Available online at www.sciencedirect.com

ScienceDirect

Agriculture and Agricultural Science Procedia 8 (2016) 769 – 773

Agriculture and Agricultural Science Procedia

Florence "Sustainability of Well-Being International Forum". 2015: Food for Sustainability and not just food, FlorenceSWIF2015

The Wine: typicality or mere diversity? The effect of spontaneous fermentations and biotic factors on the characteristics of wine

Nicola Francesca[a,*]*, Raimondo Gaglio[a], Antonio Alfonzo[a], Luca Settanni[a], Onofrio Corona[a], Pierluigi Mazzei[b,c], Raffaele Romano[b], Alessandro Piccolo[c], Giancarlo Moschetti[a]

[a]*Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy*
[b]*Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici, NA, Italy*
[c]*Centro Interdipartimentale per la Risonanza Magnetica Nucleare (CERMANU), Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy*

Abstract

Wine is probably one of the main fermented beverages for which the recognition of the "territoriality" is fundamental for its appreciation. The sensory profile of wine is significantly affected by microbial activities, and indigenous microorganisms may significantly contribute to the expression of wine typicality.

The microbial ecology of wines is complex and includes several species and strains of yeasts, bacteria and molds. Several works showed the positive effects of spontaneous fermentations on the quality of wine as a consequence of the growth of different species and/or strains together at high levels. Furthermore, a new style of "natural" winemaking is gaining importance, since the resulting wines are obtained thanks to the action of spontaneous autochthonous agents and the use of chemical addition is not allowed. In this contest, natural winemaking could provide enhanced opportunities for products with unique characters and popularly recognized as typical.

The present work reports on microbial ecology and molecular profile characterizing natural large-scale vinifications, and an innovative procedure, named "fortified pied de cuve", to accelerate the alcoholic fermentation performed spontaneously is also reported. Furthermore, this work reports on how the biotic factors, such as migratory birds, contribute in disseminating of wine-related yeasts over long distances, opening up new fields of research that will allow to unravel connection between wine and environmental factors.

Peer-review under responsibility of Fondazione Simone Cesaretti

* Corresponding author.
E-mail address: nicola.francesca@unipa.it

Peer-review under responsibility of Fondazione Simone Cesaretti
doi:10.1016/j.aaspro.2016.02.064

Keywords: wine; spontaneous fermentation; autochthonous yeasts; *Saccharomyces cerevisiae*; lactic acid bacteria; *Lactobacillus plantarum*; migratory birds; new species

1. Introduction

Several economic and social factors, such as international competition within the wine market and consumer demands for wines with innovative styles, are providing new challenges in winemaking. In this contest, numerous wine producers are convinced that the premium wine quality is made by "traditional" methods based on spontaneous fermentation that could yield wines of unique and innovative characters that are particularly appreciated by specialized consumers. Furthermore, the new life-style trend known as "green consumerism", with people demanding more foods that are organic and with reduced levels of chemical preservatives for food production and preservation (Leite et al. 2006), has led to a re-discovery of traditional food products (Settanni et al. 2012). At the same time, a traditional product associated with a given geographical area is positively perceived.

To this purpose, a new style of natural winemaking is gaining importance, since the resulting wines are obtained thanks to the action of spontaneous autochthonous agents and the use of chemical addition is not allowed. Many factors are known to affect the microbial ecology of wine production and yeasts, as well as lactic acid bacteria are involved in different ways in winemaking. Several works showed the positive effects of spontaneous fermentations on the organoleptic complexity of wine as a consequence of the growth of different species and/or strains together at high levels (La Jeune et al. 2006; Wondra and Boveric 2001).

In the present work, the most recent advancements in the microbial ecology of natural wine and spontaneous alcoholic fermentation by using fortified pied de cuve method will be presented. Furthermore, the role of biotic factors such migratory birds will be considered in the dissemination of wine-related yeasts across Mediterranean basin. Understanding the microbial diversity and ecology can allow a better control of winemaking process and resulting in final products with high quality and unique sensory profile.

