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Function of yeast species and strains in wine flavour

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Abstract

The diversity and the composition of the yeast micropopulation significantly contribute to the sensory characteristics of wine. The growth of each wine yeast species is characterized by a specific metabolic activity, which determines concentrations of flavour compounds in the final wine. However, it must be underlined that, within each species, significant strain variability has been recorded. The wide use of starter cultures, mainly applied to reduce the risk of spoilage and unpredictable changes of wine flavour, can ensure a balanced wine flavour, but it may also cause a loss of characteristic aroma and flavour determinants. Thus, the beneficial contribution from the yeast increases when starter cultures for winemaking are selected on the basis of scientifically verified characteristics and are able to complement and optimise grape quality and individual characteristics. Here we report the characterization of a large number of strains of different wine yeast species, isolated from spontaneous wine fermentations and included in the culture collection of the Basilicata University.

Keywords: Wine flavour; *Saccharomyces cerevisiae*; Non-*Saccharomyces* yeasts; Strain diversity

1. Introduction

Under the generic term of ‘‘wine’’, there is a diversity of quality which is quite unique among the products and determined mainly by interaction between grapes, yeasts and technology. Wine is a natural product resulting from a number of biochemical reactions, which begin during ripening of the grapes and continue during harvesting, throughout the alcoholic fermentation, clarification and after bottling. Many of these reactions are left to nature and microorganisms present on the grapes (Torija et al., 2001). The surface of the grapes contains a large variety of moulds, bacteria and yeasts. In particular, a great variety of yeasts are present on grapes, but only a minor portion can participate in alcoholic fermentation.

Apart from the principal wine yeast, *Saccharomyces cerevisiae*, spontaneous alcoholic fermentation of grape must is a complex process carried out by the sequential action of different yeast genera and species (Heard and Fleet, 1988), found on the grapes, in the must and in the wine, contributing to the flavour of wines (Lambrechts and Pretorius, 2000). The early stages of the alcoholic fermentation are dominated by the growth of non-*Saccharomyces* yeasts, characterized by a low fermentative power. Of these, *Hanseniaspora* (*Kloeckera)* and *Candida* (e.g. *Candida stellata*, *C. pulcherrima*) (Heard and Fleet, 1986) are more frequently the principal yeasts both in spontaneous and inoculated fermentation (Fleet et al., 1984;

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Heard and Fleet, 1985; Pardo et al., 1989). Their growth is significant and can influence the chemical composition of the wine. Sensitivity to ethanol, however, limits the growth of these yeasts to the first 2–3 days of fermentation and at ethanol concentrations above 5% to 6% (v/v) their growth declines rapidly (Margalith, 1981; Kunkee, 1984). Under these conditions, strains of *S. cerevisiae* and related species, which are more tolerant to ethanol and more competitive for growth in media with high sugar concentration (Querol et al., 1990), become the dominant yeasts and complete the process (Fleet and Heard, 1993). Other yeasts, such as species of *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulaspora*, *Zygosaccharomyces* and *Saccharomycodes*, may be present during the fermentation or ageing of the wine and sometimes, due to modifications in the fermentation parameters, they may dominate part of the process and affect adversely wine quality.

The non-*Saccharomyces* yeasts contribute to the fermentation since they can reach populations of about up 10^6–10^7 cells/ml, affecting both the kinetics of growth and metabolism of *Saccharomyces* (Lema et al., 1996). These yeasts are capable of anaerobic as well as aerobic growth and may persist during the fermentation, competing with *Saccharomyces* for nutrients, and may produce secondary compounds affecting the bouquet of the final wine.

The characterization of wine yeasts of different species for by-product formation has underlined that the yeast species itself is a prominent factor in determining the wine composition (Houtman et al., 1980; Benda, 1982; Herraiz et al., 1990; Romano, 1997; Brandolini et al., 2002). In particular, the characterization of *S. cerevisiae*, the principal actor of the winemaking, has revealed that, in addition to the production of ethanol, this yeast generates many secondary metabolites that are key determinants of wine quality (Fleet, 1990; Lema et al., 1996; Lambrechts and Pretorius, 2000; Fleet and Heard, 1993).

Over the years, much work has been devoted to investigating biochemical mechanisms, which today enables us to describe routes by which the flavour compounds in alcoholic beverages are formed. The successful use of sophisticated instrumental methods has shown that the flavour of alcoholic beverages is composed of a very large number of compounds. More than 1000 volatile compounds have been identified and of these, more than 400 are produced by yeasts during fermentation (Nykänen, 1986). The nature and concentrations of these end-products are determined by the yeast species that participate in the fermentation.

