10th International Symposium

Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation.

ŒNOVITI INTERNATIONAL network

18th to 21st May 2021

Coordinators
Pierre-Louis Teissedre
Olga Titlova
Oksana Tkachenko

ISVV INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN BORDEAUX AQUITAINE

université de BORDEAUX

Academic Partners

Universidad Nacional de Cuyo Mendoza, Argentina
Charles Sturt University, Australia
Adelaide University, Australia
University of Natural Resources and Applied Life Sciences, Austria
University of British Columbia, Canada
Chinese Academy of Sciences, China
Northwest A&F University, China
Pontificia Universidad Católica de Chile, Chile
Universidad de Chile, Chile
University of Zagreb, Croatia
Université de Bordeaux, France
Kedge Business School, Bordeaux, France
Bordeaux Sciences Agro, France
Montpellier SupAgro, France
Ecole Supérieure d'Agricultures d'Angers, France
INP Toulouse, France
Hochschule Geisenheim University, Germany
Agricultural University of Athens, Greece
University of West Attica, Greece
University of Pécs, Hungary
Università degli Studi di Milano, Italy
Università degli studi di Padova, Italy
Università degli studi di Torino, Italy
Università degli studi di Udine, Italy
Università degli studi di Verona, Italy
University of Yamanashi, Japan
Graduate School of Agriculture, Kyoto University, Japan
University of Niigata, Japan
University Saint Joseph, Lebanon
CEVIT-CETYS Universidad, Mexico
UTNA - Universidad Tecnologica del Norte de aguascalientes, Mexico
Instituto Superior de Agronomia, Universidade de Lisboa, Portugal
Universidade de Tras-os-Montes e Alto Douro, Portugal
Universidade de Porto, Portugal
University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
University of Applied Life Sciences Iasi, Romania
University of Nova Gorica, Slovenia
Stellenbosch University, South Africa
Universidad de la Rioja, Instituto de Ciencias de la Vid y del Vino, Spain
Universitat Rovira i Virgili, Spain
Haute Ecole de Viticulture et Œnologie de Changins, Switzerland
Odessa National Academy of Food Technologies, Ukraine
University of California, Davis, USA
Texas A&M AgriLife, USA

ŒNOVITI INTERNATIONAL

Research Centers

AWRI, Australia
Centro di Ricerca per la Viticoltura, Italy
Agricultural Institute of Slovenia KMETIJSKI, Slovenia
Agroscope, Switzerland
Louis Pasteur Center for medical Research, Japan

Industrial Members

Concha y Toro, Chile
Biolaffort, France
GIE Les 9, France
Inno'vin, France
Lallemand, France
Preparatori d'Uva, Italy
Vason/JU.CLAS, Italy
Sogrape Vinhos, SA, Portugal
Miguel Torres SA, Spain
SERESCO, Spain
FEUGA, Spain
VITEC, Spain

ŒNOVITI INTERNATIONAL

OENOVITI INTERNATIONAL Network

Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation.

[Forward]

The OENOVITI International network is 11 years old and the first and only international network for training and research in oenology and viticulture. It aims to promote exchanges of expertise and know-how between stakeholders in the academic and industrial winemaking worlds. The network offers its members a high level of visibility on the international scene, enabling them to maximize opportunities via joint research and training projects, as well as discussions. The OENOVITI International network gathers 61 partners across the globe in 2021, forming an international consortium of institutions known for their excellence in the field. Its members are organized into seven interdisciplinary and thematic working groups. Born from the network, the joint OENODOC doctoral program was created with the aim of developing an international doctorate specific to the oenology and viticulture sector. The OENODOC consortium includes in particular members of VINTAGE and EMaVE, which offer the VINTAGE and VINIFERA and recently WINTOUR (oenotourism) Erasmus Mundus Master Courses respectively. Two new programs have been developed: Erasmus + OENOBIO (Training on organic vines and wines), RISE vWISE a research project that focuses on the consequences and solutions to the impact of climatic change on vines, grapes and wines: abiotic stress on vines and grape quality and ripening with genetic exploitation, reduction of alcohol level in wine by adapted yeasts and engineering process, adapted microorganisms to prevent spoilage, wine quality changes and sensorial preservation.

The different working groups deal with the subject areas of climate change, extended oenology, extended viticulture, wine tourism and wine management, industrial transfer, development, strategic monitoring and international relations, and wine and health. The OENOVITI International network's innovative approach is based on staff and student mobility exchanges of experience and good practices between disciplines, and the establishment of a common educational and training framework. The programs also bring together numerous partners in industry and the socioeconomic world. In addition to financial support, they also offer their expertise in conducting R&D to high standards of excellence and provide professional opportunities for young graduates.

This network, coordinated by the University of Bordeaux, enables the academic and industrial worlds to come together to take up the many challenges of oenology and viticulture research.

This 10th network symposium is dedicated to "**Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation.**": This topic is of great importance for the wine sector to organize, adapt and preserve wine quality production. Several questions are approached during this symposium:

- **Overview of winemaking and implications for appellations and authenticity,**
- **Wine Appellations and Wine Tourism with resources preservation, typicality and system,**
- **Authenticity with analytical approaches,**
- **Winemaking and winegrowing innovations and novelties including vine and modelling, genetical approaches, vine-wine chain and consumers, vine protection and new processes, digital and marketing.**

Wine market, wine tourism economy, consumers expectations, innovations for the wine industry sector in the context of climate change, with new consumers demands, as well as product identity certification are the major points and questions of this 10th symposium with recognized scientists and researchers in this field.
OENOVITI INTERNATIONAL network along with the wine industry will encourage projects including adaptation, innovations, alternatives and research to develop wine market and tourism, wine authenticity, quality and identity.

The network enjoys particular support from Château Pichon-Baron (AXA Millésimes group), the Foundation Bordeaux University (original interface between the university and socioeconomic worlds), IdEx Bordeaux (an investment programme supporting the University of Bordeaux's transformation and development drive) and all of the network's academic, research and industrial partners. We would like to thank Christian Seely (Managing Director of Château Pichon Baron), and all the partners involved in the support, life and progress of the network, which will benefit the entire vine and wine sector. We wish all the best for the Network and the wine industry for the next 10 years!

Pierre-Louis Teissedre
Professor
OENOVITI International Network Coordinator

OENOVITI INTERNATIONAL Network

Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation.

[Contents]

Session IV. Winemaking and Winegrowing Innovations and Novelties

10th International Symposium

Challenges in viticulture and oenology:
Wine Appellations, Authenticity and Innovation.

ŒNOVITI INTERNATIONAL network

Session I. Overview of winemaking

Winemaking of Ukraine: current state and perspectives

Volodymyr Pechko [1]*
[1]*Public Association "UKRSADVINPROM", Vasylkivska Str., 37, office 414, Kyiv, 03022,* Ukraine

Abstract: It is analysed the development state of Ukrainian winemaking. In Ukraine, viticulture and winemaking is one of the important branches of the agro-industrial complex. The current state of winemaking in Ukraine is considered, the positive and negative aspects of its development and functioning are determined. The ways of development are suggested.

Keywords: winemaking, wine, wine production, vine, viticulture

1. Introduction

Viticulture in Ukraine has a centuries-old history. The largest areas of vineyards were in the 60s of the last century and amounted to about 400 thousand hectares. However, today the area of the vineyards has decreased by almost 5 times and continues to decline. According to statistics, in 2000 the area of vineyards was 150 thousand hectares, and in 2020 about 40 thousand hectares (excluding vineyards of the Autonomous Republic of Crimea). The vineyards are mainly concentrated in Odesa, Kherson, Mykolaiv, Zaporizhia and Zakarpatia regions. Geographically, there are 6 main wine-producing regions of Ukraine, 15 macrozones (wine regions) and 58 microzones, although these zones are not legally defined. The wine industry of Ukraine is represented by the enterprises of primary and secondary winemaking. The main type of raw materials for wine production is wine materials.

2. Current state

The introduction of advanced grape production technologies by domestic enterprises began only in 2000, unlike the systematic improvement of such technologies abroad since the early 70's of the last century. There are also young wineries that are contributing to the development of the culture of consumption and wine production in Ukraine.

Table 2.1 shows the production volumes of winemaking. In total, in 2019 the number of production decreased, the maximum volume for the last 5 years was in 2016 and amounted to 18187 thousand dal. (see Table).

Table 2.1. Wine production 2015-2019 (thousand dal)

Type	Year				
	2015	2016	2017	2018	2019
Wine	7144	7520	7915	8569	7832
Vermouth	2308	2717	2216	1828	1822
Sparkling wine	2692	3799	3090	2863	2224
Brandy	3111	3104	2796	2693	2835
Carbonated wine	1139	1047	654	472	441

In 2019, 124226.6 tons of grapes were processed into wine materials , including their own produced - 34247.4 tons. The average mass concentration of sugars in

*Corresponding author. E-mail: info@ukrsadvinprom.com

grapes is 195 g / dm3. The main varieties are Alligote, Bastardo Magaray, Cabernet Sauvignon, Merlot, Muscat, Odessa Black, Pinot, Riesling and more. Last year, about 300 hectares were planted of vine.

In the context of the globalization of the wine market and Ukraine's accession to the WTO, competition is exacerbated, which requires the restructuring of the industry so that its products meet the high demands of the market. In 2009, the Branch Program of Viticulture and Winemaking of Ukraine for the period up to 2025 was developed and approved. Unfortunately, it is not fully operational. The purpose of the Program was to realize the state policy of Ukraine to regulate the development of this sector of the economy, to concentrate financial, logistical and other resources, production, scientific and technical potential for solving the main problems of the industry.

Many of the most important concepts are not legislated at all, or existing laws do not meet the current requirements of the world market of wine production.
The laws and regulations governing the labeling of wines, the classification methods, the control of appellations of origin, the law on the quality control system and the supervisory authority or institute are essential.

It is necessary to increase the planting, restore and lay new grape nurseries, and most importantly to improve the quality of wine from recognized international varieties that grow well in Ukraine. Examples: Alligote, Cabernet Sauvignon, Chardonnay, Merlot, Sauvignon, Riesling, and develop local indigenous varieties, which in Ukraine enough.

However, there are general industry issues:

1. Planting material of low breeding categories.
2. The mismatch of varietal composition of grape plantations to the requirements of winemaking.
3. Low efficiency of grape production, which is caused by the high proportion of old and liquefied vineyards, which increases the cost and reduces the competitiveness of table grapes and domestic wine products.

3. Perspectives of development

It is now necessary to determine the priorities for the development of viticulture, taking into account the zonal and regional features. Pay attention to the development of special state programs for the conservation of the best grape varieties. The Government needs to renew the Law of Ukraine "On Collection for the Development of Viticulture, Horticulture and hop Growing" (587-14) by 2025 and improve the mechanism of providing state support to viticulture and ensure the development of winemaking in accordance with WTO requirements, to develop the Grapes inventory within the general land cadastre. An open question remains the regulation of land relations and rationalization of land use, the development of legal and technical registration of the right to lease land for vineyards, the formation of a market for agricultural land.

Also the ways of development of the wine industry are:

- attracting large investments in the industry;
- choosing the right marketing policy;
- implementation of measures to ensure the development of the infrastructure of the market for wine products, diversification of channels of sales and quality assurance of products, regulation of supply and demand, protection of the market from imported wine production and raw materials of low quality;

- implementation of measures to increase the capacity of the grape market and products of its processing, taking into account the needs of the population, its purchasing power and maximum export opportunities;
- innovation and investment strengthening of the material and technical base of the wine-making industry, introduction of environmentally safe, resource- and energy-saving technologies;
- improvement of insurance and tax policy in the field of wine production;
- enhancing the role of science and education, advancing development;
- improving the management system in the wine industry; development of sectoral regulatory and technological documentation that meets the requirements and requirements of the European Community.

4. Conclusions

There are favorable soil and climatic conditions, potential of domestic and foreign wine market, availability of intellectual and production capital for introduction of innovative technologies. This all necessitates the improvement and development of viticulture and winemaking in Ukraine. Fierce competition in the wine market with world leaders dictates the need to improve the quality of wine production in Ukraine, which must be accompanied by a decrease in its cost. An essential reserve for reducing the cost of wine in Ukraine is to increase the area under the vineyards and use them effectively.

Microbiological control of alcoholic fermentation

Albert Mas*, Gemma Beltran and María Jesús Torija

Oenological Biotechnology, Department of Biochemistry and Biotechnology, Faculty of Oenology, University Rovira i Virgili. Marcel·lí Domingo, 1. 43007 Tarragona. Spain.

Abstract: Alcoholic fermentation and the production of wine has accompanied humanity for more than 10000 years. However, it has been only in the last 50 years when the winemakers have had the tools to manage and control the process. The methodology to analyze and monitor the succession of the microorganisms that participate in the process along with the effective use of antimicrobial compounds (for instance sulfur dioxide), the control of the temperature and, above all, the use of cellar-friendly fermentation starters (mostly as Active Dry Wine Yeast) have provide the appropriate conditions for that control. However, the use of a limited number of commercial presentations of the starters has generated an unwanted uniformity of the wines produced. Furthermore, new tendencies in wine making with limited or no human intervention have considered these tolls as a negative aspect in the wine quality, although most of these concerns are only philosophical, without clear scientific evidence. We present a revision of the present state of the art in these methodologies where our research group has been working for the last 25 years.

Keywords: Yeast, Fermentation starters, Sulfur dioxide, Molecular methods, Spoilage.

1. Introduction

The production of fermented products has been used by humans for at least 10000 years or even more. Among them, the fermented grape vine must, or wine, has been considered one of the most prestigious in many different cultures. Since its appearance in the Caucasian region around 7000 years ago has been found on the tables of kings, nobles, religious authorities and almost all the humanity, especially in Europe and America. It was considered the result of a kind of miracle that is why in some cultures was closely associated to religion and priests. For ancient humans to understand how a sweet liquid started boiling without the application of any heat and got transformed into a beverage leading to desinhibition was much beyond their comprehension. Humanity had a previous experience already, by producing beer, knowing that those fermented products were healthy and the best way to drink water. At that time water was known to be the source of many diseases and drinking water as wine or beer was known to be safe.