2. Microbial ecology and molecular profile of natural wine

Sannino et al. (2013) and Francesca et al. (2014) provided complete overviews on yeast and LAB populations and chemical compounds characterizing a natural large-scale vinification of white wine. The yeast distribution as well as the sensorial and chemical analysis showed the possibility to carry out a natural winemaking also without commercial strain starters and oenological additives. *Saccharomyces cerevisiae* was the species found at the highest concentrations in all samples analysed, and 16 strains were registered at high levels during the alcoholic fermentation and/or ageing of wine. Interestingly, LAB strains, in particular those of *Lactobacillus plantarum*, was found at high concentrations during the alcoholic fermentation phase. In this contest, natural winemaking could provide enhanced opportunities for products with unique characters and popularly recognised as typical.

Wine is probably one of the main fermented beverages for which the recognition of the "territoriality" is fundamental for its appreciation. The term "terroir", defined as an ecosystem in which the grapevine interacts with the environmental factors (soil and climate) affecting the quality and typicality of the wine produced in a particular location, refers to a concept basic during tasting. Many analytical methods have been used to investigate the correlation between chemical composition of wine and environmental factors related to winemaking. Mazzei at al. (2010) used the combination of Nuclear Magnetic Resonance Spectrometry (NMR) with multivariate analysis based on principal component, hierarchical component and discriminant analyses to investigate the molecular quality of red wines. The wines were obtained from three different vineyards characterized by different microclimatic and pedological characteristics. Despite the wines were produced employing the same grape variety ("Aglianico di Taurasi") and the same winemaking procedures, the multivariate data elaboration of their 1H NMR spectra was able to significantly recognize dissimilarities among their molecular properties, as shown by the content of lactic acid, α-fructose, glycerol, succinic acid, β-D-glucuronic acid and α-hydroxyisobutirate. Since the diversity of the three

wines could be only reconciled to differences in soils, these results suggest that bio-pedo-climatic factors determined the ultimate wine quality. However, some metabolites (lactic and succinic acid, as glycerol) may have derived by must and wine microbial activity, thus suggesting that indigenous microorganisms may significantly contribute to the expression of terroir.

3. An innovative method to accelerate spontaneous alcoholic fermentation

Several studies showed that spontaneous alcoholic fermentation might improve quality of final product providing wines with unique regional characters (Fleet 2008), but the spontaneous alcoholic fermentation is still recognized as an uncontrolled biological process during which some spoilage yeast and/or bacterial strains could rapidly increase in concentrations and negatively affect the quality of the final products. Thus, during the spontaneous fermentation the risk of off-odour and off-flavour generation is high. Even though the use of selected autochthonous strains could significantly contribute to the expression of varietal characteristics of wines (Zott et al. 2010), they have to face the indigenous microbial communities of grapes whose composition at species and/or strain level changes during different vintages (Fleet 2008).

With these regards, in order to reduce the risk of spoilage and/or stack of alcoholic fermentations, Moschetti et al. (in press) studied an innovative procedure to use the “pied de cuve” method to accelerate the alcoholic fermentation performed spontaneously. Some wineries traditionally carry out wines by using the pied de cuve method (Ubeda Iranzo et al. 2000, Clavijo et al. 2011, Li et al.2012). This technique is based on the inoculum of a given amount of must previously subjected to a partial alcoholic fermentation, into a new must. Generally, the pied de cuve is inoculated with commercial starter, in order to start its alcoholic fermentation. In this way, the amount of starter inoculum is reduced and, overall, it is possible to transfer desirable oenological features of yeast strains from a successful fermentation to a new must (Li et al. 2012). In case the pied de cuve is spontaneously fermented, the use of pied de cuve might limit but not exclude the risks, such as growth of spoilage microorganisms, stuck of the alcoholic fermentation, formation of off-flavours related to the winemaking processes carried out without the inoculum of starter cultures.