Wine flavour is composed by a wide variety of compounds with different aromatic properties. It includes flavour compounds originating from the grapes (varietal flavour), compounds formed during operations of extraction and conditioning of must (prefermentative flavour), other compounds produced by yeasts and bacteria during alcoholic and malolactic fermentation (fermentative flavour) and compounds that appear during the ageing process (post-fermentative flavour), as reviewed by Schreier (1979), Boulton et al. (1995), and Rapp (1998). Volatiles identified in wines are usually dominated by fermentation products, since these compounds are present in the highest concentrations. Therefore, conversion of grape sugars to alcohol and other end-products by specific yeast populations may yield wines with distinct organoleptic quality. The various yeast species and strains that develop during the overall fermentative process metabolise grape juice constituents, principally the sugars, to a wide range of volatile and non-volatile end-products, which influence and determine the types and concentrations of many products that contribute to the aroma and flavour characteristics of the wine. The major volatile products of yeast metabolism, ethanol and carbon dioxide, make a relatively small contribution to wine flavour. Conversely, organic acids, higher alcohols and esters and to a lesser extent acetaldehyde constitute the main group of compounds that form the "fermentation bouquet" (Rapp and Versini, 1991). When present in excess concentrations, these compounds may also be regarded as undesirable.

Here we report by-product formation in a large number of natural wine yeasts in order to emphasize the function of yeast species and strains in wine flavour.

2. Materials and methods

2.1. Yeasts

Strains of different wine species, collected during the years and belonging to the collection of the Basilicata University, were used. In total, 127 *S.*

cerevisiae, 84 *Hanseniaspora uvarum*, 25 *C. stellata*, 25 *Zygosaccharomyces fermentati* and 25 *Saccharomycodes ludwigii* were included. All strains were grown on YEPD (1% (w/v) yeast extract 2% (w/v) peptone, 2% (w/v) glucose; OXOID, Milan, Italy).

2.2. Micro-fermentation

To determine the capacity of yeast to produce secondary metabolites, different grape musts were used. Fermentations were carried out in 130-ml Erlenmeyer flasks filled with 100 ml of grape must, autoclaved at 100 °C for 20 min. Each sample was inoculated with 10^4 cells from 48-h pre-cultures in the same must. The grape must surface was covered with a thin layer of sterilized paraffin oil in order to avoid air contact. This practice avoids the evaporation of liquid and mass losses and the weight loss recorded is a function of the carbon dioxide evolved. The determination of weight loss is used as parameter to follow the fermentation process. The samples were incubated at 25 °C until fermentation was completed, then refrigerated for 1 day at 2 °C, racked and stored at − 20 °C until required for analysis.

2.3. Pilot scale fermentation

The experiments aimed to study the strain behaviour in the traditional vinification process were performed in the experimental cellar of the Basilicata University, using red grape must from Aglianico cultivar of the Basilicata region (19% (w/v) fermentable sugar, 0.70% (w/w) titratable acidity, pH 3.15). Fermentations were carried out in 1-hl tank inoculated with 10^4 cells/ml of 48-h pre-cultures grown in the same must. The fermentation was performed following the traditional technology used to produce the Aglianico wine. Commercial wines of Aglianico were collected before malolactic fermentation from different local cellars that had not used selected yeasts.

2.4. Analytical determinations

By-products (higher alcohols, acetaldehyde, ethyl acetate, acetic acid, acetoin) were determined by gas-chromatographic analysis as reported by Romano et al. (1998b).

2.5. Statistical analysis

Data about by-products underwent statistical analysis by ANOVA and descriptive Box and Whiskers plots, using the software ‘‘Statistica for Windows’’, version 5.0, 97 Edition (Statsoft).

3. Results and discussion

3.1. Effect of wine yeast species on wine flavour

More recently, wine makers and wine researchers have come to realize that also non-*Saccharomyces* yeasts contribute in significant measure to the flavour and quality of wine than previously thought (Romano et al., 1997a). This has led to the studying of the presence and evolution of these non-*Saccharomyces* yeasts on grapes and in must, for determining their potential effects on the organoleptic qualities of the final product. The differences in the composition of wines made from different yeast species appear to be quantitative rather than qualitative (Romano, 1997). The products of fermentation are usually identical, but the relative amounts of the various compounds differ between different yeast species.