The nature of the "miracle" took very long to be understood. Although the chemical transformation was defined at the end of the 18th Century (the transformation of sugar into ethanol and water), it was in the 19th Century when living microorganisms were defined to be responsible of the process and it was not until the second half of the 20th Century to completely describe and control it. Cagniard de Latour in 1836 mentioned

*Corresponding author. E-mail: albert.mas@urv.cat

living organisms during the alcoholic fermentation. However, this observation was ignored for 30 years. It was Louis Pasteur, considered the start of the modern biochemistry and microbiology, who devoted several years to understand the production of wine and beer, describing beyond any doubt its microbiological nature. He described a succession of microorganisms that he named mycoderma (defined as "fungi that were growing on the surface"), which were later named yeasts. Additionally, some of those microorganisms were identified the responsible for the wine spoilage and also for the production of vinegar. Some years later, when the microbiological methods were more developing, including the isolation and the study of isolated species and strains, researchers and winemakers understood enough the process to start to have the appropriate control tools. For instance, in 1899 Hansen started the development of selected starters for the beer production. However, in the wine cellars took longer to acquire this novelty. Among other reasons, we can consider the importance that the yeast has in the beer characteristics or the concentration of brewers in big companies, although the main cause is that brewing is process that can is done all the yearlong, whereas wine is a seasonal product. The starters in the form of liquid media, for instance, was not the most appropriate for the wine sector due to its strong demand in a very short period (four-six weeks). The real outbreak of inoculation in wine making came with the development of Active Dry Yeast and the commercial offer available. Although many winemakers have used and still continue to use the commercial presentations, recent movements in wine making have challenged this practice by returning to old fashion and uncontrolled wine making yielding what are self-named "natural wines" (how the inoculation of a living organism, as wine yeasts, domesticated for centuries by humans and coming from such natural habitats as grapes or fermentations turns into "unnatural" or "non-natural"?).

2. Methods of analysis: Classical and molecular methods

The classical microbiological approaches to detect and quantify different wine microorganisms are generally supported by plating and observation under the microscope. Basically, a first approach consists on morphological tests, which are complemented with several physiological tests. Furthermore, the isolation of microorganisms is required to properly identify and quantify the given microorganisms. Barnett et al. (2000) described identification protocols to identify yeasts. One of the hurdles is the number of tests needed for the identification of yeast at species level. Thus, this methodology is time-consuming, and the interpretation has to be done by experts with considerable experience.

At bacterial level, initial tests are the Gram stain and Catalase test, which can be used to discriminate between Lactic Acid Bacteria (LAB) and Acetic Acid Bacteria (AAB) present in wines. However, to identify at species level becomes much more difficult and often the physiological tests are not enough. The growth of microorganisms in different specific culture media produces colonies with diverse morphologies, which can be useful (Fugelsang and Edwards 2007).

The observation under a phase-contrast microscope is a first step to analyse the microorganisms' morphology. This observation provides information about size, shape, and arrangements of the cells. However, this can be misleading, as the morphology of the microorganisms is age- and culture-dependent.

The monitoring of the density and diversity of the microbiological population gives important information about the evolution of the winemaking process. Oenologists use counting under the microscope and direct plating to have an idea about the population densities. A limitation of microscope counting is the minimal population that is required, although it is a very fast approach. Low microbial population can be tackled by concentration by filtration. Direct plating methods are also a good alternative in these cases, although it takes longer to get the results. Both methods combined can be a good approach to those wines presenting low population or viability of the microorganisms. Microscope counting chambers, for instance Neubauer or Thoma, are needed for appropriate quantification. The main limitation can be low detection limits and lack of discrimination between alive and dead cells.

On the other side, counting the colonies grown on different media allows plate enumeration of microorganisms. Some non-selective media allow the growth of all microorganisms. However, as there are different that are mixed, the fastest growing and more prevalent species dominate on the plate, which will not allow the detection of those in low proportion. The use of selective media can circumvent this problem, because these media can limit or impede the growth of the dominant microorganisms. For instance, Lysine agar is a selective medium that reduces the detection of *Saccharomyces cerevisiae* because it hardly grows with lysine as single nitrogen source. This medium is often used to study the non-*Saccharomyces* yeasts. Another alternative is the addition of antibiotics that inhibit microorganisms. Also media enriched with different nutrients can favour the growth of different microorganisms are common to study microorganisms involved in winemaking. Lactic Acid Bacteria are commonly isolated in MRS agar (De Man, Rogosa and Sharpe agar) an Acetic Acid Bacteria in GYC agar (glucose, yeast extract, and calcium carbonate agar). This last medium should also be considered differential medium AAB produce acid gluconic or acetic, which dissolves the calcium carbonate precipitates and develops a clear halo around the colony. A selective medium can be obtained after changes of temperature, pH, aerobiosis/anaerobiosis condition, etc in a generic medium. Generally, the different conditions are used together for a more efficient enumeration.
The incorporation of DNA analysis methodology has been an important step forward in the identification microorganisms. The application of these methodologies together with isolation after plating has allowed a deep understanding of the ecology of grape and/or wine. The analysis of the polymorphism in the ribosomal RNA coding regions is the most usual method for the identification of wine microorganisms. The ribosomal genes of all living beings are grouped in tandem. These tandems form transcription units that have many copies in the genome. In each transcription unit exist the coding regions that express the ribosomal genes (external transcript spaces ETS), the internal transcriber spacers (ITS) and the rRNA codifying genes. The ribosomal genes allow the establishment of the phylogenetic relations and are used to identify species (Kurtzman and Robnett 1998). The ribosomal genes are highly conserved regions and, thus, their sequences can be aligned with the sequences available in the databases allowing the identification of microorganisms. Instead, the ITS are not coding regions that present higher polymorphism, which allow the differentiation of closely related species that cannot be differentiated by the analysis of the ribosomal genes. A phylogenetic tree is generated by comparison with the sequences available in the databases and used for the identification of microorganisms.

The main regions for sequencing ribosomal genes of yeast are the domain D1 and D2 in the 26S gene (Kurtzman and Robnett 1998). For bacteria, the main gene is 16S rRNA (Cole et al. 2005). In wine these regions have been used to differentiate among yeast species (Montrocher et al. 1998) and bacterial species (Le Jeune and Lonvaud-Funel 1997). However, for routine analysis of large number of samples as it is required in ecological studies, a cheaper alternative has been the Restriction analysis of ribosomal genes (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, PCR-RFLPs). This technique uses specific endonucleases to generate fragments that can be species-specific. The regions used for wine yeast identification are the regions comprised between the 18S and 26S rRNA genes for yeast, which includes the intergenic spacers ITS1 and ITS2, and the 5,8S rRNA gene. The most RFLP used for bacteria is the 16S rRNA gene, which has been denominated Amplified Ribosomal DNA Restriction Analysis (ARDRA). The application to wine species was initiated by Guillamón et al. (1998) and Esteve-Zarzoso et al. (1999) and several studies have used this technique later on (Torija et al. 2001, Beltran et al. 2002). ARDRA has been used to identify LAB (Rodas et al. 2005) and AAB (Poblet et al. 2000, Ruiz et al. 2000, González et al. 2006a, Gullo et al. 2006, Vegas et al. 2010). Additional species discrimination has been done with the 16S - 23S intergenic spacer region (Ruiz et al. 2000, González and Mas, 2011).

Sequencing has become more accessible and affordable after the effort to fulfil the Human Genome made during this last two decades. Nowadays, only sequencing, alignment with sequences in databases and elaboration of genetic trees should be accepted as criteria for the identification of microbial species. However, when a large number of samples is to be processed, grouping through RFLP of the appropriate ribosomal genes or ITS has to be considered an initial step, assuming that all the isolates that present the same identification or banding pattern will belong to the same species. A minimum of two or three representatives of each grouping should be sequenced.

The application of molecular-based methods on plate isolates has allowed also the discrimination at strain level. The polymorphism and repeated sequences along the genome have been used as methods for strain genotyping. The most basic technique is based on the random amplification of genomic DNA with a single primer sequence of 9 or 10 bases of length (RAPD). Each strain present different amplification fragments, in size and number. The amplification is followed by agarose gel electrophoresis, which yields a band pattern that should be characteristic of a given strain. This technique has been used to genotype wine yeasts (Cocolin et al. 2004), LAB strains of *Oenococcus oeni* (Cappello et al. 2008) and AAB strains (Bartowsky et al. 2003). Other methods have used the repetitive elements of the genome, all of them based on the design of oligonucleotides homologous to these repeated sequences that allow the amplification of these regions, obtaining a pattern of electrophoretic bands for each species or strain. For the identification of different wine microorganisms several different techniques for yeast and bacteria have been applied. For instance, microsatellites are tandem repeat units of short DNA sequences, typically 1-10 nucleotide length in eukaryotic cells. The number of repeated sequences along the genome is very variable, making the distances between sequences highly polymorphic in size. Thus, the technique consists in the amplification of the parts of the genome that are flanqued by these microsatellites; which yields an amplicon pattern that allows to differentiate strains. The most common oligonucleotides used are $(GACA)_4$, (GAG)$_5$, (GTG)$_5$ and others. *S. cerevisiae* strains were differentiated by Lieckfeldt et al. (1993) and then it was applied to wine strains by Maqueda et al. (2010). Gevers et al. (2001) used of (GTG)$_5$-PCR (also named rep-PCR in bacteria) to differentiate a wide range of food associated lactobacilli and other LAB species. Nowadays, (GTG)$_5$-PCR are extensively used to genotype AAB in wine vinegar production (Hidalgo et al. 2010, Vegas et al. 2010).

Different methods have been used to genotype *S. cerevisiae* as main microorganism in the alcoholic fermentation. For instance, delta elements are conserved sequences that flank transposable Ty elements. The separation distance between these elements is variable and does not exceed 1-2kb, which determines that are appropriate to amplify the region comprised between them. The separation by size of these bands can be used to differentiate *S. cerevisiae* strains. This method was developed for Ness et al. (1993) and Masneuf and Dubourdieu (1994) to genotype strains of *S. cerevisiae*. The facility to perform the PCR analysis without extraction of the DNA (using directly the colony) has made this technique the most widely used to differentiate *S. cerevisiae* strains. The other main technique to differentiate *S. cerevisiae* strains is the Restriction analysis of mitochondrial DNA (mtDNA-RFLP). The basis of this technique is to use specific restriction endonucleases to fragment the DNA into specific sites, generating fragments of variable size. These fragments are separated on agarose gel showing pattern strain specific. This technique was firstly applied to brewer's yeast and wine strains of *S. cerevisiae* by Aigle et al. (1984) and Dubourdieu et al. (1987), respectively. Querol et al. (1992) simplified the protocol by using a unique characteristic of the mtDNA with high proportion of AT. Then, the restriction pattern DNA with enzymes that target sequences such as GCAT will cut less frequently the mtDNA than the nuclear DNA. So far, this was the most used technique to genotype the strains of *S. cerevisiae* (Torija et al. 2001, Beltran et al. 2002), although it still has the need to extract the DNA and it is more time consuming than the direct PCR that can be performed with delta elements.

Finally, the most traditional technique for typing is the Pulsed-Field Gel Electrophoresis (PFGE), based on the electrophoretic separation of the entire set of chromosomes with alternating electrical fields. The chromosomes should change their migration direction, which enables the separation of large fragments of DNA. This technique has been used to genotype wine strains of *S. cerevisiae* (Guillamón et al., 1996), some non-*Saccharomyces* (Esteve-Zarzoso et al. 2001) and *O. oeni* (Vigentini et al. 2009).

However, the main drawback of the methods based on plating is that they only quantify the microorganisms that are able to grow, and thus, the cells that are able to form colonies (colony forming units, abbreviated as CFU or cfu). The population enumerated by this method is considered as the "culturable" population, which sometimes is considered representative of the viable population. Despite the extension of its use, this limitation together with the time required for some microorganisms to grow (2-5 days in yeasts, 2-10 days in bacteria) is a main handicap for the wine industry. However, one of the main challenges of the wine microbial ecology is that many microorganisms undergo states that are defined as Viable But Not Culturable (VBNC, Millet and Lonvaud-Funel 2000). Microorganisms that are VBNC state are those that lose the ability to grow in a culture medium but still maintain some metabolic activity. This is one of the responses of many microorganisms when the environmental conditions are not optimal. The previous assumption was that these microorganisms were dead. However, these microorganisms are alive, but they are not able to form colonies. The VBNC state involves the microorganism ability to recover and grow if they are allowed recovering in media without the stress that originates this status (Oliver 2005). Although they have reduced metabolism, they are still able to spoil wines. In fact, during long periods when wines are settled (ageing, bottles, etc.) the chances of spoilage are greater even in the absence of culturable spoiling microorganisms. Even when the metabolism of the microorganisms is slow and the population is low, they have a lot of time to act and, thus, they can alter the wine properties. These microorganisms maintain the basal metabolism to keep active the main cellular functions.

Thus, there are live cells, dead cells, and several cells in transient states in all microbial mixtures, as during the wine making process. These transient cells could be old cells that still retain the ability to grow under optimal conditions; old cells that have impaired the ability to grow on regular plates but still fully viable with active metabolism and finally cells that have already entered the lytic process. The old cells that have lost the ability to grow on plates can often be recovered by providing a very rich medium, normally using liquid medium with strong aeration to resume their growth again (Wang et al. 2016). Thus, culture independent techniques have used the molecular techniques to identify and/or quantify wine microorganisms without previous cultivation of these microorganisms (Rantsiou et al. 2005). These methods provide a better knowledge of the population, avoiding the biases that represent the microorganisms that are absent or not grow well on a plate.