In this contest, Moschetti et al. (in press), studied the performances of two fortified pied de cuve (FPdC) that were prepared with the addition of wine at 1.5 and 3.0% (v/v) of ethanol to the musts and let to be spontaneously fermented. FPdCs were then added to fresh bulk musts in order to accelerate the alcoholic fermentation performed spontaneously. Thus, the addition of ethanol into pied de cuve, before the beginning of the spontaneous alcoholic fermentation, improved the development of several *S. cerevisiae* strains detected at high concentration during the entire vinification process. The high strain diversity of *S. cerevisiae* population, as well as its annual variability, might positively affect the quality of final wine. All conventional chemical parameters of experimental wines were in agreement with those reported for the production regulations of commercial wines, and undesired off-odours and off-flavours were not detected. In addition, data obtained by volatile organic compounds and sensory analysis showed that wines produced by FPdC, in particular that of FPdC fortified with addition of wine at 1.5% (v/v), were characterized by the highest scores of sensory intensity and complexity. Thus, an addition of wine to must till 1.5 % (v/v) ethanol is suggested for pied de cuve preparation in order to accelerate the spontaneous alcoholic fermentation and to influence the sensory profile of red wines.

4. Biotic factors and wine-related yeasts

The assumption that geographical location and microclimatological changes may affect the microbial species/strain diversity during winemaking (Raspor et al. 2006), as well as the idea of ‘terroir’ on the expression of wines, also at the microbial-level (Renouf et al. 2006) have been supported by several scientific results. For some authors, the autochthonous microbiota is linked to vineyard and/or cellar habitat in a given area (Lopes et al. 2002; Schuller et al. 2005), and for these reasons, they are found in consecutive years (Schuller et al. 2005; Torija et al. 2001).

However, *is the vineyard microbiota stable over time? Are the autochthonous strains associated to a specific environment over time and in places?*

Probably, it is still unclear the role of many biotic and abiotic factors in disseminating of microorganisms in wine environment. On the other hand, recent researches (Francesca et al. 2010, 2012) focused on the role of migratory birds in the environmental dissemination of wine-related yeasts highlighted the hypothesis of new ecological niches for wine microorganisms. The diversity of yeasts (Francesca et al. 2010, 2012) and filamentous fungi (Alfonzo et al.2013) isolated from migratory birds in the island of Ustica (Sicily, Italy) was evaluated. Several species of wine yeasts such as *S. cerevisiae*, *Hanseniaspora guilliermondii*, *Metschnikowia pulcherrima, Debaromyces hansenii*, *Pichia kudriavzevi*, *Pichia terricola* and *Aureobasidium pullulans* were isolated from many individuals of migratory birds. Moreover, Francesca et al. (2012) showed the persistence of a *S. cerevisiae* strain, with oenological aptitude, in migratory birds for a period of 12 hours, suggesting that birds can act as long-distance vectors of living yeasts related to wine environment. Such recent findings are promoting the interest on the dissemination of microorganisms by animals that could even include transcontinental displacement. Francesca et al. (2013, 2014) studied several strains belonging to two new ascomycetous yeast species (*Wickerhamomyces sylviae* and *Phaffomyces usticensis*) isolated from migratory birds. Contrary to their closest relatives, the new yeast species showed a unique phenotypic characteristics suggesting an adaptation to the gastro-intestinal tract of birds. Up to now, most studies on yeasts transported by animals have been focused on *S. cerevisiae* due to its relevance in human activities (Goddard et al. 2010; Stefanini et al. 2012) and it has been shown that social wasps have a preferential role in the dissemination of this yeast (Stefanini et al. 2012). Although important findings have been obtained, it is still unclear how *Saccharomyces* or other yeasts could be transported between distant places since social wasps as well as other animal vectors do not travel long distances. On the contrary, migratory birds can move between continents and therefore can act as yeast carriers over long distances. In the Mediterranean basin, bird migrations involve millions of individuals that twice a year, in spring and autumn, move between Africa and North Europe. The body fat represents the main energy source during flight and the birds with a very low value of sub-cutaneous fat amount need to stop in resting sites in order to replenish their fat reserves (Kaiser, 1993). During the flight, and in the places where they stop, birds can ingest yeasts present in their diets such as insects and fruits. On the basis of these results, the working hypothesis of Francesca et al. (2012, 2014) was that birds can transport and disseminate yeasts able to withstand the conditions of the animal's gastrointestinal tract across long distances, during their annual migrations.