Fig. 1 reports the metabolic profiles obtained for strains belonging to some of the most frequently encountered wine yeast species: *S. cerevisiae, H. uvarum, C. stellata, S. ludwigii, Z. fermentati*. It is seen that the profile exhibited by the predominant yeasts of the early fermentation phases, *H. uvarum* and *C. stellata,* is quite similar, being characterized by a high production of acetoin and ethyl acetate (higher in *C. stellata* than in *H. uvarum*) and a general low production of higher alcohols, whereas *S. cerevisiae* is characterized by high production of isoamyl alcohol and 2,3-butanediol and low production of acetoin. The profile of *Z. fermentati* is characterized by a general low production of the compounds considered, with only a high 2,3-butanediol production. *S. ludwigii*, a well-known wine spoilage yeast, can be considered a general high producer, in particular of acetoin, ethyl acetate, isoamyl alcohol and isobutanol. The metabolites formed by yeasts can be divided into two classes. In the first class, one can consider by-products formed with a minimum level of variation within each species, which are metabolites differentiating among the

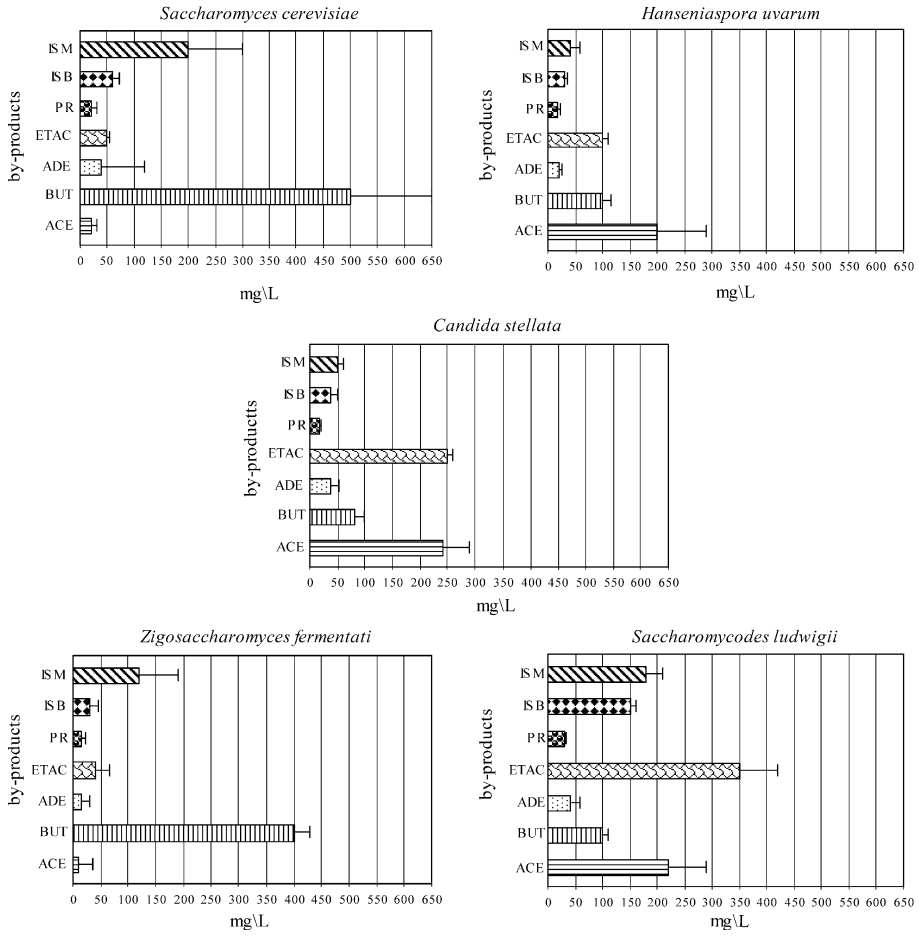

Fig. 1. Aromatic profile characterizing different wine yeast species: ISM = isoamyl alcohol; ISB = isobutanol; PR = *n*-propanol; ETAC = ethyl acetate; ADE = acetaldehyde; BUT = 2,3-butanediol; ACE = acetoin.

various species. As examples, ethyl acetate or the total content in higher alcohols can be mentioned. As another example, there is a correlation between acetoin and 2,3-butanediol, which are always produced by wine yeasts with an inverse pattern, i.e. yeasts producing high levels of acetoin always produce low levels of 2,3-butanediol and vice versa (Romano et al., 1998a, 2000).