As consequence, most of the consolidated knowledge on wine microbiology has emerged from the use of plating and the analysis of the microorganisms that could be recovered on the plates. However, the enumeration and identification of the microorganisms recovered on plates underwent a strong change from the extension of the molecular biology techniques that targeted DNA as main element, which meant a quick and big step toward the determination of grape and wine ecology. The expansion of these molecular biology techniques for identification and typing allowed a step further: the use of those techniques directly from grapes or wines, without the steps of culturing the microorganisms on plates. These "culture-independent" techniques have been used quite extensively since the beginning of this Century and still they are very common. Many of these culture-independent techniques have some limitations, though. If the main target is DNA, this molecule is rather stable with time, and it does not allow the differentiation between live and dead cells. Several alternatives have been proposed to circumvent this limitation: targeting more labile molecules, such as RNA; quantification and identification through hybridisation of non-DNA molecules with short life, etc. For instance, a solution for appropriate differentiation between dead cells, VBNC cells and culturable alive cells has been the use of culture independent techniques with some modifications to eliminate the DNA from dead cells or use RNA. Several studies used RNA instead of DNA to quantify or detect the viable population, since this molecule is rapidly degraded in the dead cells (Cocolin and Mills 2003, Hierro et al. 2006). However, it is very tedious to work with RNA because it is unstable and can be degraded during the purification or analysis. Furthermore, rRNA might be more stable than required (Hierro et al. 2006, Andorrà et al. 2011, Sunyer-Figueres et al, 2018). Successful alternatives to use RNA have been developed with DNA binding dyes that only penetrate in the dead cells (damaged membranes) and block the amplification of this DNA (Rudi et al. 2005, Nocker and Camper 2006). Ethidium monoazide bromide (EMA) and propidium monoazide bromide (PMA) were proposed by Nogva et al. (2003) and Nocker et al. (2006), respectively, to detect bacterial viable cells. Both chemicals penetrate only into dead cells; in fact, into cells with compromised membrane integrity but not into live cells with fully functional cell membrane. Upon binding to the DNA of dead cells, the photo-inducible azide group allows these dyes to be covalently cross-linked by exposure to bright light and precipitate the DNA (Nocker and Camper 2006). Thus, only the DNA from live cells will be detected and quantified after the treatment with these dyes. This methodology has been applied successfully to wine microorganisms (Andorrà et al. 2010a).

The control of wine making process requires the identification of the microorganisms present as well as the quantification of each species in the different stages. The quantification is based on the correlation of the amount of the target molecules with the amount of biomass. This is true for DNA, but it is not completely valid for other molecules such as RNA or proteins, as they are more related to the physiological statuses of the cells, which present strong changes during wine making. In fact, almost all the relevant microorganisms in wine making undergo complete life and growing cycles during the process.

3. Grape microbiome

Grapes support microorganisms that are mostly epiphytes (that grow on the grape surface). The substrates that allow the growth of microorganisms are normally the exudates from grapes, rich in saccharides. The yeast population on sound grapes can go from 10^2 cfu/berry to 10^5 cfu/berry depending on the ripening state (Renouf et al. 2005). Interestingly, population quantity also changes during ripening of grapes, being the highest at the end of ripening (Renouf et al. 2005). The increased population at the harvest time is mostly due to the increased nutrient availability, because the berry cuticle becomes soften and might have some microfissures not easily visible (Barata et al. 2012). At full ripening, grape musts obtained from healthy grapes contain yeast populations varying from 10^4 to 10^6 cfu/ml (Beltran et al. 2002, Padilla et al. 2016). Damaged grape berries can sustain growth of many microorganisms, increasing considerably the population at least one log cycle of population (to 10^6 or 10^8 cfu/berry) due to nutrient availability (Barata et al. 2012).

The yeasts present on the grape surface are mostly *Ascomycetous* moulds (yeast-like), *Basidiomycetous* and *Ascomycetous*. As main species of the *Ascomycetous* moulds, *Aureobasidium pullulans* is the most common yeast-like mould occupying grape surface. *Basidiomycetous* yeasts are also abundant on grape surface and the most frequent species are from genera *Cryptococcus*, *Rhodotorula* and *Sporodiobolus*. Although *Ascomycetous* yeasts generally colonize intact grape berries, a great diversity is found in the worldwide surveys. Common *Ascomycetous* yeasts on grape surface include the genera *Hanseniaspora*, *Candida* (most of those found on grapes have been later reclassified within *Starmerella)*, *Issatchenkia*, *Debaryomyces*, *Metschnikowia* and *Pichia*. Species diversity of *Ascomycetous* yeasts is even higher depending on a series of variations (climatic conditions, vineyard treatments, biotic factors, geographic location and vineyard factors including size, age, variety of grape and vintage year) (Barata et al. 2012). However, some species from *Ascomycetous* have been found worldwide such as *Hanseniaspora* *uvarum*, *Metschnikowia pulcherrima, Issatchenkia terricola* and *Issatchenkia orientalis*. *Saccharomyces cerevisiae* has hardly been found on sound grape berries, similar to some spoilage species such as *Zygosaccharomyces bailii*. However, damaged or rotted berries can provide high nutrient to favour the growth of *Ascomycetous* yeast. When whole bunch is harvested, some damaged berries may yield high numbers of the *Ascomycetous* yeast. Therefore, the isolation of *S. cerevisiae* and other spoilage species from grape berry is suspected to be related with grape health and sampling approach (Barata et al. 2012). *Ascomycetous* moulds and *Basidiomycetous* yeasts are considered residents on grape berries. These oligotrophic residents are thought to be adapted to the environment with poor nutrient availability (Loureiro et al. 2012). However, *Ascomycetous* yeasts are classified as copiotrophic opportunists, because they are rarely detected on immature grape berries but detected on grape berries with high nutrient availability (veraison, harvest or damaged grape berries). This is supported by the uneven distribution of *Ascomycetous* yeasts: microcolonies gather around the sites with most likely nutrient leaking from the berries (Loureiro et al. 2012). Although all *Ascomycetous* yeasts are opportunist, it is difficult to isolate some species on sound berries even at harvest time and the classical representative is *S. cerevisiae*. *S. cerevisiae* and its close relatives (other *Saccharomyces* yeast species) reside primarily in tree barks and soils as spores, where they are detected all year long. Only in the two months with grape growing from veraison to harvest or decay, the spores are dispersed onto grape berries by some vectors such as insects (Loureiro et al. 2012).

4. The succession of microorganisms during alcoholic fermentation: Yeast interactions

Yeasts on grape berries could survive and grow in grape must during alcoholic fermentation. Yeasts metabolize the main nutrients (sugars) to ethanol but also to other volatile compounds giving the wine its particular character. According to their fermentation capacity, competitiveness and contribution to wine, two main types of yeast can be considered in spontaneous wine fermentation: non-*Saccharomyces* yeasts and *Saccharomyces* yeasts. Non-*Saccharomyces* yeasts have lower fermentative capacity and are less competitive than *Saccharomyces* yeasts. However, today they are considered to increase wine complexity (Jolly et al. 2014, Mas et al. 2016).

The transformation of grape must into wine is a complex process that involves the sequential development of microbial species: mostly fungi, yeast, LAB and AAB. The microorganisms present in the berry surfaces are mainly yeasts. The microbiota associated to grapes varies constantly in response to grape variety, climatic conditions, viticultural practices, stage of ripening, physical damage (caused by moulds, insects and birds) and fungicides applied to vineyards (Pretorius et al. 1999). Although grape must is rather complete in nutrient content, its low pH and high sugar content, yields a selective media where only a few bacteria and yeast species can grow. Furthermore, the oenological practice of adding sulphur dioxide as antioxidant and antimicrobial preservative supposes an additional selection. This practice is meant to limit the growth of undesirable oxidative microbes and to prevent oxidation of grape must. Another important factor derives from the anaerobic conditions created during fermentation, especially at the start due to massive production of carbon dioxide

(Henschke 1997). As a result, the alcoholic fermentation of grape juice into wine can be regarded as a heterogenous microbial process. The number of yeasts on the grape berry and grape must change depending on the geographical situation of the vineyard, climatic conditions, sanitary state of the berries and pesticide treatments of the vineyard (Beltran et al. 2002, Romano et al. 2006, Padilla et al. 2016). At harvest time, the yeast population is quite complex and the major fermenting yeast, *S. cerevisiae*, is not very abundant (Beltran et al. 2002, Torija et al. 2001). Therefore, the non-*Saccharomyces* population is expected to be dominant in the early stages of grape must processing. Thus, non-*Saccharomyces* yeasts predominate during the early stages of wine fermentation (Fleet 2003), and finally the *S. cerevisiae* yeast species, the most alcohol tolerant yeast, dominates the fermentation. Besides, some species of non-*Saccharomyces* may be also be present during fermentation and in wine. Some of these yeast species should be considered as spoilage microorganisms because they produce metabolites with an undesirable impact (Pretorius 2000).

4.1. Non-Saccharomyces yeasts

The term of non-*Saccharomyces* has no taxonomical significance. According to Jolly et al. (2014), only yeast with a positive role in wine production is included in this description whereas spoilage yeasts such as *Dekkera*/*Brettanomyces* should not be included in this denomination. However, this is not a widespread concept and many authors refer to all species regardless their effects as non-*Saccharomyces*. In fact, many of those species considered as having a positive role in wine fermentation may have spoilage activity if their activity is prolonged during wine fermentation. Non-*Saccharomyces* yeasts are commonly known as wild yeasts, because they are mostly present in grapes and at the beginning of the fermentation (Fugelsang and Edwards 2007).

There are around 15 non-*Saccharomyces* yeast genera involved in wine fermentation. These are: *Dekkera* (anamorph *Brettanomyces*), *Candida/Starmerella*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora* (anamorph *Kloeckera*), *Kluyveromyces/Lachancea*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomycodes*, *Schizosaccharomyces*, *Torulaspora* and *Zygosaccharomyces* (Pretorius et al. 1999). Most of the non-*Saccharomyces* wine-related species show limited oenological aptitudes, such as low fermentation activity and low SO_2 resistance (Ciani et al. 2010). However, these species play an important role in the metabolic impact and aroma complexity of the final product. Furthermore, this species contribute to the enzymatic reactions, the main enzymatic activities described for some non-*Saccharomyces* species are protease, β-glucosidase, esterase, pectinase and lipase (Esteve-Zarzoso et al. 1998). Thus, the metabolic activities of various non-*Saccharomyces* yeast species during alcoholic fermentation have been matter of interest. Some yeast species such as *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia kluyveri* and *Lachancea thermotolerans* are currently sold as commercial starters for wine production. The assessment of *Hanseniaspora uvarum*, *Starmerella bacillaris* (previously *Candida zemplinina*) and other species are still on the way to balance their positive contribution and negative impact on wine (Masneuf-Pomarede et al. 2016). Another species, *Hanseniaspora vineae* has been successfully used in wines from Uruguay and Spain (Lleixà et al. 2016, Martín et al. 2016), although it is not present as commercial product yet.

The negative impact of non-*Saccharomyces* is mainly the low fermentative activity and high level of undesirable flavours. The low fermentative activity can be overcome by mixed fermentation with *Saccharomyces* yeasts. The undesirable flavours are solved by olfactive perception experiments to screen acceptable or neutral strains (Bely et al. 2013). The genetic and phenotypic performance of 115 *Hanseniaspora uvarum* strains were fully assessed by Albertin et al. (2016), as well as 63 *Starmerella bacillaris* strains by Englezos et al. (2015), both being designed for exploitation of the two common non-*Saccharomyces* yeast species isolated in wine fermentation.

4.2. Saccharomyces yeasts

Saccharomyces is the most useful and widely exploited yeast genus at industrial level. The taxonomy of the genus *Saccharomyces* has undergone many revisions and reclassifications. In fact, many species considered as non-*Saccharomyces* were initially classified as *Saccharomyces*. According to Barnett et al. (2000) and Naumov et al. (2000), *Saccharomyces* yeasts were taxonomically separated into three groups: *Saccharomyces sensu stricto* group, containing *S. cerevisiae*, *S. bayanus*, *S. paradoxus*, *S. pastorianus*, *S. cariocanus*, *S. mikatae* and *S. kudriavzevii*, *Saccharomyces sensu lato* group, including *S. dairensis*, *S. exiguus*, *S. unisporus*, *S. servazzi* and *S. castelli* and the third group with only *S. kluyveri*. Later, *Saccharomyces* genus involved four species isolated from natural habitats, *S. cariocanus, S. kudriavzevii*, *S. mikatae* and *S. paradoxus* and three species associated with industrial fermentation processes, *S. bayanus*, *S. cerevisiae* and *S. pastorianus* (Barrio et al. 2006). Nowadays only *S. arboricolus* (not a wine species), *S. eubayanus* and *S. uvarum* are considered pure species, and the other "species" are considered hybrids (Borneman and Pretorius, 2015). Physiological tests are not useful to differentiate the species of *Saccharomyces* and only their DNA sequences are reliable (Ribéreau-Gayon et al. 2006). In fact, the *Saccharomyces* species of oenological interest are *S. cerevisiae* and *S. bayanus*. *S. cerevisiae* is the main species in alcoholic fermentation, responsible for the metabolism of grape sugar to alcohol and carbon dioxide, but also important in the formation of secondary metabolites and conversion of grape aroma precursors to varietal wine aromas. *S. bayanus* has been used for alcoholic fermentation at low temperature since they are cryotolerant (Tamai et al. 1998); *S. bayanus* var. *uvarum* (synonym *S. uvarum*) is proved to be a good starter culture due to its reduced ethanol production, psychrophilism and acetate ester production (Masneuf-Pomarede et al. 2010, Bely et al. 2013, Csernus et al. 2014). In addition to these species, it is important to remember that haploid cells or spores from the *Saccharomyces sensu stricto* species are able to mate with each other resulting in viable hybrids (Querol et al. 2003). Hybrid strains of *S. bayanus* and *S. cerevisiae* and of *S. cerevisiae* and *S. kudriavzevii* have been isolated in alcoholic fermentations (González et al. 2006b). This phenomenon is a great possibility for the development of new species or strains. However, it is a source of taxonomic confusion due to the molecular and phenotypic classification analysis. For example, *S. cerevisiae* and *S. bayanus* are thought to be either two separate species, or the same species, that differ slightly from physiological aspects (Fugelsang and Edwards 2007). It is also known the physiological instability of strains belonging to *Saccharomyces sensu stricto* group (Ribéreau-Gayon et al. 2006).
Saccharomyces genus possesses series of unique characteristics that are not found in other genera. *Saccharomyces* yeasts have the ability to produce and accumulate ethanol even under aerobic conditions (Crabtree effect) (Marsit and Dequin 2015). Also, they have a high capacity to ferment sugars quickly and efficiently. This ability allows them to colonize sugar-rich media and efficiently overgrow other yeasts, which

are not so tolerant to alcohol (Barrio et al. 2006). However, the competition between *Saccharomyces* and non-*Saccharomyces* is more complex than the production of ethanol. In fact, there are many interactions, among them probably the most relevant cell-to-cell contact, nutrient limitation or the secretion of antimicrobial peptides (Wang et al. 2016). Although most of these mechanisms of interactions have been shown by analysing the growth on plates, recent findings relate that they induce the VBNC states that can end with the cell death (Branco et al. 2015, Wang et al. 2016). Nissen and Arneborg (2003) described also cell-to-cell contact as a possible inducer of lack of cultivability, although the reported mechanism seems to be limited to S101 *S. cerevisiae* strain, as other strains did not show the same mechanism (Wang et al. 2015).

