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A Methodological Approach to the Selection of *Saccharomyces cerevisiae* Wine Strains

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Summary

Yeast strains, producing different amounts of secondary compounds, exert a definite influence on the flavour and aroma of the wines and impart their characteristics. This suggests that the use of a single strain for different types of wines is not appropriate, due to a potential uniformity of aromatic characteristics in the final products. In order to typify each product for the varietal and geographic characteristics, it becomes necessary to isolate natural autochtonous strains, which, in addition to the desirable technological characteristics, exhibit a metabolic profile corresponding to each wine.

Thirty strains of Saccharomyces cerevisiae, isolated from different Aglianico grape cultivars, were tested for fermentation power, SO_2-resistance, Cu-resistance and the production of secondary compounds. The results for each strain were transformed into individual functions of desirability (d_i), i.e. dimensionless values between 0 and 1, and then combined to obtain a response of total desirability (D_{tot}). The form of the transformation was subjectively selected according to the level of knowledge of the desired optimal response. The strains were tested in Aglianico fermentations and only three showed a D_{tot} value higher than 0.7. By comparing D_{tot} values of selected strains with D_{tot} values of experimental wines, an evident correspondence was found. This demonstrates the value of the selection method utilised.

Keywords: strain selection, wine characteristics, metabolic profile, wine

Introduction

The recognition that formation of various pleasant compounds in wine is associated with certain yeasts *(1–5)* has stimulated numerous studies on the effect of yeast strain on wine organoleptic characteristics. Nowadays it is ascertained that yeast strains, producing different amounts of secondary compounds, impart specific and definite characteristics on the flavour and aroma of the wines *(6–8)*. Uncontrolled growth of yeasts can significantly alter the wine sensory properties, aroma and flavour, whereas the use of pure yeast cultures results in more predictable control of fermentation and quality *(9)*. The main advantages of wine fermentation inoculated with yeast starters are a more rapid and even rate of fermentation and more consistent quality *(10)*. Most active dry wine yeasts that are produced commercially are selected strains of the »true« wine yeasts. They belong to the species *Saccharomyces cerevisiae* and have been isolated by a particular winery. The use of these strains for different types of wines could be inappropriate, due to a potential uniformity of aromatic characteristics in the final product. Selected strains can be used as inoculum in wine fermentation only if the major characteristics of wine flavour remain essentially unchanged.

In order to characterise each product for the varietal and geographic characteristics, it is presumed more advantageous to use natural autochtonous strains, which, in addition to having the desirable technological characteristics, exhibit a metabolic profile corresponding to each wine. Thus, the choice of starter cultures may be more profitably based on the idea of typicity, *i.e.* the special wine produced comes from the special wine yeasts located on grapes of that particular vineyard.

The aim of this study was to establish a relationship between strains and wine individual characteristics in order to define a method oriented toward the selection of autochtonous strains.

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Materials and Methods

Choice of grape variety

Our study was concentrated on the typical grape variety of the Basilicata region, Aglianico of Vulture, which is mainly produced in a special geographical location, Venosa, where Orazio Flacco was born. Aglianico represents an ancient grape variety and gives a red wine of esteemed characteristics.

Strain isolation and identification

Samples of grapes were collected from 3 different vineyards located in the area of Aglianico production. In the vineyards, from ten vines per vineyard, 500 g of grapes per vine were collected in separate plastic bags. The grape clusters were chosen healthy and bird-damaged alike. The samples were transported to the laboratory and crushed under aseptic conditions in the original collection plastic bags. The musts obtained were transferred into sterile flasks and underwent spontaneous fermentation at room temperature. Appropriate dilutions of wines at the end of the fermentation process were plated on YPD agar for single colony isolation. The plates were incubated at 25 °C. From plates presenting well-isolated colonies, large numbers of yeast colonies were examined under the microscope, and five clones, tentatively recognised as *Sacch. cerevisiae*, were isolated at random from each sample, purified in YPD agar and maintained as stock cultures on YPD slants until analysis. Each clone derives from a single cell-colony.

Strain identification was carried out according to general methods *(11,12)*.