Strong polymorphism has been observed within some species, with by-products formed with a high variability, resulting in an individual strain characteristic. These by-products can distinguish between strains of the same species and represent the second class of secondary compounds, that is by-products, which tend to be formed with a wider variability within the species. They will be described in the following examples.

3.2. Effect of wine yeast strain on wine flavour—S. cerevisiae

A strain diversity has been observed for *S. cerevisiae* wine yeasts isolated from a given fermentation: i.e. fermentations often comprise different strains of *S. cerevisiae* (Frezier and Dubourdieu, 1992; Querol et al., 1992; Schütz and Gafner, 1993; Polsinelli et al., 1996), all of which participate actively in the fermentation process and contribute to the chemical composition and sensory qualities of the resulting wine (Lurton et al., 1995). Collection of

S. cerevisiae wine strains from natural fermentations has demonstrated the existence of such strong polymorphism within this species (Cabrera et al., 1988; Giudici et al., 1990; Romano et al., 1993, 1994; Henschke, 1997; Pretorius, 2000) and it is widely accepted that *S. cerevisiae* strains, producing different amounts of secondary compounds, impart desirable or undesirable characteristics on the flavour and aroma of the wines.

Thus, the examination of 115 *S. cerevisiae* autochthonous strains, isolated from Aglianico del Vulture, a typical wine of Basilicata region (Southern Italy), emphasized the important role played by the *S. cerevisiae* strain in determining the final composition of the wine (Romano et al., 2002). Important variables for strain differentiation were the different levels of isobutanol, isoamyl alcohol, acetaldehyde and acetic acid production.

Fifty-two *S. cerevisiae* strains, isolated from different grape varieties, were characterized for the production of secondary compounds in Aglianico of Vulture grape must. Fig. 2 shows the behaviour of the strains for the production of *n*-propanol, isobutanol and isoamyl alcohol, acetaldehyde, ethyl acetate and acetic acid. The strains exhibited a low variability in the levels of *n*-propanol, acetaldehyde and ethyl acetate, whereas the other compounds were formed with significant strain variability. In this study, the main variables for the differentiation of the strains were isobutanol and isoamyl alcohol. As regards acetic acid, all the strains produced less than 600 mg/l, that is considered the threshold value of acceptability in wine (Romano, 1990).

These results lead to consider the production level of these compounds as an individual strain characteristic and underline the importance of characterizing strains for industrial purpose.

3.3. Effect of wine yeast strain on wine flavour—*H. uvarum*

The apiculate yeasts represent often the dominant microbial population of the early stages of grape must fermentation. Of these yeasts, *H. uvarum* (*Kloeckera apiculata*) is the most frequently encountered species, which can survive longer and can grow to maximum populations of 10^6–10^7 cell/ml. Even if the growth of these yeasts is limited only to the first 2–3 days of fermentation, the activity of *Hanseniaspora* (*Kloeckkera*) wine strains can influence significantly the chemical composition of the final wine (Romano et al., 1992, 1993, 1997a,b; Comi et al., 2001). In contrast to the species *S. cerevisiae*, the non-*Saccharomyces* yeasts have revealed the capability to produce and secrete several enzymes (esterases, β-glucosidases, proteases, etc.) in the medium, thus interacting with grape precursor compounds to produce aroma active compounds and consequently playing an important role in varietal aroma (Charoenchai et al., 1997). These findings have stimulated an increasing interest of wine researchers towards these yeasts with the aim to identify the differences between strains in the production of potentially detrimental or beneficial flavour-active compounds.

Fig. 3 reports the data regarding by-products formed in inoculated fermentations by 59 strains of *H. uvarum* strains of wine origin. By analysing the figure, it is possible to note a significant strain variability for the compounds determined, with the exception of isobutanol, which is very similar in all

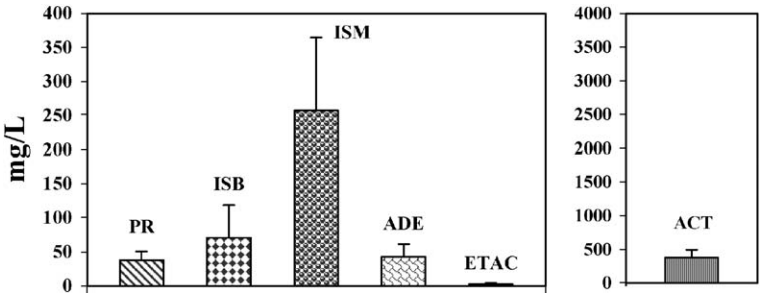

Fig. 2. By-products formed by *S. cerevisiae* wine strains in grape must fermentation: PR = *n*-propanol; ISB = isobutanol; ISM = isoamyl alcohol; ADE = acetaldehyde; ETAC = ethyl acetate; ACT = acetic acid. □ Mean, ⊤ S.D.