Hence, the origin of wine-related strains, in particular of *S. cerevisiae*, is still hard to retrieve. Some researchers stated that *S. cerevisiae*, even at extremely low cell numbers are present in the vineyard environment, while others claimed that a natural origin for *S. cerevisiae* should be excluded, pointing to a direct association with artificial, man-made environments, such as wineries and fermentation plants. On the basis of those findings a further question needs to be posed: *could be "autochthonous" strains defined "indigenous"?*

5. Conclusion

Several scientific results support the idea of a "terroir" which is defined as high complex ecosystem in which the vine interacts with the environmental factors (soil, climate, insects, birds, humans, etc.) affecting the quality and typicality of the wine produced in a particular location. From this perspective, the dissemination of yeasts by migratory birds might contribute to biodiversity of many geographical locations, as well as to explain the differences observed in vineyard ecosystem.

If "*Biodiversity*" means the richness and variety of life including genes, species and ecosystems; if "*Sustainability*" means the ability to maintain something (ecosystem) over a long period of time; thus, the assumption "*higher biodiversity will increase the ecosystem's sustainability*" might be clearly stated.

References

Alfonzo, A., Francesca, N., Sannino, C., Settanni, L., Moschetti, G., 2013. Filamentous fungi transported by birds during migration across the Mediterranean Sea. Current Microbiology 66, 236–242.

Clavijo, A., Calderon, I.L., Paneque, P., 2011. Yeast assessment during alcoholic fermentation inoculated with a natural ''pied de cuve'' or a commercial yeast strain. World Journal of Microbiology and Biotechnology 27, 1569–1577.

Fleet, GH., 2008. Wine yeasts for the future. FEMS Yeast Research 8, 979–995.

Francesca, N., Chiurazzi, M., Romano, R., Settanni, L., Moschetti, G., 2010. Indigenous yeast communities in the environment of "Rovello bianco" grape variety and their use in commercial white wine fermentation. World Journal of Microbiology and Biotechnology 26, 337–351.

Francesca, N., Canale, D.E., Settanni, L., Moschetti, G., 2012. Dissemination of wine related yeasts by migratory birds. Environmental Microbiology Reports 4, 105–112.

Francesca, N., Carvalho, C., Miguel Almeida, P., Sannino, C., Settanni, L., Sampaio, J.P., Moschetti, G., 2013. *Wickerhamomyces sylviae* f.a., sp. nov., an ascomycetous yeast species isolated from migratory birds in Sicily, Italy. International Journal of Systematic and Evolutionary Microbiology 63, 4824–4830.

Francesca, N., Carvalho, C., Sannino, C., Guerreiro, M.A., Almeida, P.M., Settanni, L., Massa, B., Sampaio, J.P., Moschetti, G., 2014. Yeasts vectored by migratory birds collected in the Mediterranean island of Ustica and description of *Phaffomyces usticensis* f.a. sp. nov., a new species related to the cactus ecoclade. FEMS Yeast Research 6, 910–921.

Francesca, N., Sannino, C., Settanni, L., Corona, O., Barone, E., Moschetti, G., 2014. Microbiological and chemical monitoring of Marsala base wine during the large-scale production obtained by spontaneous fermentation. Annals of Microbiology 64, 1643–1657.

Goddard, M.R., Anfang, N., Tang, R., Gardner, R.C., Jun, C., 2010. A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-aided global dispersal in oak barrels. Environmental Microbiology 12, 63–73.