4.3. Population dynamics of wine yeasts during spontaneous fermentation

The contribution of yeasts to wine is affected by their participation during the alcoholic fermentation (Comitini et al. 2011). Yeast species commonly found in spontaneous fermentation can be divided into three groups: aerobic yeast (*Pichia*, *Debaryomyces*, *Rhodotorula*, *Candida/ Starmerella*, *Cryptococcus*), apiculate yeast (*Hanseniaspora*) and fermentative yeast (*Kluyveromyces*, *Torulaspora*, *Metschnikowia*, *Zygosaccharomyces* and *Saccharomyces*). Generally, the succession of yeast involves the initial domination of aerobic and apiculate yeasts which are present on grape surface, their decrease and then the increase of fermentative yeasts during fermentation, and finally the domination of the *Saccharomyces* yeasts (Schütz and Gafner 1993, Torija et al. 2001, Beltran et al. 2002). The main yeast species isolated at the beginning of the fermentation generally belong to *Hanseniaspora*, *Metschnikowia* and *Starmerella* genera. The dominance of *S. cerevisiae* is needed to finish the alcoholic fermentation (Jolly et al. 2014). However, distinct fermentation dynamics are the result of the fermentation conditions and the relative levels of the main yeast species present. For instance, *Hanseniaspora* persists longer in fermentations at low temperature (Andorrà et al. 2010b); *Zygosaccharomyces bailii* leads botrytis-affected spontaneous fermentation (Nisiotou et al. 2007); *Pichia kudriavzevii* emerges along with *Saccharomyces* when relative low ethanol (9%) was obtained at the end of fermentation (Wang and Liu 2013); *Starmerella (Candida)* has been reported to codominate at late stages of fermentation (Llauradó et al. 2002) or to finish alcoholic fermentation (Clemente-Jimenez et al. 2004).

Furthermore to the succession of different yeast species during wine fermentation, a dynamic change of strains within each species is also evident, based on molecular techniques for strain differentiation (Fleet 2003). For *S. cerevisiae*, some dominant or codominant strains have been found (Sabate et al. 1998, Torija et al. 2001), and in some cases where a single strain dominates the killer phenotype may be present (Schuller et al. 2005). Strain diversity of non-*Saccharomyces* species has also been reported but focused on their oenological interest rather than in the dynamic changes (Capece et al. 2005, Masneuf-Pomarede et al. 2015, Albertin et al. 2016).

5. Control of fermentation: from spontaneous to inoculated fermentations

Winemakers have traditionally seen non-*Saccharomyces* yeast as a source of wine spoilage. The main way for microbiological control in fermentations is the use of starter cultures. In winemaking, the most common yeast used as starter culture is *S. cerevisiae.* The development of cellar-friendly Active

Dry Wine Yeast (ADWY) has extended its use in wine production, helping the winemaker to control of fermentation. The selection of yeast to be used as starter cultures has been developed using different tests and criteria. Nowadays, many different ADWY are commercially available. These yeasts are meant to increase aromatic expression, resistance to ethanol, low or high temperature, etc., but all of them with good fermentation potential and generally sufficient to complete the alcoholic fermentation.

Furthermore, yeasts not only lead the alcoholic fermentation, but also have an important role in wine quality. The activity of different yeast species and strains has an important effect on the organoleptic profiles of wine increasing its complexity and sensory richness (Ribereau Gayon et al. 2006). Presently, wine producers use commercial starters of *S. cerevisiae* to ensure the control of fermentation and produce a predictable and reproducible wine. A side effect of the widespread practice is the elimination of the participation of native microbiota. This limited participation might result in wines with similar sensory and analytical properties, depriving them from the, complexity, variability and personality, which define the typicality of a wine (Fleet 1993). Thus, the use of indigenous or native yeasts can be a tool to protect the authenticity; since it has been presented that microbial diversity is distinctive for a given area (Bokulich et al. 2014, Setati et al. 2015). The microbial population characteristic of a given area can be defined as the **microbial fingerprint**. This microbial population will develop a distinctive character in the wine, measurable by the various components (molecules) that each microorganism leaves that we can define as the **microbial footprint**.

The different microbial footprint will be related to the presence of these microorganisms during the winemaking process. The knowledge on the evolution of yeast populations during alcoholic fermentation has been going on, since the microbiology got the appropriate methods. Obviously, as techniques have evolved, knowledge has been completed. Despite the fact that the populations of *Saccharomyces* are very low in grapes (Beltran et al. 2002), their development during the alcoholic fermentation and the extensive use of ADWY have turned *S. cerevisiae* as the most common “cellar-resident yeast” (Beltran et al. 2002, Bokulich et al. 2014). Thus, the populations associated with the grapes change with the cellar environment (presses, pumps, tanks) contact, where they joint the resident microbiota. This microbiota is not usually found in new wineries with equipment without previous use (Constanti et al. 1997).

In spontaneous fermentations, the native microbiota proliferate for several days and produce various compounds that could improve the organoleptic quality of the wines or at least give the wines a specific flavour. When the activities of these yeasts have been analysed, it has been detected the presence of enzymatic activities of great interest: esterases, beta-glucosidase, pectinases, etc. (Jolly et al. 2014). Additionally, they may cause ethanol reduction (Gonzalez et al. 2013, Contreras et al. 2014), which has been proposed as a key objective in the current winemaking due to the increased concentration of sugars, among other effects, derived from climate change (Mira de Orduna 2010). Despite these favourable aspects, the traditional bias of winemakers against non-*Saccharomyces* yeast has limited their use. However, in recent years, there is an increasing interest in selecting non-*Saccharomyces* yeasts to be used with *S. cerevisiae.* Thus, the key role of *S. cerevisiae* during alcoholic fermentation has been challenged (Fleet 2003, Jolly et al. 2014).

The positive effects on wine quality are the main goal for the selection of non-*Saccharomyces* yeast. These include either the production of new aromas or the removal of detrimental compounds that would decrease the wine quality. *Torulaspora delbrueckii* reduces the volatile acidity that is normally produced during winemaking (Renault et al. 2009) and has proved appropriate for the fermentation of botrytised grapes (Bely et al. 2008). Nowadays, it is possible to find various commercial preparations of this yeast. Another commercially available non-*Saccharomyces* yeast is *Metschnikowia pulcherrima*, which is recommended for the production of some aromas based on thiols and terpenes in white wines (González-Royo et al. 2015). Finally, another yeast available is *Lachancea thermotolerans*, for its production of lactic acid and glycerol (Gobbi et al. 2013). Although there are still few commercial preparations of non-*Saccharomyces* yeasts, they will probably increase in the near future. These include *Starmerella bacillaris* that produces large amounts of glycerol (Ciani and Ferraro 1996) and also because of its fructophilic character, which favours the end of fermentation (Soden et al. 2000). Other non-*Saccharomyces* species that can be expected in commercial preparations are the typical apiculate yeasts from the *Hanseniaspora* genus, such as *H. uvarum* (Andorrà et al. 2010c), *H. vinae* (Medina et al. 2013) and *H. guilliermondii* (Moreira et al. 2008). Other species that can have some oenological interest are species of the genera *Hansenula, Pichia, Schizosaccharomyces, Zygosaccharomyces,* etc., although its possible commercial development seems unlikely (Jolly et al. 2014). Nevertheless, pure culture fermentations with non-*Saccharomyces* wine yeast generally increase metabolite contributions to noticeable negative levels and poor fermentation activities that generally exclude their use as single starter cultures. The most important spoilage metabolites produced by non-*Saccharomyces* yeast are acetic acid, acetoin, acetaldehyde and ethyl acetate (Ciani et al. 2010).

However, the use of non-*Saccharomyces* yeast in the production of wine has the goal to increase some characteristics of the final product, yet it does not solve the main problem induced by the massive use of ADWY: the uniformity observed in inoculated wines. Some winemakers have eliminated or reduced the amount of starter cultures used in the production of "natural" wine to increase the effect of the native microbiota. This practice increases the risks of uncontrolled fermentations, which may lead to economical losses as these wines may have much higher risks of presenting different levels of spoilage that will not be acceptable for the consumer.

The recommended solution to fight this uniformity is to exploit indigenous yeasts. Some years ago, different yeast producers developed commercial "local selection" yeasts in an attempt to protect the genuineness and authenticity of wines. However, in all cases the focus was on strains of *S. cerevisiae*. This solution defends the policy of *terroir* and typicality by using these starter cultures from local selection. Therefore, the use of oenologically competent indigenous yeasts as suitable inocula for the production of conventional or organic wines can achieve this goal.

6. Spoilage microorganisms in wine making

In the wine industry, where alcoholic fermentation is conducted by many microorganisms, it is difficult to distinguish between beneficial fermenting activity and spoilage activity. Microorganisms can spoil wines at several stages during production. Any inappropriate grow of microorganisms may produce undesirable flavours.

Wine that is exposed to air may develop fermentative or oxidative yeasts on its surface, usually species of *Candida* and *Pichia* (Fleet 2003). These species oxidise ethanol, glycerol and acids, giving wines with unacceptably high levels of acetaldehyde, esters and acetic acid. Other wines can also be spoiled by fermentative species of *Zygosaccharomcyes*, *Dekkera* (anamorph *Brettanomyces*), *Saccharomyces* and *Saccharomycodes*. In addition to causing excessive carbonation, sediments and haze, these species produce estery and acid off-flavours (Sponholz 1993).

The winemaker's most feared spoilage yeast is *Dekkera*/*Brettanomyces*. This yeast produces off-flavours due to the synthesis of tetrahydropyridines and volatile phenols (4-ethylguaiacol and 4-ethylphenol). Generally the production of these phenolic off-odours is noticed under a broad range of descriptors such as "barnyard-like, mousy, horsey, leather and pharmaceutical" (Grbin and Henschke 2000, Du Toit and Pretorius 2000). Among the species of this genus, *Dekkera bruxellensis* is the most representative in wines (Rodrigues et al. 2001). Furthermore, it has been found that other species are able to produce volatile phenols, such as *Pichia guilliermondii*, which has the ability to produce 4-ethylphenol with efficiencies as high as those observed in *D. bruxellensis* (Dias et al. 2003).

Pichia anomala, *Metschnikowia pulcherrima* and *H. uvarum* are known for producing high levels of ethyl acetate and acetic acid before and during initial fermentation steps, leading to serious wine deterioration (Romano et al. 1992, Plata et al. 2003).

Spoilage species of LAB and AAB may grow in different stages of wine making, wines during storage in the cellar and after bottling (Sponholz 1993, Fuselsang 1997, Fleet 1998, Du Toit and Pretorius 2000). LAB can spoil wine during winemaking or during maturation and bottle aging. In the first case, bacteria can start performing the malolactic fermentation too early, before all the sugars have been consumed by yeasts. The fermentation of these carbohydrates by LAB leads to the production of lactic acid as major metabolite, but acetic acid, ethanol and CO_2 are also produced. Ideally during wine aging, no yeasts or bacteria should survive in wine. Not all the strains spoil wine, most depreciations and diseases are related to lactobacilli and pediococci, but they are normally destroyed during wine production. However, some strains demonstrate abnormal tolerance to the medium, especially to the ethanol concentration. Other undesirable compounds which are consequence of the LAB metabolism are the biogenic amines and ethylcarbamate (Lonvaud-Funel 1999). These metabolites do not have an impact on the aroma of the wine, but they are considered as pernicious for the health of the wine consumer.

The AAB can also spoil wines at many stages during the winemaking process. AAB that are naturally occurring in grape can survive in winemaking processes, depending on the environmental conditions and the technological practices carried out. Moreover, equipment and instruments used during wine making could be a good vehicle of AAB to contaminate the product in which the hygienic conditions are disregarded. The AAB isolated from grapes of different origins include the species of *Acetobacter, Ameyamaea, Asaia, Gluconobacter* and *Komagataeibacter* genus (Joyeux et al. 1984, González et al. 2005, Prieto et al. 2007, Valera et al. 2011, Barata et al. 2012, Mateo et al. 2014). On the other hand, the present view of microbial species associated with grapes, must and wines is much more complex than it has been previously described in early studies based on culture-dependent methods (Portillo and Mas 2016).