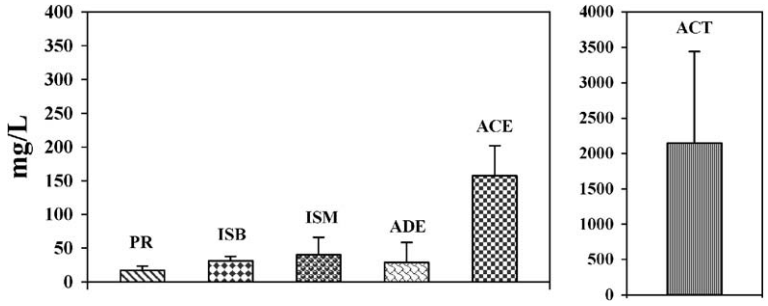

Fig. 3. By-products formed by *H. uvarum* wine strains in grape must fermentation: PR = *n*-propanol; ISB = isobutanol; ISM = isoamyl alcohol; ADE = acetaldehyde; ACE = acetoin; ACT = acetic acid. □ Mean, ⊤ S.D.

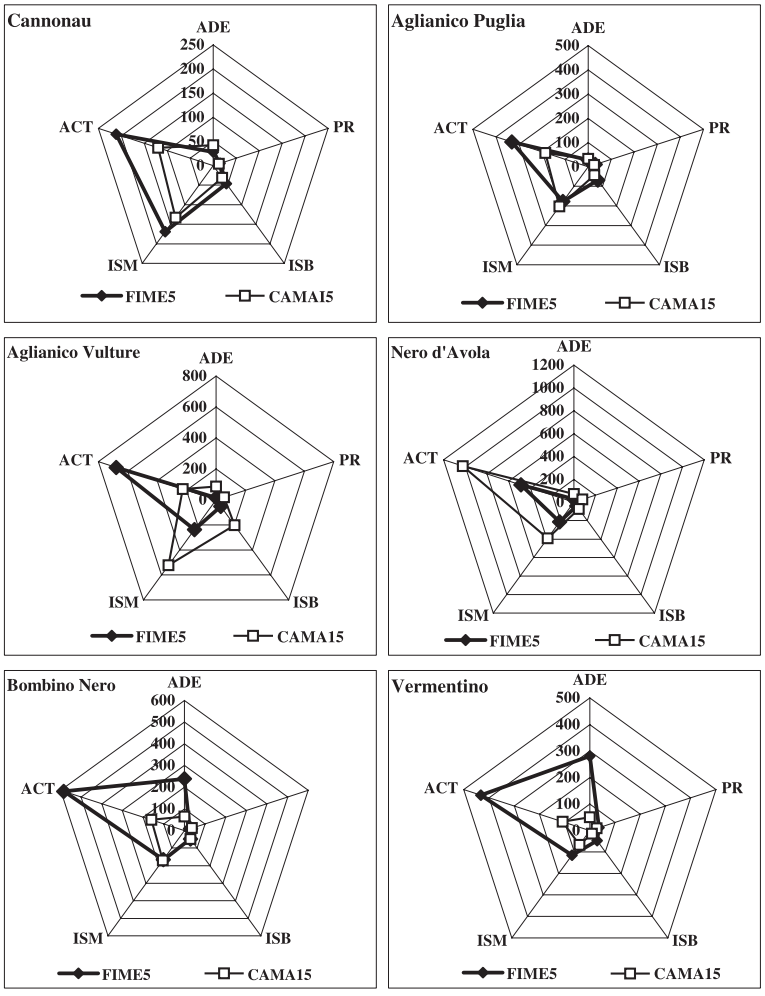

Fig. 4. Metabolic profiles of experimental wines in function of the grape must and strain of *S. cerevisiae*. By-products: ISM = isoamyl alcohol; ISB = isobutanol; PR = *n*-propanol; ADE = acetaldehyde; ACT = acetic acid; *S. cerevisiae* strains: FIME5, CAMA15.

the strains tested. Of particular interest is acetic acid, which was produced with a considerable variability, from about 0.6 g/l until more than 3.4 g/l. The high production of acetic acid is recognized as a common pattern in apiculate yeasts and for this characteristic, they have been considered for long time as spoilage yeasts.

