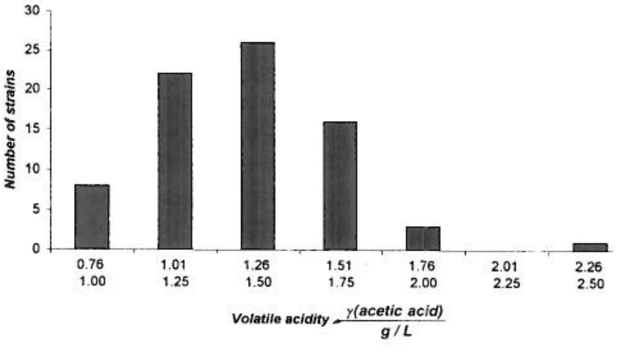

Fig. 5. Distribution of volatile acidity in meads fermented by tested strains

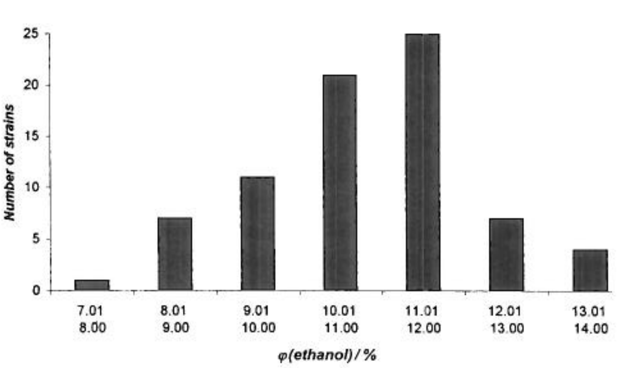

Fig. 6. Distribution of ethanol in meads fermented by tested strains

Ethanol: Forty-two strains had a value above the average of 10.80 % (volume fraction). An intense biodiversity is evident again, with variation of the alcoholic content from 7.20 to 13.50 % (volume fraction). The most numerous frequency class, with 25 of the 76 tested strains, was that where values varied between 11.01 and 12.00 % (volume fraction) (Fig. 6). The 40 strains included in the 1st, 2nd, 3rd, and 4th frequency classes that were able to produce a maximum of only 11 % (volume fraction) of ethanol did not pass this test.

Discussion

The selection shows, on the whole, a remarkable heterogeneity among the tested strains. This behaviour, that is index of phenotypical biodiversity, is particularly important for some characteristics, that are particularly useful for the selection. The majority of the yeasts did not show the ability to grow in the honey must during preliminary screening. Many of those strains that passed the preliminary screening manifested some defects when tested for fermentation performance. For some fermentation characteristics, such as fermentation vigour and ethanol production, the strains showed remarkable differences, which were particularly useful in the subsequent selection. Only three yeasts exhibited good general performance; to these strains was added the strain Sc2535, re-included for its excellent oenological characteristics, with the exception of the low fermentation vigour. It is important to remember that the fermentation vigour can be radically increased by the addition of proper growth factors to the honey must. The result of this selection is represented by these four strains. Their fermentation characteristics compared with those of two commercial strains for oenological use (*Sacch. cerevisiae* VL1 and *Sacch. bayanus* Fermichamp) are reported in Table 1. The 4 strains each have different characteristics:

1. *Sacch. cerevisiae* p.r. *chevalieri* strain Sc46 possesses a particularly high fermentation vigour;

2. *Sacch. cerevisiae* p.r. *chevalieri* strain Sc251 produces a mead with high content of fixed acids, which corresponds to a low *pH* value;

3. *Sacch. cerevisiae* p.r. *cerevisiae* strain Sc2535 gives a high production of ethanol; it must be used with addition of nutrients to the honey must, to improve its rather low fermentation vigour;

4. *Sacch. cerevisiae* p.r. *cerevisiae* strain 220 produces a mead with low content of volatile acidity.

The results obtained from this research confirm that it is important to carry out a specific choice of the strains to be employed for the production of mead. It may be preferable to perform a selection for each merceological category of honey, since, for each variety, there are different compounds that hinder microbial growth. The research will go on to employ the four selected strains for the production of meads on an experimental scale, with or without the addition of SO_2 and nutrients, and using different varieties of honey. The meads thus produced will be examined for their chemical, physico-chemical and sensory profile. These data will be compared with those obtained from the best known strains of selected yeasts available on the market.

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Odabir kvasaca *Saccharomyces sensu stricto* za proizvodnju medovine

Sažetak

Svrha rada bila je odabir kvasaca, ekološkog podrijetla, sposobnih da provedu alkoholno vrenje u razrijeđenom medu. Ispitano je preko stotinu sojeva Saccharomyces sensu stricto. Prethodni odabir proveden je s otopinom pripravljenom miješanjem 3 dijela vode i jednog dijela meda dobivenog od citrusa. Evaluirani su intenzitet rasta i fermentacijska aktivnost.

Na temelju dobivenih rezultata izabrani su sojevi s boljim osobinama kako bi se testirala njihova sposobnost fermentacije u otopini meda s većim udjelom šećera. Većina kvasaca nije bila sposobna rasti u otopini meda tijekom prethodnog odabira. Mnogi od sojeva koji su prošli prethodni odabir pokazali su neke nedostatke prilikom testiranja sposobnosti fermentacije. Uočene su neke značajne razlike u jačini vrenja i proizvodnji etanola kod pojedinih sojeva, što je bilo osobito korisno pri daljnjem odabiru. Četiri su soja imala dobra opća svojstva.

Autori će nastaviti daljnje istraživanje primjenom najboljih sojeva za proizvodnju medovine u eksperimentalnom mjerilu, s hranjivim dodacima ili bez njih, koristeći različite vrste meda. Tako dobivena medovina ispitat će se kemijski, fizikalno-kemijski i senzorski.