Strain characterisation

Strain resistance to sulphur dioxide and copper was tested directly by replica plating. Copper resistance was scored as the ability of a strain to grow on synthetic complete plates containing different amounts of added copper sulphate (100, 200, 300, 400, 500 μmol/L), whereas resistance to sulphur dioxide was tested in grape must agarised and added with different amounts of SO_2 (50, 75, 100, 125, 150, 175 ppm). The strain resistance to copper and sulphur dioxide was evaluated on the basis of positive growth after 24 h at 30 °C in comparison with a control without compound addition. The resistance degree of each strain was reported as minimal dose, which allowed growth.

To study the strain performance, as fermentation power and ability to produce secondary compounds of fermentation, red grape must from Aglianico cultivar of the Basilicata region (fermentable sugar 19%, pH = 3.15) was used. Strain fermentation vigour was evaluated as weight loss (CO_2 evolution) after 3 days of fermentation (Δp3). Fermentation power was measured as strain capacity to complete fermentation with exhaustion of sugars in ten days (Δp10).

Microvinification

The strains were tested for fermentation performance in flasks containing 100 mL of must with 50 mg/L of SO_2. The must samples were inoculated with 10^4 cell/mL of 48 h precultures grown in the same must. The flasks were plugged with a glass valve containing sulphuric acid to allow only CO_2 to evolve from the system *(13)* and incubated at 25 °C. The fermentation was followed by determining the weight loss caused by CO_2 production. The quantity (in grams) of CO_2 produced was used to express strain fermentation vigour after 3 days (Δp3) and strain fermentation power at the end of the fermentation (Δp10). When the CO_2 production ceased, the fermentation was considered completed and the samples were refrigerated for 2 days at 4 °C, racked and stored at –20 °C until analysis.

Analytical determination

Higher alcohols (n-propanol, isobutanol, active amyl alcohol, isoamyl alcohol), acetaldehyde, acetic acid and ethyl acetate were analysed by injection of 2 μL of fermented grape must into a 180 cm x 2 mm glass column packed with 80/120 Carbopack B/5% Carbowax 20M (Supelco). A gas chromatograph Varian (Vista 6000) equipped with a flame ionisation detector was used. The column was run from 60 °C to 198 °C at a rise rate of 5° min^{-1}. The carrier gas was nitrogen at a flow rate of 20 mL min^{-1}. Each sample was preloaded with n-butanol at a concentration of 100 mg L^{-1}.

Sugar concentrations in must and wine were determined by the method of total reducing sugars *(14)*.

Total desirability index

The study of the correspondence of metabolic and technological variables to ideal strain characteristics was carried out by a desirability-functions method.

The desirability method allows the association of different trends of the technological and metabolic parameters chosen into one response. By this procedure, in agreement with the results previously obtained *(15–17)*, the above-mentioned variables were transformed into individual functions of desirability (d_i), *i.e.* dimensionless values between 0 (very poor result) and 1 (extraordinarily good result). Transformations associated with desirability equations can be linear and non-linear *(15)*. The form of the transformation is arbitrary, selected according to the level of knowledge of the process and the desired optimal response. Individual d_i functions represent an external evaluation of the goodness of each response. Intermediate values of d_i are obtained thereafter by interpolations of the transformation functions. An arbitrary scale for d_i can be set as d>0.8 (excellent), 0.8–0.6 (good to acceptable), 0.6–0.4 (acceptable to fair), 0.4–0.3 (fair to poor), d<0.3 (poor to very poor).

A global index, total desirability, (D_{tot}) can be obtained as a geometric mean of individual functions of desirability (d_i):

$$D_{tot} = (d_1\ d_2\ d_3 \ldots\ldots d_n)^{1/n}$$

As individual desirability (d_i), D_{tot} values vary between 0 and 1. The observations with D_{tot} values close to 1 represent an optimal combination of the individual desirabilities (d_i).

Results and Discussion

In this study, in order to carry out the strain selection, technological variables (SO_2 and Cu^{2+} resistance, fermentation power and vigour, volatile acidity and methanol content) and metabolic variables (production of acetaldehyde, ethyl acetate, n-propanol, isobutanol, D-amyl alcohol, isoamyl alcohol) were chosen as selective parameters (Table 1). Real values of each variable were transformed into individual desirability values (d_i) by using specific desirability functions. For each strain, the individual desirability values were combined in a global index, D_{tot}. Strains with D_{tot} values close to 1 were considered as ideal strains, *i.e.* possessing potentially more suitable characteristics for Aglianico fermentation.