The large variability among *H. uvarum* wine strains, with amounts less than 1 g/l acetic acid produced by some strains, allows the identification of strains suitable to be used as starter cultures in mixed fermentation with *S. cerevisiae*.

Although differences in acetic acid production may be expected between different media, depending on sugar concentration, nitrogen source and pH, it must be stressed that strains of *H. uvarum* (*K. apiculata*) forming low amounts of acetic acid in different grape musts have been found (Comi et al., 2001). A positive interaction with *S. cerevisiae* has been reported for *H. uvarum* in several studies (Herraiz et al., 1990; Zironi et al., 1993; Romano, 2002).

3.4. Interactions between S. cerevisiae strains and grape must

The beneficial contribution of yeast becomes more significant when starter cultures for winemaking are able to optimise grape quality. During wine fermentation, yeast metabolic activity operates at two different levels: by producing new aromatic compounds and by transforming aromatic precursors present in the grape must. In grape must, there are certain compounds which are transformed to aromatic compounds only by yeast metabolic pathways. In fact, it is demonstrated that by fermentation of grape must of different origin, the same yeast produces different

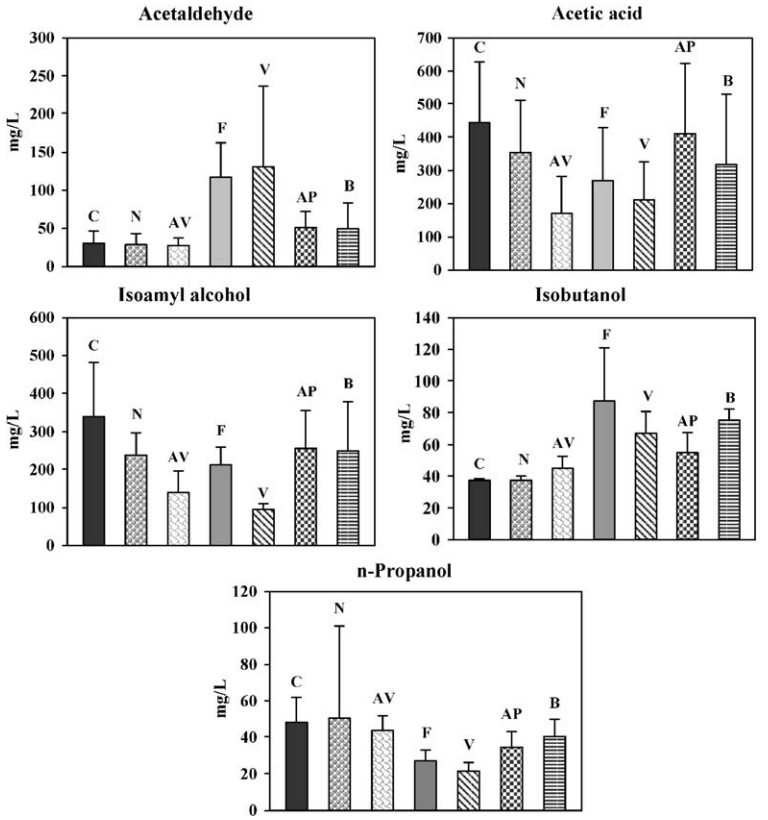

Fig. 5. Variability in *S. cerevisiae* strains for the formation of by-products in seven different grape musts (C = Cannonau; N = Nero d'Avola; Av = Aglianico del Vulture; F = Fiano; V = Vermentino; AP = Aglianico Puglia; B = Bombino Nero). □ Mean, ⊤ S.D.

wines in consequence of the qualitative and quantitative differences in the grape must composition (Delfini and Bardi, 1993).

In order to evaluate the effect of grape must on the production of secondary metabolites, fermentations were performed by inoculating grape musts of different varieties (Cannonau, Aglianico Puglia, Aglianico Vulture, Nero d'Avola, Bombino Nero, Vermentino) with two *S. cerevisiae* strains. The determination of the levels of matabolies showed significant differences in the wines obtained (Fig. 4). Such differences were recorded also in the case of the same grape variety, but of different regional origin ("Aglianico Vulture" and "Aglianico Puglia").

The same experiment was performed for 20 *S. cerevisiae* strains in musts of different grape varieties to obtain more information on strain variability. The strains exhibited remarkable differences of metabolites (Fig. 5). The strains exhibited a similar behaviour for the production of *n*-propanol and isobutanol in the majority of musts, with the exception of Nero d'Avola (*n*-propanol) and Fiano (isobutanol), whereas a considerable strain variability was determined for acetic acid production in all the grape musts.