Kaiser, A., 1993. A new multi-category classification of subcutaneous fat deposits of songbirds. Journal of Field Ornithology 64, 246–255.

Le Jeune, C., Enry, C., Demuyter, C., Lollier, M., 2006. Evolution of the population of Saccharomyces cerevisiae from grape to wine in a spontaneous fermentation. Food Microbiology 23, 709–716.

Leite, S.E., Montenegro, S.T.L., de Oliveira, L.E., 2006. Sensitivity of spoiling and pathogen food-related bacteria to *Origanum vulgare* L. (Lamiaceae) essential oil. Brazilian Journal of Microbiology 37, 527–532.

Li, E., Liu, C., Liu, Y., 2012. Evaluation of yeast diversity during wine fermentations with direct inoculation and pied de cuve method at an industrial scale. Journal of Microbiology and Biotechnology 22, 960–966.

Lopes, C.A., van Broock, M., Querol, A., Caballero, A.C., 2002. *Saccharomyces cerevisiae* wine yeast populations in a cold region in Argentinean Patagonia. A study at different fermentation scales. Journal of Applied Microbiology 93, 608–615.

Mazzei, P., Francesca, N., Moschetti, G., Piccolo, A., 2010. NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. Analytica Chimica Acta 673, 167–172.

Moschetti, G., Corona, O., Gaglio, R., Squadrito, M., Parrinello, A., Settanni, L., Barone, E., Francesca, N. The use of fortified pied de cuve as an innovative method to start spontaneous alcoholic fermentation for red winemaking. Australian Journal of Grape and Wine Research (in press).

Raspor, P., Milek, D.M., Polanc, J., Mozina, S.S., Cadez, N., 2006. Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. International Journal of Food Microbiology 109, 97–102.

Renouf, V., Miot-Sertier, C., Strehaiano, P., Lonvaud-Funel, A., 2006. The wine microbial consortium: a real terroir characteristic. Journal International des Sciences de la Vigne et du Vin 40, 209–216.

Sannino, C., Francesca, N., Corona, C., Settanni, L., Cruciata, M., Moschetti, G., 2013. Effect of the natural winemaking process applied at industrial level on the microbiological and chemical characteristics of wine. Journal of Bioscience and Bioengineering 116, 347–356.

Schuller, D., Alves, H., Dequin, S., Casal, M., 2005. Ecological survey of Saccharomyces strains from vineyards in the Vinho Verde Region of Portugal. FEMS Microbiology Ecology 51, 167–177.

Settanni, L., Di Grigoli. A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G., Bonanno, A., 2012. Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a Caciocavallo type cheese. International Journal of Food Microbiology 155, 73–81.

Stefanini, I., Dapporto, L., Legras, J.L., Calabretta, A., Di Paola, A.M., De Filippo, C., Viola, R., Capretti, P., Polsinelli, M., Turillazzi, S., Cavalieri, D., 2012. Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. Proceedings of the National Academy of Sciences of the United States of America 109, 13398–13403.

Torija, M.J., Rozes, N., Poblet, M., Guillamon, J.M., Mas, A., 2001.Yeast population dynamics in spontaneous fermentations: comparison between two different wine-producing areas over a period of three years. Antonie Van Leeuwenhoek 79, 345–352.

Ubeda Iranzo, J.F., Gonzalez Magana, F., Gonzalez Vinas, M.A., 2000. Evaluation of the formation of volatiles and sensory characteristics in the industrial production of white wines using different commercial strains of the genus Saccharomyces. Food Control 11, 143–147.

Wondra, M., Boveric, M., 2001. Analyses of aroma components of Chardonnay wine fermented by different yeast strains. Food Technology and Biotechnology 39, 141–148.

Zott, K., Claisse, O., Lucas, P., Coulon, J., Lonvaud-Funel, A., Masneuf-Pomarede, I., 2010. Characterization of the yeast ecosystem in grape must and wine using real-time PCR. Food Microbiology 27, 559–567.