The AAB species found on grapes or in grape must show differences from those in wine, depending on the differences in environmental conditions. Recent studies based on next generation sequencing technologies suggest that AAB are more abundant than previously thought during wine fermentations, independently of the grape variety (Portillo and Mas 2016). AAB that are usually involved in the wine spoilage are strains belonging to the genera *Acetobacter, Gluconobacter, Gluconacetobacter, Komagataeibacter* and *Asaia*.

Finally, filamentous fungi can also impact on wine production at several stages: spoilage of the grapes in the vineyard, production of mycotoxins in grapes and their transfer to wines, production of metabolites that enhance or inhibit the growth of wine yeast and malolactic bacteria, and cause the earthy, corky taints in wines after grow in grapes, corks and wine barrels (Fleet 2003). In order to prevent wine spoilage, hygienic conditions should be controlled during wine production. Although high hygienic conditions lead to limit the contaminant microorganisms, additional applications are mostly necessary to decrease the risk of spoilage. Sulphur dioxide (SO_2) is the one of the most efficient additives used for the prevention of wine spoilage. The effects of SO_2 depend on the kinds of organism to be suppressed and also pH value and sugar content of wine. 75 to 200 ppm sulfur dioxide is enough to inactivate spoilage microorganisms in must, while low concentrations of sulfur dioxide have minimal effect on *A. pasteurianus* strain (Du Toit et al. 2005). On the other hand, some metabolites synthesized by AAB, such as acetaldehyde from ethanol and dihydroxyacetone from glycerol, bind SO_2 and reduce the antimicrobial effect of this compound (Ribéreau-Gayon et al. 2000, Valera et al. 2017). In recent years, there has been a growing interest to develop emerging preservation technologies that can replace or complement the action of SO_2, since it might cause negative effects on health. These alternatives include the addition of antimicrobial agents (silver nanoparticles, bacteriocins, polyphenols etc.) and the application of physical methods (high pressure, low electric current, pulsed electric field, pulsed light, ultrasound, UV and e-beam irradiation, etc.) (García-Ruiz et al. 2015, Morata et al. 2017).

Acknowledgements

This manuscript has been prepared a within the framework of the SUMCULA and OENOBIO Erasmus + Programme Projects (2017-1-SE01-KA203-034570 and 2018-1-FR01-KA203-047839 respectively).

References

Aigle, M., Erbs, D. and Moll, M. (1984). Some molecular structures in the genome of larger brewing yeast. American Society of Brewing Chemists 42: 1-7.

Albertin, W., Miot-Sertier, C., Bely, M. and Mostert, T.T. (2016). *Hanseniaspora uvarum* from winemaking environments show spatial and temporal genetic clustering. Frontiers in Microbiology 6: 1569.

Andorrà, I., Esteve-Zarzoso, B., Guillamón, J.M. and Mas, A. (2010a). Determination of viable wine yeast using DNA binding dyes and quantitative PCR. International Journal of Food Microbiology 144: 257-262.

Andorrà, I., Landi, S., Mas, A., Esteve-Zarzoso, B. and Guillamón, J.M. (2010b). Effect of fermentation temperature on microbial population evolution using culture-independent and dependent techniques. Food Research International 43: 773-779.

Andorrà, I., Berradre, M., Rozés, N., Mas, A., Guillamón, J.M. and Esteve-Zarzoso, B. (2010c). Effect of pure and mixed cultures of the main yeast species on grape must fermentations. European Food Research and Technology 231: 215-224.

Andorrà, I., Monteiro, M., Esteve-Zarzoso, B., Albergaria, H. and Mas, A. (2011). Analysis and direct quantification of *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* populations during alcoholic fermentation by fluorescence in situ hybridisation, flow cytometry and quantitative PCR. Food Microbiology 28: 1483-1491.

Barata, A., Malfeito-Ferreira, M. and Loureiro, V. (2012). Changes in sour rotten grape berry microbiota during ripening and wine fermentation. International Journal of Food Microbiology 154: 152-161.

Barnett, J. A., Payne, R.W., Yarrow, D. (2000). Yeasts: Characteristics and Identification (3rd ed.). Cambridge University

Press Cambridge, UK.

Barrio, E., González, S.S., Arias, A., Belloch, C. and Querol, A. (2006). Molecular Mechanisms Envolvedin the Adaptative Evolution of Industrial Yeasts. In: Yeast in Food Beverages (Ed. A. Querol and G. H. Fleet). Springer, Berlin, pp. 153-174.

Bartowsky, E.J., Xia, D., Gibson, R.L., Fleet, G.H. and Henschke, P.A. (2003). Spoilage of bottled red wine by acetic acid bacteria. Letters in Applied Microbiology 36: 307-14.

Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamon, J.M., Rozes, N. and Mas, A. (2002). Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. Systematic and Applied Microbiology 25: 287-293.

Bely, M., Stoeckle, P., Masneuf-Pomarède, I. and Dubourdieu, D. (2008). Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. International Journal of Food Microbiology 122: 312-320.

Bely, M., Renault, P., da Silva, T., Masneuf-Pomarede, I., Albertin, W., Moine, V., Coulon, J., Sicard, D., de Vienne, D. and Marullo, P. (2013). Non conventional yeasts and alcohol level reduction. Conference "Alcohol Level Reduction in Wine" 3-37.

Bokulich, N.A., Thorngate, J.A., Richardson, P.M. and Mills, D.A. (2014). Microbial biogeography of wine grapes in conditioned by cultivar, vintage, and climate. Proceedings of the National Academy of Sciences 111(1): 139-148.

Borneman, A.R. and Pretorius, I.S. (2015). Genomic insights into the *Saccharomyces sensu stricto* complex. Genetics, 199: 281-291.

Branco, P., Viana, T., Albergaria, H. and Arneborg, N. (2015). Antimicrobial peptides (AMPs) produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora guilliermondii* cells. International Journal of Food Microbiology 205: 112-118.

Capece, A., Fiore, C., Maraz, A. and Romano, P. (2005). Molecular and technological approaches to evaluate strain biodiversity in *Hanseniaspora uvarum* of wine origin. Journal of Applied Microbiology 98: 136-144.

Cappello, M.S., Stefani, D., Grieco, F., Logrieco, A. and Zapparoli, G. (2008). Genotyping by Amplified Fragment Length Polymorphism and malate metabolism performances of indigenous *Oenococcus oeni* strains isolated from Primitivo wine. International Journal of Food Microbiology 127: 241-245.

Ciani, M. and Ferraro, L. (1996). Enhanced glycerol content in wines made with immobilized *Candida stellata* cells. Applied and Environmental Microbiology 62: 128-132.

Ciani, M., Comitini, F., Mannazzu, I. and Domizio, P. (2010). Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Research 10: 123-133.

Clemente-Jiménez, J., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Heras-Vázquez, F.J.L. and Rodríguez-Vico, E. (2004). Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. Food Microbiology 21: 149-155.

Cocolin, L. and Mills, D.A. (2003). Wine yeast inhibition by sulfur dioxide: a comparison of culture-dependent and independent methods. American Journal of Enology and Viticulture 54(2): 125-130.

Cocolin, L., Pepe, V., Comitini, F., Comi, G. and Ciani, M. (2004). Enological and genetic traits of *Saccharomyces cerevisiae* isolated from former and modern wineries. FEMS Yeast Research 5: 237-245.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M. and Tiedje, J.M. (2005). The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Research 33: 294-296.

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. and Ciani, M. (2011). Selected non-*Saccharomyce*s wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiology 28: 873-882.

Constantí, M., Poblet, M., Arola, L., Mas, A. and Guillamón, J.M. (1997). Analysis of yeast populations during alcoholic fermentation in a newly established winery. American Journal of Enology and Viticulture 48: 339-344.

Contreras, A., Hidalgo, C., Henschke, P.A., Chambers, P.J., Curtin, C. and Varela, C. (2014). Evaluation of non *Saccharomyces* yeasts for the reduction of alcohol content in wine. Applied and Environmental Microbiology 80(5): 1670-1678.

Csernus, O., Pomázi, A. and Magyar, I. (2014). Isolation, characterization, and selection of wine yeast strains in etyek-buda wine district, Hungary. Acta Alimentaria 43(3): 489-500.

Dias, L., Dias, S., Sancho, T., Stender, H., Querol, A., Malfeito-Ferreira, M. and Loureiro, V. (2003). Identification of yeasts originated from wine related environments and capable of producing 4- ethylphenol. Food Microbiology 20: 567-574.

Du Toit, M. and Pretorius, I.S. (2000). Microbial spoilage and preservation of wine: Using weapons from Nature's own arsenal—A Review. South African Society for Enology & Viticulture 21: 74-96.

Du Toit, W.J., Pretorius, I.S. and Lonvaud-Funel, A. (2005). The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of *Acetobacter pasteurianus* and a strain of *Brettanomyces bruxellensis* isolated from wine. Journal of Applied Microbiology 98: 862-871.

Dubourdieu, D., Sokol, A., Zucca, J., Thalouarn, P., Datee, A. and Aigle, M. (1987). Identification the souches de levures isolées de vins par l'analyse de leur DNA mitochondrial. Conn Vigne Vin 4: 267-278.

Englezos, V., Rantsiou, K., Torchio, F., Rolle, L., Gerbi, V. and Cocolin, L. (2015). Exploitation of the non-Saccharomyces yeast *Starmerella bacillaris* (synonym *Candida zemplinina*) in wine fermentation: physiological and molecular characterizations. International Journal of Food Microbiology 199: 33-40.

Esteve-Zarzoso, B., Manzanares, P., Ramón, D. and Querol, A. (1998). The role of non-*Saccharomyces* yeasts in industrial winemaking. International Microbiology 1: 143-148.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. International Journal of Systematic Bacteriology 49: 329-337.

Esteve-Zarzoso, B., Peris-Torán, M.J., Ramón, D. and Querol, A. (2001). Molecular characterisation of *Hanseniaspora* species. Antonie Van Leeuwenhoek 80: 85-92.

Fleet, G.H. (1993). Wine microbiology and biotechnology. Harwood, Chur, Switzerland.

Fleet, G.H. (1998). Microbiology of alcoholic beverages. In: Microbiology and Fermented Foods (Ed. B. J. Wood). Blackie Academic & Professional, London, England, pp. 217-262.

Fleet, G.H. (2003). Yeast interactions and wine flavour. International Journal of Food Microbiology 86: 11-22.

Fugelsang, K.C. (1997). Wine Microbiology. Chapman & Hall, New York, USA.

Fugelsang, K.C. and Edwards, C.G. (2007). Wine microbiology. Practical Applications and Procedures. Springer Science Business Media, LLC, New York, USA.

García-Ruiz, A., Crespo, J., Lopez-de-Luzuriaga, J.M., Olmosi M.E., M. Monge, M., Rodríguez-Alfaro, M.P., Martín-Alvarez, P.J., Bartolome, B. and Moreno-Arribas, M.V. (2015). Novel biocompatible silver nanoparticles for controlling the growth of lactic acid bacteria and acetic acid bacteria in wines. Food Control 50: 613-619.

Gevers, D., Huys, G. and Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. FEMS Microbiology Letters 205: 31-36.

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. and Ciani, M. (2013). *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: a strategy to enhance acidity and improve the overall quality of wine. Food Microbiology 33: 271-281.

González, A., Hierro, N., Poblet, M., Mas, A. and Guillamón, J.M. (2005). Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. International Journal of Food Microbiology 102: 295-304.

González, A., Guillamón, J.M., Mas, A. and Poblet, M. (2006a). Application of molecular methods for routine identification of acetic acid bacteria. International Journal of Food Microbiology 108: 141-146.

González, S.S., Barrio, E., Gafner, J. and Querol, A. (2006b). Natural hybrids from *Saccharomyces cerevisiae, Saccharomyces bayanus,* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Research 6: 1221-1223.

González, A., Mas, A. (2011) Differentiation of acetic acid bacteria based on sequence analysis of 16S-23S rRNA gene internal transcribed spacer sequences. International Journal of Food Microbiology, 147, 217-222,

Gonzalez, R., Quiros, M. and Morales, P. (2013). Yeast respiration of sugars by non-*Saccharomyces* yeast species: A promising and barely explored approach to lowering alcohol content of wines. Trends in Food Science and Technology 29: 55-61.

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., EsteveZarzoso, B., Mas, A., Miquel Canals, J. and Zamora, F. (2015). Oenological consequences of sequential inoculation with nonSaccharomyces yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. European Food Research and Technology 240: 999-1012.

Grbin, P.R. and Henschke, P.A. (2000). Mousy off-flavour production in grape juice and wine by *Dekkera* and *Brettanomyces* yeasts. Australian Journal of Grape and Wine Research 6: 255-262.

Guillamón, J.M., Barrio, E. and Querol, A. (1996). Characterization of wine yeast strains of the *Saccharomyces* genus on the basis of molecular markers: relationships between genetic distance and geographic or ecological origin. Systematic and Applied Microbiology 19: 122-132.

Guillamón, J.M., Sabate, J., Barrio, E., Cano, J. and Querol, A. (1998). Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. Archives of Microbiology 169: 387-392.

Gullo, M., Caggia, C., De Vero, L. and Giudici, P. (2006). Characterization of acetic acid bacteria in ‘‘traditional balsamic vinegar’’. International Journal of Food Microbiology 106: 209-212.

Henschke, P.A. (1997). Yeast Sugar Metabolism. In: Wine yeast (Ed. K. D. Entian). Zimmermann FK, Technomic Publishing: Lancaster, Pennsylvania, USA, pp. 527-560.

Hidalgo, C., Vegas, C., Mateo, M., Tesfaye, W., Cerezo, A.B., Callejón, R.M., Poblet, M., Guillamon, J.M., Mas, A. and Torija, M.J. (2010). Effect of barrel design and the inoculation of *A. pasteurianus* in wine vinegar production. International Journal of Food Microbiology 141: 56-62.