Individual d_i functions for technological variables

In order to evaluate the 30 strains for peculiar technological characteristics, each technological variable (SO_2 resistance, Cu^{2+} resistance, fermentation power, fermentation vigour, volatile acidity and methanol content) was transformed into a dimensionless index (function of desirability).

In agreement with literature data *(18–20)*, the individual desirability functions were parametrized as follows: the maximum of desirability for SO_2 resistance (1 value) was attributed to 175 ppm, 0.5 value to 75 ppm, and 0.15 value to 25 ppm; the maximum of desirability (1 value) for Cu^{2+} resistance was attributed to 500 μmol/L, 0.5 value to 100 μmol/L, and 0 value to 25 μmol/L. The maximum of desirability (1 value) for the fermentation power (Δp10 days) was attributed to 11.11 g, 0.5 value to 10 g, and 0 value to 9.24 g, whereas the maximum of desirability (1 value) for fermentation vigour (Δp3 days) was attributed to 6 g, 0.6 value to 4 g, and 0.4 value to 3 g. The maximum of desirability (1 value) for acetic acid concentration *(20)* was attributed to 200 ppm, 0.5 value to 700 ppm, and 0 value to 1200 ppm. According to the regulation *(21)* for methanol amount in wine the maximum of desirability (1 value) was attributed to 100 mg/L, 0.5 value to 175 mg/L, and 0 value to 250 mg/L. Individual functions of desirability for technological variables are reported in Table 2.

No differences were found for individual desirability of Cu^{2+}, Δp10 days, methanol and acetic acid: these variables exhibited values all close to maximum of desirability. As these parameters do not distinguish among strains, they were not taken into account in strain selection. On the contrary, strains showed differences in SO_2 resistance and fermentation vigour (Δp3 days). Consequently, only these two technological variables were taken into account in the D_{tot} construction.

Table 1. Strain selection: technological and metabolic variables for the 30 strains tested