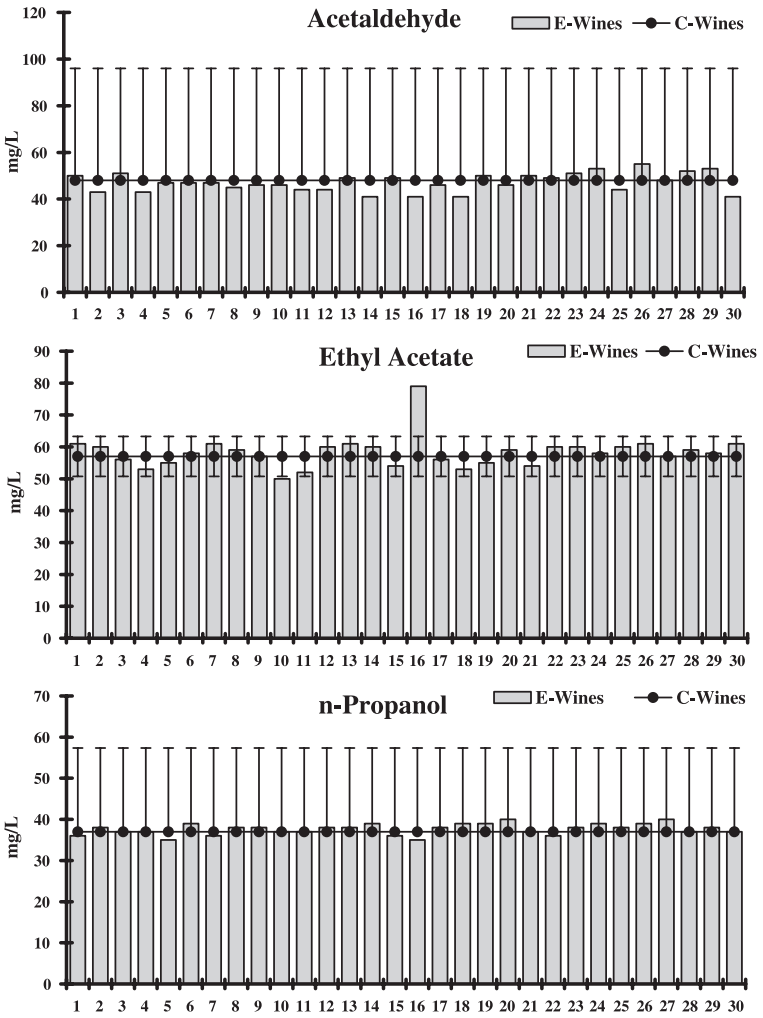

Fig. 6. Comparison of the concentrations in some secondary compounds between experimental wines, produced by thirty selected *S. cerevisiae* strains (E-Wines), and commercial wines of Aglianico (C-Wines: —●— mean ± S.D.).