Hierro, N., Esteve-Zarzoso, B., González, A., Mas, A. and Guillamón, J.M. (2006). Real-time quantitative PCR (QPCR) and reverse transcription-QPCR (RT- QPCR) for the detection and enumeration of total yeasts in wine. Applied and Environmental Microbiology 72: 7148-7155.

Jolly, N.P., Varela, C. and Pretorius, I.S. (2014). Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Research 14: 215-237.

Joyeux, A., Lafon-Lafourcade, S. and Ribéreau-Gayon, P. (1984). Evolution of acetic Acid bacteria during fermentation and storage of wine. Applied and Environmental Microbiology 48: 153-156.

Kurtzman, C.P. and Robnett, C.J. (1998). Identification and phylogeny of ascomycetous yeast from analysis of nuclear large subunit 26S ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73: 331-371.

Le Jeune, C. and Lonvaud-Funel, A. (1997). Sequence of DNA 16S/23S spacer region of *Leuconostoc oenos* (*Oenococcus oeni*): application to strain differentiation. Research in Microbiology 148: 79-86.

Lieckfieldt, E., Meyer, W. and Börn, T. (1993). Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. Journal of Basic Microbiology 33: 413-426.

Llauradó, J., Rozés, N., Bobet, R., Mas, A. and Constantí, M. (2002). Low temperature alcoholic fermentations in high sugar concentration grapemusts. Journal of Food Science 67: 268-273.

Lleixà, J., Martín, V., Portillo, M.C., Carrau, F., Beltran, G. and Mas, A. (2016). Comparison of fermentation and wines produced by inoculation of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. Frontiers in Microbiology 7: 338.

Lonvaud-Funel, A. (1999). Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek 76: 317-331.

Loureiro, V., Ferreira, M.M., Monteiro, S. and Ferreira, R.B. (2012). The microbial community of grape berry. In: The Biochemistry of the grape berry (Ed. H. Gerós, M. M. Chanves and S. Delrot). Bentham Science publishers, Sharjah, United Arab Emirates, pp. 241-268.

Maqueda, M., Zamora, E., Rodríguez-Cousiño, N. and Ramírez, M. (2010). Wine yeast molecular typing using a simplified method for simultaneously extracting mtDNA, nuclear DNA and virus dsRNA. Food Microbiology 27: 205-209.

Marsit, S.M. and Dequin, S. (2015). Diversity and adaptive evolution of *Saccharomyces* wine yeast: a review. FEMS Yeast Research 15: fov067.

Martín, V., Mas, A., Carrau, F., Dellacasa, E. and Boido, E. (2016). Effect of yeast assimilable nitrogen on the synthesis of phenolic aroma compounds by *Hanseniaspora vineae* strains. Yeast 33: 323-328.

Mas, A., Padilla, B., Esteve-Zarzoso, B., Beltran, G., Reguant, C. and Bordons, A. (2016). Taking advantage of natural biodiversity for wine making: The WILDWINE Project. Agriculture and Agricultural Science Procedia, 8: 4-9.

Masneuf, I. and Dubourdieu, D. (1994). Comparaison de deux techniques d’identification des souches de levures de vinification basées sur le polymorphisme de l’ADN génomique: réaction de polymérisationen chaine (PCR) et analyse des caryotypes (electrophorèse en champ pulsé). Journal International des Sciences de la Vigne et du Vin 28: 153-160.

Masneuf-Pomarede, I., Bely, M., Marullo, P., Lonvaud-Funel, A. and Dubourdieu, D. (2010). Reassessment of phenotypic traits for *Saccharomyces bayanus var. uvarum* wine yeast strains. International Journal of Food Microbiology 139: 79-86.

Masneuf-Pomarede, I., Juquin, E., Miot-Sertier, C., Renault, P., Laizet, Y.H., Salin, F., Alexandre, H., Capozzi, V., Cocolin, L., Colonna-Ceccaldi, B., Englezos, V., Girard, P., Gonzalez, B., Lucas, P., Mas, A., Nisiotou, A., Sipiczki, M., Spano, G., Tassou, C., Bely, M. and Albertin, W. (2015). The yeast *Starmerella bacillaris* (synonym *Candida zemplinina*) shows high genetic diversity in winemaking environments. FEMS Yeast Research 15(5): fov045.

Masneuf-Pomarede, I., Bely, M., Marullo, P. and Albertin, W. (2016). The genetics of non-conventional wine yeasts: current knowledge and future challenges. Frontiers in Microbiology 6: 1563.

Mateo, E., Torija, M.J., Mas, A. and Bartowsky, E.J. (2014). Acetic acid bacteria isolated from grapes of South Australian vineyards. International Journal of Food Microbiology 178: 98-106.

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M.E., Barquet, M., Gaggero, C., Dellacassa, E. and Carrau, F. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. Food Chemistry 141: 2513-2521.

Millet, V. and Lonvaud-Funel, A. (2000). The viable but non-culturable state of wine micro-organisms during storage. Letters in Applied Microbiology 30: 136-141.

Mira de Orduna, R. (2010). Climate change associated effects on grape and wine quality and production. Food Research International 43: 1844-1855.

Montrocher, R., Verner, M.C., Briolay, J., Gautier, C. and Marmeisse, R. (1998). Phylogenetic analysis of the *Saccharomyces cerevisiae* group based on polymorphisms of rDNA spacer sequences. International Journal of Systematic and Evolutionary Microbiology 48: 295-303.

Morata, A., Loira, I., Vejarano, R., Gonzalez, C., Callejo, M.J. and Suarez-Lepe, J.A. (2017). Emerging preservation technologies in grapes for winemaking. Trends in Food Science & Technology 67: 36-43.

Moreira, N., Mendes, F., Guedes de Pinho, P., Hogg, T. and Vasconcelos, I. (2008). Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. International Journal of Food Microbiology 124: 231-238.

Naumov, G.I., James, S.A., Naumova, E.S., Louis, E.J. and Roberts, I.N. (2000). Three new species in the *Saccharomyces sensu stricto complex: Saccharomyces cariocanus, Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. International Journal of Systematic and Evolutionary Microbiology 50: 1931-1942.

Nisiotou, A.A., Spiropoulos, A.E. and Nychas, G.E. (2007). Yeast community structures and dynamics in healthy and *Botrytis*-affected grape must fermentations. Applied and Environmental Microbiology 73(21): 6705-6713.

Nissen, P. and Arneborg, N. (2003). Characterization of early deaths of non- *Saccharomyces* yeasts in mixed cultures with *Saccharomyces cerevisiae*. Archives of Microbiology 180: 257-263.

Nocker, A. and Camper, A.K. (2006). Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied and Environmental Microbiology 72: 1997-2004.

Nocker, A., Cheung, C.Y. and Camper, A.K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. Journal of Microbiological Methods 67: 310-320.

Nogva, H.K., Drømtorp, S.M., Nissen, H. and Rudi, K. (2003). Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR. BioTechniques 34: 804-813.

Oliver, J.D. (2005). The viable but nonculturable state in bacteria. The Journal of Microbiology 43: 93-100.

Padilla, B., García-Fernández, D., González, B., Izidoro-Pacheco, I., Esteve-Zarzoso, B., Beltran, G. and Mas, A. (2016). Yeast biodiversity from DOQ priorat uninoculated fermentations. Frontiers in Microbiology 7: 930.

Plata, C., Millán, C., Mauricio, J.C. and Ortega, J.M. (2003). Formation of ethyl acetate and isoamylacetate by various species of wine yeasts. Food Microbiology 20: 217-224.

Poblet, M., Rozès, N., Guillamón, JM. and Mas, A. (2000). Identification of acetic acid bacteria by restriction fragment length polymorphism analysis of a PCR-amplified fragment of the gene coding for 16S rRNA. Letters in Applied Microbiology 31: 63-67.

Portillo, M.C. and Mas, A. (2016). Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing. LWT - Food Science and Technology 72: 317-321.

Pretorius, I.S., van der Westhuizen, T.J. and Augustyn, O.P.H. (1999). Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry. South African Journal of Enology and Viticulture 20: 61-70.

Pretorius, I.S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancientart of winemaking. Yeast 16: 675-729.

Prieto, C., Jara, C., Mas, A. and Romero, J. (2007). Application of molecular methods for analysing the distribution and diversity of acetic acid bacteria in Chilean vineyards. International Journal of Food Microbiology 115: 348-355.

Querol, A., Barrio, E., Huerta, T. and Ramón, D. (1992). Molecular monitoring of wine fermentations conducted by active dry yeast strains. Applied and Environmental Microbiology 58: 2948-2953.

Querol, A., Fernández-Espinar, M.T., del Olmo, M. and Barrio, E. (2003). Adaptive evolution of wine yeast. International Journal of Food Microbiology 86: 3-10.

Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P. and Comi, G. (2005). Culture dependent and independent methods to investigate the microbial ecology of Italian fermented sausages. Applied and Environmental Microbiology 71: 1977-1986.

Renault P, Miot-Sertier C, Marullo P, Hernandez-Orte P, Lagarrigue L, Lonvaud-Funel, A. and Bely, M. (2009). Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. International Journal of Food Microbiology 134: 201-210.

Renouf, V., Claisse, O. and Lonvaud-Funel, A. (2005). Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. Australian Journal of Grape and Wine Research 11: 316-327.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. and Lovaud, A. (2000). Handbook of Enology. vol. 1. The Microbiology of Wine and Vinifications. John Wiley & Sons Ltd., Chichester, England.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. and Lovaud, A. (2006). Handbook of Enology vol. 1. The Microbology of wine and Vinifications (2nd ed). John Wiley & Sons Ltd., West Sussex, England.

Rodas, A.M., Ferrer, S. and Pardo, I. (2005). Polyphasic study of wine *Lactobacillus strains*: taxonomic implications. International Journal of Systematic Evolutionary Microbiology 55: 197-207.

Rodrigues, N., Gonçalves, G., Malfeito-Ferreira, M. and Loureiro, V. (2001). Development and use of a differential medium to detect yeasts of the genera *Dekkera/Brettanomyces*. International Journal of Food Microbiology 90: 588-599.

Romano, P., Suzzi, G., Comi, G. and Zironi, R. (1992). Higher alcohol and acetic acid production byapiculate wine yeasts. Journal of Applied Bacteriology 73: 126-130.

Romano, P., Capece, A. and Jespersen, L. (2006). Taxonomic and ecological diversity of food and beverage yeasts. In: Yeast in Food Beverages (Ed. A. Querol and G. H. Fleet). Springer, Berlin, Germany, pp. 55-82.

Rudi, K., Naterstad, K., Dromtorp, S.M. and Holo, H. (2005). Detection of viable and dead *Listeria monocytogenes* on gouda-like cheeses by real-time PCR. Letters in Applied Microbiology 40: 301-306.

Ruiz, A., Poblet, M., Mas, A. and Guillamon, J.M. (2000). Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S–23S rDNA intergenic spacer. International Journal of Systematic and Evolutionary Microbiology 50: 1981-1987.

Sabate, J., Cano, J., Querol, A. and Guillamon, J. M. (1998).

Diversity of *Saccharomyces* strains in wine fermentations: analysis for two consecutive years. Letters in Applied Microbiology 26: 452-455.

Schuller, D., Alves, H., Dequin, S. and Casal, M. (2005). Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde region of Portugal. FEMS Microbiology Ecology 51: 167-177.

Schütz, M. and Gafner, J. (1993). Analysis of yeast diversity during spontaneous and induced alcoholic fermentations. Journal of Applied Microbiology 75: 551-558.

Setati, M.E., Jacobson, D. and Bauer, F.F. (2015). Sequence-based analysis of the *Vitis vinifera* L. cv Cabernet Sauvignon grape must mycobiome in three south African vineyards employing distinct agronomic systems. Frontiers in Microbiology 6: 1358.

Soden, A., Francis, I.L., Oakey, H. and Henschke, P.A. (2000). Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. Australian Journal of Grape and Wine Research 6: 21-30.

Sponholz, W. (1993). Wine spoilage by microorganisms. In: Wine Microbiolgy and Biotechnology (Ed. G. H. Fleet). Harwood Academic Publishers, Chur, Switzerland, pp. 395-420.

Sun, S.Y., Gong, H. S., Liu, W.L. and Jin, C.W. (2016). Application and validation of autochthonous *Lactobacillus plantarum* starter cultures for controlled malolactic fermentation and its influence on the aromatic profile of cherry wines. Food Microbiology 55: 16-24.

Sunyer-Figueres M, Wang C, Mas A. 2018. Analysis of RNA stability for the detection and quantification of wine yeast by quantitative PCR. International Journal of Food Microbiology, 270, 1-4

Tamai, Y., Momma, T., Yoshimoto, H. and Kaneko, Y. (1998). Co-existence of two types of chromosome in the bottom fermenting yeast, *Saccharomyces pastorianus*. Yeast 14: 923-933.

Torija, M.J., Rozès, N., Poblet, M., Guillamón, J.M. and 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.

Valera, M.J., Federico Laich, F., Sara, S., González, S.S., Torija, M.J., Mateo, E. and Mas, A. (2011). Diversity of acetic acid bacteria present in healthy grapes from the Canary Islands. International Journal of Food Microbiology 151: 105-112.

Valera, M.J., Torija, M.J. and Mas, A. (2017). Detrimental Effects of Acetic Acid Bacteria in Foods. Chapter 12. In: Acetic Acid Bacteria: Fundamentals and Food Applications (Ed. I. Y. Sengun). CRC Press, Taylor & Francis Group, Boca Raton, pp. 299-320.

Vegas, C., Mateo, E., González, A., Jara, C., Guillamon, J.M., Poblet, M., Torija, M.J. and Mas, A. (2010). Population dynamics of acetic acid bacteria during traditional wine vinegar production. International Journal of Food Microbiology 138: 130-136.