	Technological variables					*Metabolic variables*						
Strains	SO_2 Resist. ppm	Cu^{2+} Resist. mmol/L $CuSO_4$	Δp3 days	Δp10 days	Acetic acid ppm	Methanol ppm	Acetaldehyde ppm	Ethyl-acetate ppm	N-propanol ppm	Isobut. ppm	D-amyl alcohol ppm	Isoamyl alcohol ppm
1EII6	150	0	5.19	10.39	1111	25.5	31.0	15.5	23.5	167.5	57.5	223.5
2EII8	150	100	5.38	10.83	913	25.5	30.0	12.5	28.5	166.5	63.5	255.0
5EII10	150	100	3.67	10.57	1624	21.0	29.0	19.0	29.0	60.0	35.0	240.5
7EIII10	150	0	5.09	10.70	797	28.0	31.0	16.0	24.0	219.0	66.5	255.5
8EIII11	150	100	5.09	11.11	671	27.0	33.0	13.5	29.0	170.5	72.5	275.0
9EIII1	150	100	3.31	9.43	1555	21.0	23.5	20.5	28.0	54.5	62.5	246.0
10EIII1	150	0	5.01	10.75	1178	24.0	35.0	15.0	28.0	196.5	78.0	281.0
11EIII6	100	100	5.07	10.43	1050	24.0	27.5	19.5	38.0	92.0	68.0	339.5
12EIII6	100	200	5.37	10.86	1123	22.5	31.5	15.5	27.5	50.5	54.5	228.0
13EIII10	100	100	5.03	10.62	770	25.5	33.0	16.5	26.5	45.0	46.0	202.5
16EIII5	100	100	4.74	10.02	983	25.5	21.0	13.0	21.0	44.5	49.0	234.5
18EIII3	125	0	5.11	10.69	1200	26.5	25.5	21.5	26.0	50.5	40.5	184.0
1LI3	100	0	5.02	10.23	1811	23.5	29.5	21.5	29.5	80.0	61.5	286.5
2LI6	125	100	4.53	11.04	1559	22.5	22.5	17.0	28.0	76.0	56.0	313.5
3LI1	100	100	4.51	10.71	1049	23.5	31.0	20.0	30.0	59.5	44.5	204.5
4LI5	150	100	4.27	10.65	1255	24.5	23.0	19.0	31.0	61.5	70.5	432.5
5LI4	125	100	3.62	10.49	1108	25.5	26.5	20.5	27.0	55.0	50.5	219.5
6LI4	150	100	4.32	10.55	1345	27.5	27.0	20.0	34.5	69.0	59.0	306.5
7LI3	150	100	4.53	10.75	635	20.0	36.5	12.5	20.0	114.5	59.5	251.5
8LI2	150	0	4.58	9.38	1161	23.0	25.0	23.5	30.0	106.5	63.5	327.0
9LI2	150	0	5.47	10.96	344	24.5	35.5	10.5	32.0	171.0	76.0	368.5
1LBI3	125	0	4.48	9.25	775	23.5	25.5	12.5	12.5	12.5	56.0	304.0
2LBI5	125	0	4.74	9.60	1516	22.0	23.5	19.0	19.0	19.0	74.5	373.0
3LBI9	150	200	3.43	10.32	1679	20.0	23.5	19.0	19.0	19.0	54.5	244.5
4LBI3	150	100	5.68	11.03	550	17.5	29.0	13.0	13.0	13.0	54.0	299.0
5LBI4	125	0	4.78	9.70	1247	18.0	20.5	14.5	14.5	14.5	62.0	288.5
6LBI	125	0	4.49	9.24	673	14.0	28.5	23.5	23.5	23.5	80.5	303.5
7LBI1	150	0	4.53	9.28	1050	18.5	20.5	20.5	20.5	20.5	71.0	322.5
8LBI3	150	100	4.43	10.55	845	19.0	26.0	16.0	16.0	16.0	81.0	287.0
9LBI2	150	100	5.71	10.96	426	26.0	30.5	15.5	15.5	15.5	59.0	327.0

Individual d_i functions for metabolic variables

Individual functions of desirability for metabolic variables are reported in Table 2.

In order to verify correspondence between strain metabolic profile and typical Aglianico wine profile, desirability functions for metabolic variables were defined.

Therefore mean, standard deviation and variability coefficient for acetaldehyde, ethyl acetate and higher alcohols (n-propanol, isobutanol, D-amyl alcohol, isoamyl alcohol) were calculated in 10 Aglianico wines (Table 3).

As shown in Table 3, ethyl acetate and n-propanol amounts varied considerably among the ten wines, with a high variability coefficient. It was supposed they were non-differentiating variables, and consequently considered not useful in characterising Aglianico wine. On the contrary, the amounts of acetaldehyde, isobutanol and amyl alcohols varied with a variability coefficient lower than 50%. Therefore these traits were assumed as characterising variables in strain selection program for Aglianico fermentation.

For these variables the maximum desirability (1) was attributed to the mean, 0.75 value to $x\pm\sigma$, 0.5 value to $x\pm2\sigma$ and 0.25 value to $x\pm3\sigma$.

Total desirability calculation and strain selection

Total desirability (D_{tot}) was obtained by geometric mean of individual desirability functions (d_i) for the following variables: SO_2 resistance, fermentation power, (as technological variables) and acetaldehyde, isobutanol, D-amyl alcohol and isoamyl alcohol production (as metabolic variables).

Table 4 shows real values for the variables selected, which represent the values for individual functions of desirability ($d_{variables}$) and D_{tot} values. In the thirty strains tested, D_{tot} values vary from 0.29 (poor value) to 0.75 (good value), with mean and standard deviation values respectively of 0.5 and 0.16. As shown in Table 4, only seven strains show a D_{tot} value close to 0.7 and among these three strains, 18EIII3, 3LI1, 4LBI3 (D_{tot}: 0.74, 0.75, 0.74), were selected and assumed as ideal strains for Aglianico wine fermentation.