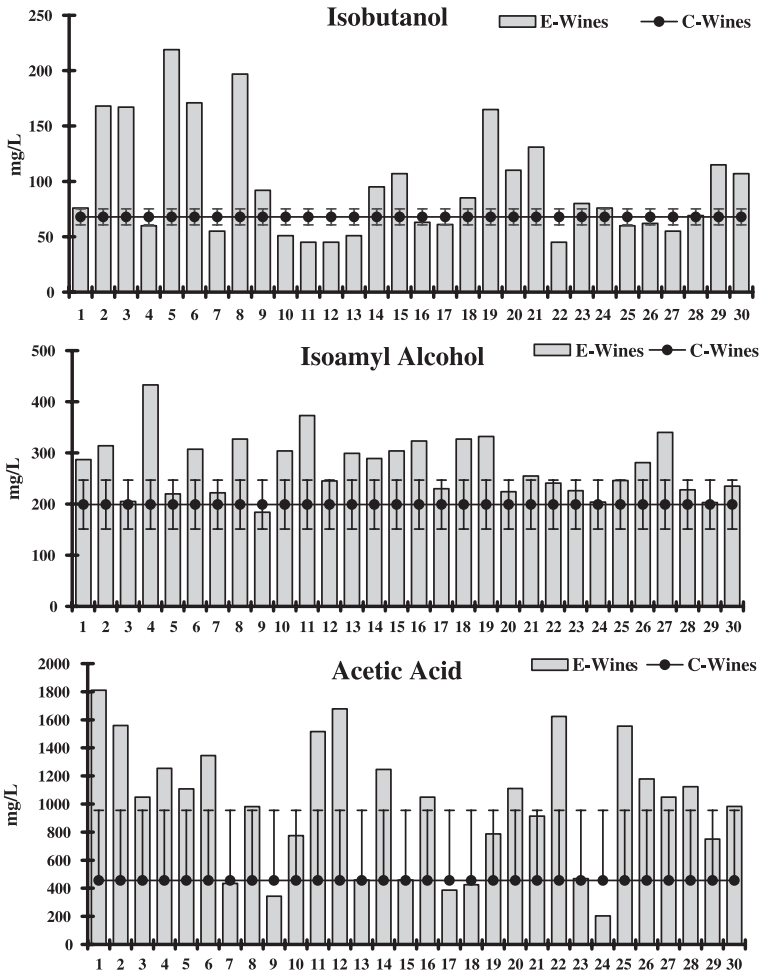

Fig. 6 (*continued*).

By the metabolic profiles described above, it is documented that the final quality of the wine is the result of the interaction between the yeast and the grape must composition. This should be considered when selecting a strain of *S. cerevisiae* for a particular grape variety and must composition.

3.5. Comparison between experimental and commercial wines

The use of commercial strains of *S. cerevisiae* is becoming a common practices in winemaking. This practice ensures a reproducible product, reduces the risk of wine spoilage and allows a more predictable control of fermentation and quality (Henick-Kling et al., 1998). However, the use of pure yeast cultures could also reduce the production of some desired metabolites. Selected strains can be used as inoculum in wine fermentation only if the major characteristics of wine flavour remain essentially unchanged. For this purpose, it is seen as more advantageous to use natural autochthonous strains, selected on the basis of desirable technological characteristics including a preferred metabolic profile (Romano et al., 1998b).

In order to identify suitable starter cultures for a specific vine cultivar, experimental wines produced with 30 *S. cerevisiae* strains in fermentation of Aglianico grape must were compared with commercial wines of the same cultivar. The comparison between the 30 experimental wines and 10 commercial wines

(Aglianico) are shown in Fig. 6. It is seen that the strains exhibited a significant variability for the production of isoamyl alcohol, isobutanol and acetic acid, whereas a very low variability was determined in the levels of acetaldehyde, *n*-propanol and ethyl acetate.

As regards the commercial Aglianico wines, the calculation of mean and standard deviation for the compounds considered revealed a considerable variation in acetic acid, acetaldehyde and *n*-propanol. On the contrary, the amounts of isobutanol, ethyl acetate and, at a lesser extent, isoamyl alcohol did not varied significantly in the commercial wines. Therefore, these traits can be assumed as characterizing variables to be used as reference in a yeast strain selection program for Aglianico fermentation.

In the experimental wines, the content of *n*-propanol, acetaldehyde and ethyl acetate was very similar to the average value determined in the commercial wines. Conversely, acetic acid in experimental wines was generally much higher than the average value determined in the commercial wines. The same behaviour was recorded for isoamyl alcohol, but with smaller differences between the values of experimental and commercial wines. In the case of isobutanol, pronounced differences were recorded between the experimental wines, whereas commercial wines exhibited a very similar content of this compound.

It is also seen from Fig. 6 that, of the strains tested, only a few exhibited both a behaviour close to that of commercial Aglianico wine and produced balanced wines.

4. Conclusions

A present trend in winemaking is the use of starter cultures of *S. cerevisiae*. The aim is to induce reliable and rapid fermentation, resulting in a wine of consistent quality. Thus, selected strains of *S. cerevisiae* characterized with regard to principal technological traits are normally applied. However, these strains are often anonymous strains, and strains should be selected which are more specific and appropriate to the individual characteristics of the particular wines. As wine is the results of the interaction of the different yeast species, in particular between non-*Saccharomyces* yeasts from the early fermentation phase and *Saccharomyces* spp., it is becoming advantageous to formulate and used mixed starter cultures. It is widely accepted that non-*Saccharomyces* yeasts survive for longer periods both in spontaneous and inoculated fermentation, that their growth is quantitatively significant and that they influence the chemical composition of the wine. In addition, it is experienced that, when *S. cerevisiae* is added to grape must partially fermented by apiculate yeasts, the wine produced is characterized by a more complex and better aroma. However, wines produced in initially mixed or sequential cultures of apiculate yeasts together with *S. cerevisiae* differ significantly in the final content of end products.

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