Vigentini, I., Picozzi, C., Tirelli, A., Giugni, A. and Foschino, R. (2009). Survey on indigenous *Oenococcus oeni* strains isolated from red wines of Valtellina, a cold climate wine -growing Italian area. International Journal of Food Microbiology 136: 123-128.

Wang, C. and Liu, Y. (2013). Dynamic study of yeast species and *Saccharomyces cerevisiae* strains during the spontaneous fermentations of Muscat blanc in Jingyang, China. Food Microbiology 33: 172-177.

Wang, C., Mas, A. and Esteve-Zarzoso, B. (2015). Interaction between *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* during alcoholic fermentation. International Journal of Food Microbiology 206: 67-74.

Wang, C., Mas, A. and Esteve-Zarzoso, B. (2016). The Interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. Frontiers in Microbiology 7: 502.

Overview and new developments of Wine technology

Prof. Dr. Monika Christmann[1]*, Dr. Matthias Schmitt[2]
[1,2] Department of Enology, Hochschule Geisenheim University, Von-Lade-Straße 1, 65366 Geisenheim, Germany

Abstract: The global wine sector has experienced important changes over the last 50 years. While the World wine production remains quite stable the number of producing countries continues to increase, which causes growing competition.
Consumption becomes more globalized and consumption patterns have evolved from a traditional model (regular and substantial consumption) to a 'modern' model ('' fun '', more occasional drinking).
Regarding trade, nearly 40% of the wine consumed in the world comes from the world market, compared to 25% in the middle of the 2000s. New expectations and concerns of consumers are emerging as they are better informed and educated, with more knowledge and increased demands.
Wine production has not only a strong economic dimension, but also a territorial dimension as well as historical/cultural. For the future it will be essential to maintain a good balance between tradition and modernity.
3 major challenges for the vine and wine sector can be identified:

- improving the economic competitiveness in a more globalized and competitive market;
- Maintaining the quality and the identity of the products in their diversity by fulfilling the expectations of consumers;
- Respecting environmental concerns in terms of preservation of natural resources and the implications of climate change.

Keywords: Globalization, changing consumer expectations, natural resources, safe products, climate change

Over the last 4-5 decades the viti-vini sector has experienced dramatic changes.

The Wine market is an increasingly globalized sector as more and more countries produce wine. Wine and grape juice production is growing and becoming more diversified. As a result competition increases.

But also Wine consumption is getting more and more globalized because consumption patterns have changed: from a traditional model to a "modern model".

Consumers and citizens have new expectations and concerns as they are better informed and more and more educated. This results in new demands concerning wine quality and also product safety.

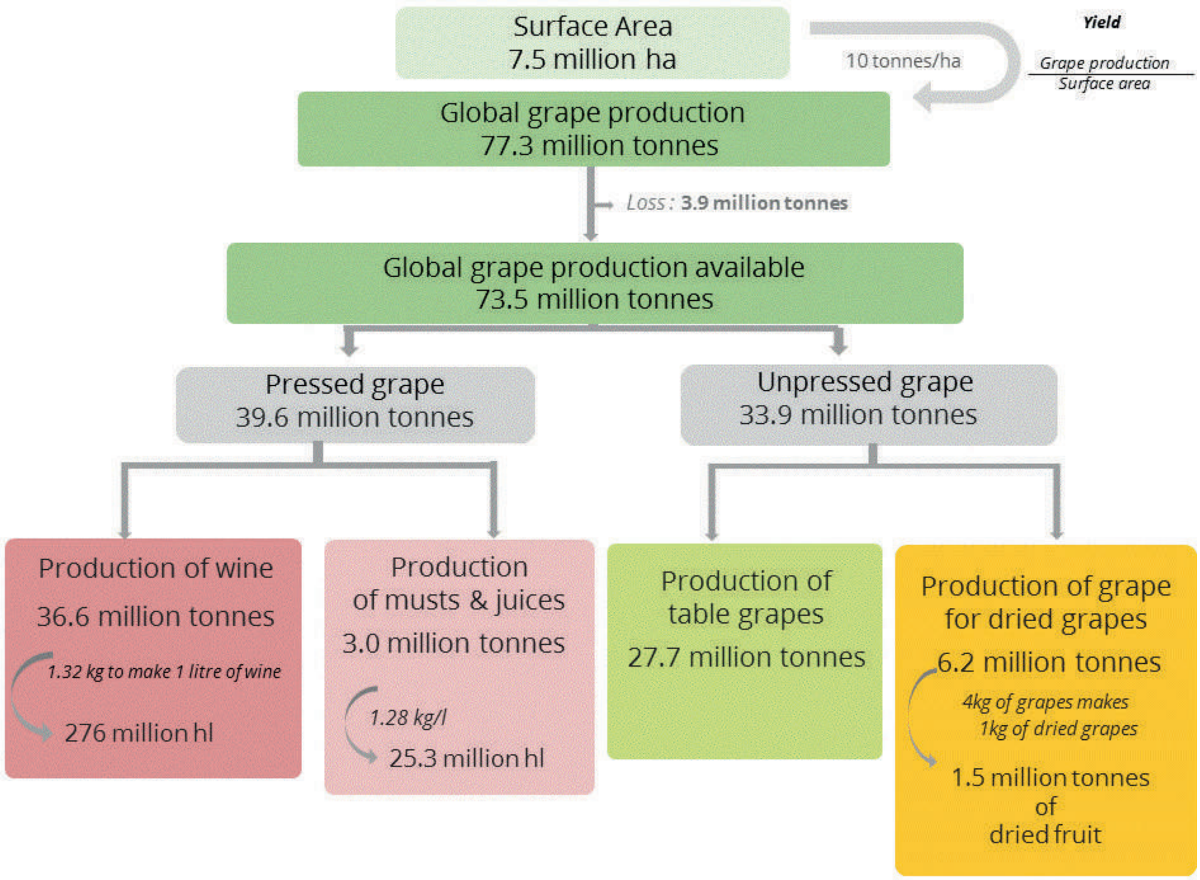
Trade is, as well, getting more and more globalized. While10 years ago 25% of the wine consumed was imported, this share reached 40% of the wine consumption nowadays.

*Corresponding author. E-mail: Monika.Christmann@hs-gm.de

Besides the market issues the global climate change is forcing wine producers to change their practices and technologies which might lead to a very emotional discussion about "industrial versus natural" wines. Particularly in view of wine being a cultural product with a long-lasting history some new technologies are often not accepted mainly by traditional producers but also by wine writers. This could be caused by a lack of information or missing fundamental enological knowledge.

Technical progress is adding to this discussion. Many players in the "wine world" are very conservative and find it difficult to accept new technologies. Quite often "traditional" is defined as "better" by overseeing that there has always been a need for treatments. Winemakers can only react to a specific need with the technical possibilities of the time. And these technical options have been optimized.

Before taking a closer look at these various topics it is interesting to see what happens to the 77 million tons of grapes grown in the world :



(Source: Internal communication OIV)

Under changing conditions it will be necessary to **optimize** all areas in the viti-vini sector. In detail this could be:

1. **Precision Viticulture**

   a. Digital technologies at the service of viticulture need to be implemented

   b. Communicating weather station

diffusing micro-climatic data (rainfall, temperature, hygrometry, etc.). which, once modeled, allow the winemaker to optimize its pest control strategy

c. Device for measuring the amount of sugar per berry for monitoring the ripening of plots, and determine the date of harvest.

- **The accountable management of water, effluent, by-products and waste**
- **New Resistant cultivars with tolerance to powdery/downy mildew or drought**
- **Rootstock breeding**
- **New fungicide or pesticides molecules**

2. **Oenology**

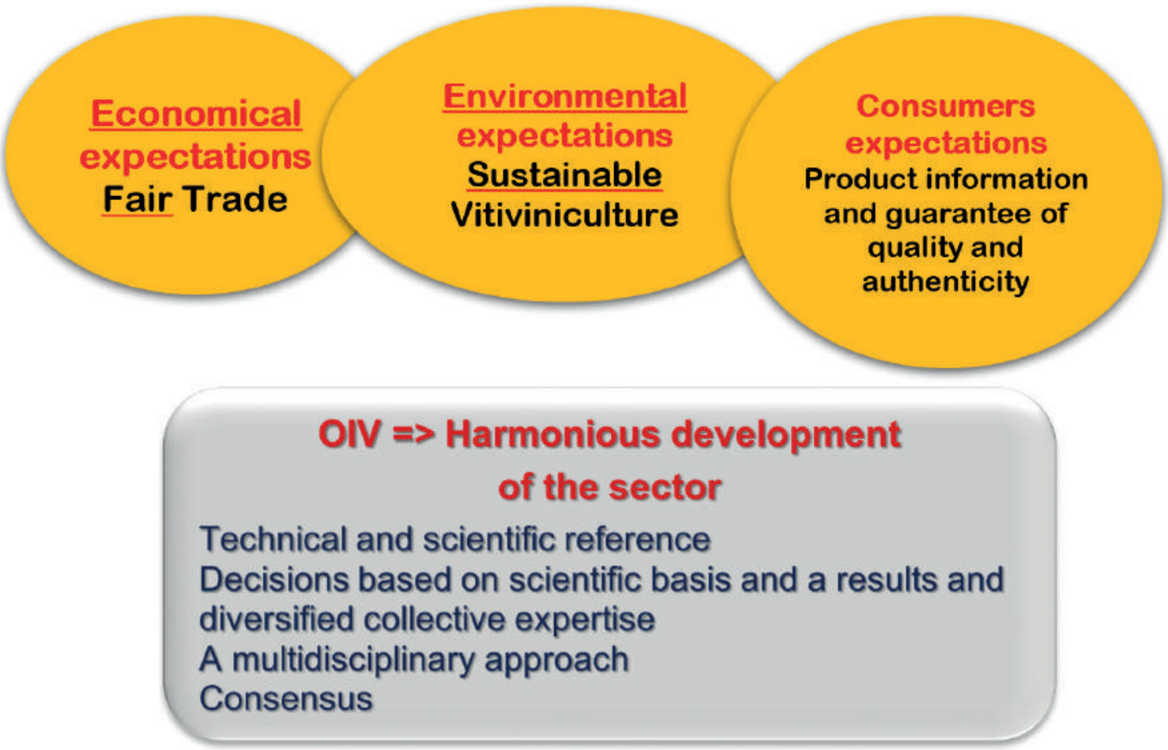
   1. **Decision making tools (Digitalization ?) according to the type of products expected**
   2. **Methods of analysis**
      - for grape variety
      - for authenticity of wine (origin)
   3. **Focus on physical process instead of chemical products**
      - de-alcoholisation, membrane techniques (acidification, gas management),
      - High Hydrostatic Pressure Technology on Wine to inactivate bacteria and yeasts for wine preservation
      - High-power ultrasounds during red wine vinification to increase the extraction of phenolic compounds
      - Pulsed electric fields technology (PEF) in wineries to control of microbial growth in grape wine
      - PEF pretreatment can increase phenolic compound and anthocyanin contents and can enhance color intensity
      - Thermovinication
      - Membrane processes

3. **Economy**

   1. **Stable and adapted regulatory framework for the**
      - Definition of the products
      - Conditions of production
      - Oenological practices
      - Taxation
      - Avoiding counterfeiting
   2. **Stable and adapted regulatory framework for the**
      - Definition of the products
      - Conditions of production
      - Oenological practices
      - Taxation
   3. **Modernisation of equipment in** Vineyards and wineries to reduce cost and meet new criteria.
   4. **Adapted Marketing**
      - Identification such as Geographical indication
      - Development of enotourism
      - Good knowledge of markets and new marketing strategies *(e-Commerce, internet….)*
   5. **Traceability for**
      - ensuring the health and safety of consumers
      - guarantying origin-logistics
      - avoiding counterfeiting.

In conclusion:

The international wine world is changing dramatically. Therefore it is increasingly important for the viti-vini-sector to work closer together in partnerships but also in the OIV, which as the international and intergovernmental organization is working hard in all of this segments to create an environment which is focusing on a sustainable and successful future for producers and consumers.



(Source: Internal communication OIV)

Wine Oxidation Measurements

Thi H. Nguyen and Andrew L. Waterhouse*
Viticulture and Enology, University of California, Davis, CA 95616, USA

Abstract: Wine oxidation appears to be mediated by iron cycling. There are two parts of the reaction, the oxidation of iron-2 by oxygen, and the reduction of iron-3 by phenolics. Here we investigate the reaction of iron-3 with phenolics. Iron-3 is reduced by pyrocgallol, but not catechol, showing that the higher reduction oxidation potential of pyrogallol alters the reaction. Catechol can reduce iron-3 when a nucleophile, here we used BSA, is present that can consume the quinone. The reduction of iron-3 accelerates at pH 3 compared to pH 4, the opposite effect observed in wine oxidation, indicating that this reaction is not rate limiting in wine oxidation. The quantity of iron reduced may reflect the oxidation potential of a wine.

Keywords: wine; oxidation; iron; nucleophiles; complexation

1. Introduction

Ingress of oxygen, whether during cellar operations or aging, changes the chemical profile of wine, consequently altering sensory attributes such as color, flavor, and mouthfeel. The cascade of chemical reactions constituting wine oxidation starts with the reduction of oxygen coupled to the oxidation of phenols through the redox cycling of iron between two oxidation states: Fe^{2+} and Fe^{3+} (Figure 1).(Danilewicz 2003, Danilewicz 2007, Danilewicz 2011, Danilewicz 2013) As Fe^{3+} is reduced to Fe^{2+}, the loss of electrons from phenols generates quinones, electrophiles that react with the nucleophilic species found in wine, namely thiols and other phenols.(Nikolantonaki and Waterhouse 2012, Waterhouse and Nikolantonaki 2015) So, an iron-free wine would not oxidize, but to date we have no means to remove iron completely.