Validation of the methodological approach

To verify the validity of the method utilised and to confirm the relationship between selected cultures and Aglianico wine characteristics, the ideal strains (18EIII3, 3LI1, 4LBI3) were tested in triplicate Aglianico fermentation. Individual desirability (d_i) and D_{tot} were calculated in the wine samples obtained with the selected strains. Using the previous results as a guide, the three selected strains were tested for d_{SO_2} and $d_{\Delta p3}$ as technological variables, and $d_{Acetald.}$, $d_{Isobut.}$, $d_{AmyL.}$, $d_{Isoamyl.}$ as metabolic ones. By applying the equations reported in Table 2, the technological and metabolic values of the selected variables were transformed into individual functions of desirability and then into total desirability for each strain and repetition. The results of this experiment are reported in Table 5.

The selected strains confirmed a good general performance and exhibited a stable behaviour with low variability in the expression of the variables chosen. In particular, the strain 4LBI3 yielded the highest value of D_{tot} (>0.80), emerging as the most suitable culture of this selection program. In addition, by comparing D_{tot} values of the three selected strains with D_{tot} values of experimental wines, an evident relation was found. This demonstrates the success of the selection method utilised.

Table 2. Individual desirability functions for transformation of technological and metabolic variables in d_i values

Variables	*Desirability functions*
Technological variables	
SO_2 resistance	$d_{SO_2} = 0.0058x^* + 0.0083$
Cu^{2+} resistance	$d_{Cu^{2+}} = 0.0022x^* + 0.0083$
$\Delta p3$	$d_{\Delta p3} = 0.2x^* - 0.2$
$\Delta p10$	$d_{\Delta p10} = 0.56x^* - 5.1239$
Methanol	$d_{Methanol} = -0.005x^* + 1.5$
Acetic acid	$d_{Acetic\ acid} = -0.001x^* + 1.2318$
Metabolic variables	
Acetaldehyde	$d_{Acetaldehyde} = -0.0005x^{2*} + 0.0454x^* - 0.2044$
Isobutanol	$d_{Isobutnol} = -0.0006x^{2*} + 0.0863x^* - 2.0908$
D-amyl alcohol	$d_{D\text{-}amyl\ alcohol} = -0.0013x^{2*} + 0.1211x^* - 1.9906$
Isoamyl alcohol	$d_{Isoamyl\ alcohol} = -0.00003x^{2*} + 0.0113x^* - 0.2768$

* = x represents real values of the technological and metabolic variables

Table 3. Mean, standard deviation and variability coefficient of metabolic variables measured in 10 Aglianico wines

	Acetald.	Ethyl acetate	N-prop.	Isobut.	D-amyl alcohol	Isoamyl alcohol
Average (ppm)	45.27	46.93	35	74.43	47.04	201.55
Standard deviation	20	24.14	20	10.63	7.45	50.57
Variability coefficient (%)	45.67	51.37	59	14.29	15.84	25.09

Table 4. Transformed (d_i) values and D_{tot} for selected technological and metabolic variables of the 30 strains

Strains	d_{SO_2}	d_{Ap3}	$d_{Acetal.}$	$d_{Isobut.}$	$d_{Amyl\ alcohol}$	$d_{Isoamyl\ alcohol}$	D_{tot}
1EII6	0.88	0.84	0.72	0.10	0.67	0.75	0.55
2EII8	0.88	0.88	0.70	0.10	0.46	0.65	0.50
5EII10	0.59	0.53	0.69	0.92	0.65	0.70	0.67
7EIII10	0.88	0.82	0.72	0.10	0.27	0.61	0.45
8EIII11	0.88	0.98	0.75	0.10	0.10	0.56	0.39
9EIII1	0.88	0.46	0.59	0.83	0.50	0.68	0.64
10EIII1	0.88	0.80	0.77	0.10	0.10	0.49	0.37
11EIII6	0.59	0.81	0.67	0.76	0.22	0.10	0.42
12EIII6	0.59	0.87	0.73	0.73	0.71	0.73	**0.72**
13EIII10	0.59	0.81	0.75	0.57	0.83	0.78	**0.71**
16EIII5	0.59	0.75	0.53	0.55	0.80	0.70	0.65
18EIII3	0.73	0.82	0.63	0.74	0.78	0.79	**0.74**
1LI3	0.59	0.80	0.70	0.97	0.54	0.50	0.66
2LI6	0.73	0.71	0.56	1.00	0.69	0.29	0.62
3LI1	0.59	0.70	0.72	0.91	0.82	0.77	**0.75**
4LI5	0.88	0.65	0.57	0.95	0.07	0.10	0.36
5LI4	0.73	0.52	0.64	0.84	0.81	0.76	**0.71**
6LI4	0.88	0.66	0.66	0.99	0.60	0.35	0.65
7LI3	0.88	0.71	0.78	0.10	0.60	0.67	0.52
8LI2	0.88	0.72	0.62	0.29	0.40	0.21	0.46
9LI2	0.88	0.89	0.78	0.10	0.10	0.10	0.29
1LBI3	0.73	0.70	0.62	0.67	0.68	0.36	0.61
2LBI5	0.73	0.75	0.58	0.21	0.10	0.10	0.29
3LBI9	0.88	0.49	0.58	0.94	0.74	0.68	**0.70**
4LBI3	0.88	0.94	0.69	0.94	0.75	0.42	**0.74**
5LBI4	0.73	0.76	0.52	0.89	0.50	0.49	0.63
6LBI	0.73	0.70	0.68	0.10	0.10	0.33	0.32
7LBI1	0.88	0.71	0.52	0.16	0.05	0.25	0.29
8LBI3	0.88	0.69	0.62	0.10	0.10	0.47	0.35
9LBI2	0.88	0.94	0.71	0.97	0.62	0.21	0.65

Table 5. Individual desirability and D_{tot} values for experimental wines

Strains	d_{Ap3}	$d_{Acetald.}$	$d_{Isobut.}$	$d_{Amyl\ alcohol}$	$d_{Isoamyl\ alcohol}$	D_{tot}
18EIII3	0.82	0.62	0.88	0.72	0.78	0.75
18EIII3	0.82	0.66	0.86	0.51	0.77	0.71
18EIII3	0.82	0.50	0.91	0.43	0.77	0.67
3LI1	0.70	0.58	0.94	0.55	0.77	0.66
3LI1	0.70	0.48	0.96	0.83	0.70	0.69
3LI1	0.70	0.55	0.96	0.74	0.77	0.71
4LBI3	0.94	0.70	1.00	0.66	0.77	0.81
4LBI3	0.94	0.70	1.00	0.83	0.75	0.84
4LBI3	0.94	0.74	1.00	0.75	0.75	0.84

Further studies will be conducted in order to verify the genetic stability in the selected strains for the characteristics which give the "typical imprinting" to Aglianico wine.

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Metodološki pristup selekciji vinskih kvasaca

Sažetak

Sojevi kvasaca, proizvodeći različitu količinu sekundarnih spojeva, bitno utječu na okus i aromu vina, određujući njihovu osobitost. To znači da nije dobro koristiti jedan soj za različite vrste vina zbog moguće jednolikosti aromatičnih značajki gotova proizvoda. Kako bi se tipizirao svaki proizvod prema varijetetskim i geografskim osobinama, potrebno je izolirati prirodne autohtone sojeve, koji osim poželjnih tehnoloških značajki imaju i metaboličke reakcije što odgovaraju svakom vinu. Ispitana je fermentacijska sposobnost, otpornost prema SO_2, Cu^{2+} te proizvodnja sekundarnih spojeva trideset sojeva Sacch. cerevisiae izoliranih iz grožđa različitih vinograda na području Aglianico. Rezultati, za svaki soj, transformirani su u pojedine funkcije poželjnosti (d_i), tj. bezdimenzionalne vrijednosti između 0 i 1, a zatim povezane kako bi se postigla ukupna poželjnost (D_{tot}). Oblik transformacije bio je subjektivno odabran u skladu s poželjnim optimalnim odgovorom. Sojevi su testirani fermentacijom, a samo su tri soja pokazala vrijednost D_{tot} višu od 0,7. Očita podudarnost utvrđena je uspoređivanjem vrijednosti D_{tot} eksperimentalnih vina i vrijednosti D_{tot} odabranih sojeva. To potvrđuje ispravnost odabranog postupka selekcije.