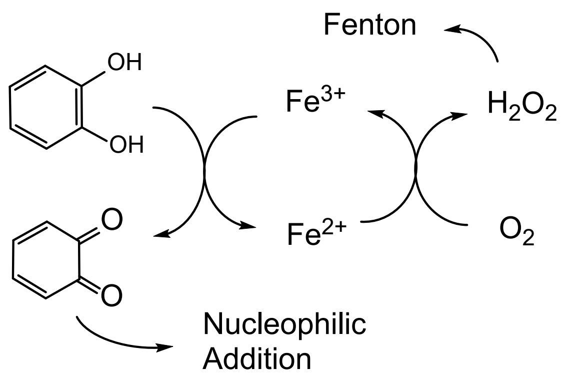


Figure 1. Reduction of oxygen coupled to phenol oxidation through the redox cycling of iron.(Nguyen and Waterhouse 2019)

The oxidation of Fe^{2+} to Fe^{3+} provides the electrons to convert oxygen into H_2O_2. (Danilewicz 2003, Danilewicz 2013, Waterhouse and Laurie 2006) Oxidation of Fe^{2+} to Fe^{3+} occurs again in the Fenton reaction, in which H_2O_2 decomposes to yield the hydroxyl radical ($HO^{\bullet}$). This highly unstable species reacts immediately with any substances present in solution in proportion to their concentrations. Given its relative abundance in wine, ethanol is a prime target for oxidation, and through a series of radical intermediates is converted

*alwaterhouse@ucdavis.edu, +1-530-752-4777

into acetaldehyde.(Buxton et al. 1988, Elias and Waterhouse 2010) In addition to impacting wine aroma, acetaldehyde can react with flavonoids such as anthocyanins and flavanols, to produce color-stable pigments and pyranoanthocyanins(Bakker and Timberlake 1997, de Freitas and Mateus 2011, Mateus et al. 2002, Timberlake and Bridle 1976) as well as ethylidene-bridged polymers.(Drinkine et al. 2007, Es-Safi et al. 1999, Es-Safi et al. 1999)

The various reactions of the wine oxidation pathway as described have been pieced together from numerous studies over several decades, though surprisingly only one study could be found that has confirmed all the steps from oxygen to acetaldehyde together. (Kreitman et al. 2013) However, in this study there was no means of regenerating Fe^{2+} from Fe^{3+}, meaning iron could not redox cycle and oxygen consumption and acetaldehyde production could not occur continuously.

This study undertakes preliminary investigation of the reaction of phenolics with iron-3 in model systems and using the FRAP assay to see if that reaction can be used to determine the oxidation potential of a wine.

2. Results

2.1. Reduction of Iron by Phenolics

The reduction of iron(III) to iron(II) was monitored spectrophotometrically(Nguyen and Waterhouse 2019) in oxygen-free model wines (12% ethanol, 8 g/L tartaric acid, and 0.1 mM iron(III) (5.5 mg/L)) comprising the experimental treatments. The effects of phenolic structure on iron reduction rates were evaluated first at pH 3.5 using the model phenolic compounds 4-methylcatechol and pyrogallol, representing odihydroxy phenols (e.g. catechin) and 1,2,3-trihydroxy phenols (e.g. epigallocatechin) respectively. The phenols were evaluated at 1.0 mM, approximating total phenol levels typical of white wines, and were used with and without equimolar benzenesulfinic acid, a model nucleophile capable of reacting with the oxidized quinone forms of phenols to “pull” the reaction forward, as do other nucleophiles in wine (e.g. SO_2, glutathione). The effect of copper was evaluated by the addition of 0.01 mM copper (0.6 mg/L).

The effects of pH were further evaluated using only the model wine system containing 4methylcatechol and benzenesulfinic acid, its pH adjusted to 3.0 and 4.0 in addition to pH 3.5. The rate of oxygen consumption was also monitored for this system at pH 3.0, 3.5, and 4.0 following aerial saturation (~8 mg/L oxygen). Pseudo-first order reaction rate constants were calculated for iron reduction and oxygen consumption and compared to ascertain the relationship between these two processes.

In model wine at pH 3.5, it was found 4-methylcatechol could not reduce iron to any significant extent without aid from the nucleophile benzenesulfinic acid (Figure 2). On the other hand, pyrogallol was able to reduce iron without benzenesulfinic acid, and even did so more rapidly in its presence. These differences in ability

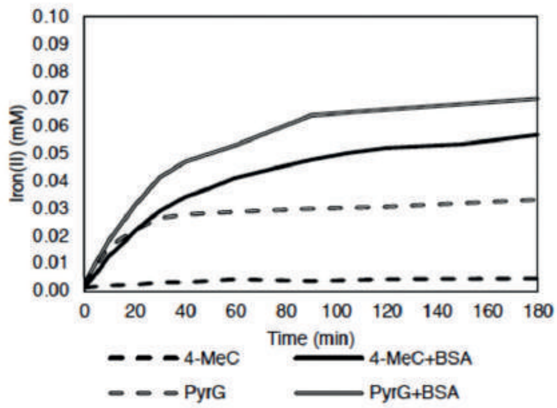


Figure 2. Iron reduction by 4-methylcatechol (4MeC) or pyrogallol (PyrG) in the absence and presence of benzenesulfinic acid (BSA).

to reduce iron indicate the phenolic compounds found in wine are not equally oxidizable. Pyrogallol has a lower reduction potential (higher oxidation potential) than 4-methylcatechol,(Danilewicz 2012) and it is likely the third hydroxyl group of 1,2,3-trihydroxy phenols increases their reactivity with iron. These findings also suggest wine oxidation can depend on the availability of nucleophiles, including other phenols, SO_2, glutathione, and thiols. Given the majority of wine phenols are odihydroxy phenols like 4-methylcatechol, it is conceivable the redox cycling of iron, and consequently wine oxidation, slows significantly or ceases altogether when the pool of nucleophiles is exhausted.

Inclusion of copper did not significantly affect iron reduction. Copper has been found in model wine experiments to facilitate the oxidation of iron(II) to iron(III) by oxygen,(Danilewicz 2011) though the results here indicate copper has no effect on the reduction half of the redox cycle.

Based on published literature showing increased oxygen consumption rates in alkaline conditions,(Singleton 1987) it is commonly believed wines with higher pH oxidize more quickly. Monitoring oxygen consumption in model wines containing 4methylcatechol and benzenesulfinic acid revealed an increase in rate as pH increased from 3.0 to 4.0. It was thus expected the rate of iron reduction would also increase with pH, given this process supplies the iron(II) necessary for oxygen consumption.

pH	Oxygen Consumption	Iron(III) Reduction
3.0	7.5E-05	1.1E-02
3.5	1.4E-04	6.0E-03
4.0	2.9E-04	5.0E-03

Table 1. Rate constants (min^{-1}) for oxygen consumption and iron reduction in model wine with 4-methylcatechol and benzenesulfinic acid at various pH.

However, this was not found to be the case: rates of iron reduction unexpectedly decreased as pH increased from 3.0 to 4.0 (Figure 3), thus pH appears to have opposite effects on the two halves of the iron redox cycle.

The rate of oxygen consumption is clearly not equal to the rate of phenol oxidation, two processes linked by iron but occurring asynchronously. It is likely observations of faster oxygen consumption with higher pH are due only to iron(II) oxidation independent of iron(III) reduction, the former half of the redox cycle having been found to increase in rate with higher pH in the presence of tartrate.(Michaelis and Smythe 1931, Smythe 1931) However, it is worth noting the rate constants for oxygen consumption never exceeded those for iron reduction (Table 1), indicating wine phenols, in the presence of sufficient nucleophiles, are able to maintain a supply of iron(II) that does not limit the rate of oxygen consumption, i.e. wine is constantly "primed" to receive more oxygen. These results explain the high iron(II):iron(III) ratios and low oxygen levels observed for wines in storage.(Danilewicz 2016, Danilewicz 2018, Ferreira et al. 2007, Lopez-Lopez et al. 2015, Nguyen and Waterhouse 2019) It would seem then wine ages at a rate limited not by the reactions therein, but by oxygen ingress, thus ageability may be conceptualized not

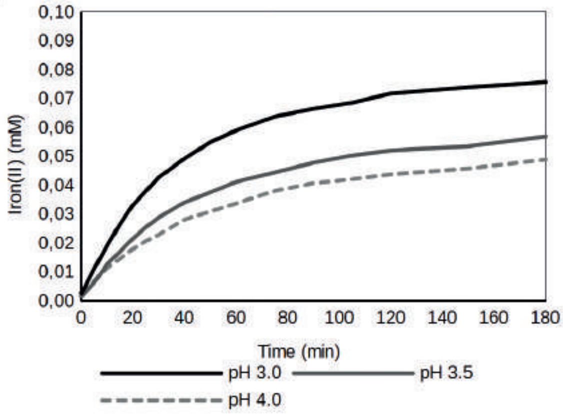


Figure 3. Iron reduction in model wine with 4methylcatechol and benzenesulfinic acid at various pH.

in terms of the rate of oxidation, but rather the capacity for oxidation. Only very rarely would oxygen be in constant excess (e.g. defective packaging).

2.2. *Quantifying wine oxidizibility using the Ferric Reducing Antioxidant Power assay*

It has been observed previously that a 2012 Cabernet Sauvignon stored in a plastic bottle was no longer able to reduce iron, unlike other much younger red wines from 2016 stored in glass bottles. Despite iron reduction not being the rate-determining step of wine oxidation, it may still be indicative of wine's aging potential, i.e. its remaining iron-reducing "lifespan" as measured using the extended FRAP assay.

A 1/10 dilution was required for the 2016 UC Davis Petite Syrah in order for measurements to fall within the limits of the calibration curve over the extended time; it is expected this dilution must be adjusted accordingly for different wines. Taken at the standard 6 min, the FRAP value of 21.5 mM iron for the Petite Syrah is within the range of values published for red wines, 19.3 – 32.3 mM iron, although the ages of these wines at time of publication are unknown.(Benzie and Strain 1999, Katalinic et al. 2004) Iron(II) levels started to plateau at 120 min, providing a FRAP value of 35.3 mM iron. Given the current understanding of the reactions of wine oxidation, this can be converted to an oxygen consumption capacity: assuming 3 moles iron(II) must be supplied in order to "process" 1 mole oxygen through to acetaldehyde formation(Danilewicz 2013, Waterhouse and Laurie 2006) the Petite Syrah might be expected to consume around 376 mg/L oxygen before oxidation reactions slow to a halt. This is equivalent to approximately 47 aerial saturations' worth of oxygen; Singleton has reported red wines start to exhibit oxidized character beyond 30 saturations.(Singleton 1987)

It was expected continuous stirring of the Petite Syrah with constant air exposure would decrease its iron-reducing power, but this oxygenation did not produce any significant changes to FRAP measurements (Table 2). Since oxygen consumption was not measured in this experiment, it is difficult to say how much oxygen was "consumed" by the wine. In any case, in six days, it could be expected that one atmosphere's worth might have been metabolized. This would have reduced the FRAP value by about 1 mM, so it appears that a longer time is needed to detect substantial change. This also points out the need to quantify oxygen consumption, a factor in planning future experiments. Treatment and analysis of additional wine samples are necessary to establish how quickly these experiments can provide data, after which work can be done towards an alternative definition of wine ageability as well as a means to measure it.

Time (min)	Initial	One Day	Six Days
6	21.5 ± 0.8	20.8 ± 0.6	20.5 ± 0.7
120	35.3 ± 0.9	33.1 ± 0.5	33.2 ± 0.8

Table 2. Ferric reducing antioxidant power (mM iron) measurements, taken at two timepoints: the standard 6 min and an extended 120 min, for the 2016 UC Davis Petit Syrah, continuously stirred under constant air exposure over several days.

However, it is also possible continuous stirring does not adequately mimic wine aging; several days of constant oxygen exposure likely does not bring about the same chemical changes as does years of storage in barrel or bottle. Oxygen ingress may be the rate-determining step of wine oxidation, as suggested the results from Objective 1, but that is not to say the cascade of reactions following oxygen consumption occurs instantaneously in an excess of oxygen. The formation of acetaldehyde, polymerization of phenols, depletion of nucleophiles, etc. may not have occurred in the short timeframe of this experiment to such an extent as to significantly affect FRAP measurements.

3. References

Bakker, J. and C.F. Timberlake. 1997. Isolation, Identification, and Characterization of New Color-Stable Anthocyanins Occurring in Some Red Wines. Journal of Agricultural and Food Chemistry 45: 35-43.

Benzie, I.F.F. and J.J. Strain. 1999. Ferric Reducing Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. Methods Enzymol. 299: 15-27.

Buxton, G.V., C.L. Greenstock, W.P. Helman and A.B. Ross. 1988. Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms, and Hydroxyl Radicals in Aqueous Solution. Journal of Physical and Chemical Reference Data 17: 513-886.

Danilewicz, J.C. 2003. Review of Reaction Mechanisms of Oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper. American Journal of Enology and Viticulture 54: 73-85.

Danilewicz, J.C. 2007. Interaction of Sulfur Dioxide, Polyphenols, and Oxygen in a Wine-Model System: Central Role of Iron and Copper. American Journal of Enology and Viticulture 58: 53-60.

Danilewicz, J.C. 2011. Mechanism of Autoxidation of Polyphenols and Participation of Sulfite in Wine: Key Role of Iron. American Journal of Enology and Viticulture 62: 319-328.

Danilewicz, J.C. 2012. Review of Oxidative Processes in Wine and Value of Reduction Potentials in Enology. American Journal of Enology and Viticulture 63: 1-10.

Danilewicz, J.C. 2013. Reactions Involving Iron in Mediating Catechol Oxidation in Model Wine. American Journal of Enology and Viticulture 64: 316-324.

Danilewicz, J.C. 2016. Fe(Ii):Fe(Iii) Ratio and Redox Status of White Wines. American Journal of Enology and Viticulture 67: 146-152.

Danilewicz, J.C. 2018. Fe(Iii):Fe(Ii) Ratio and Redox Status of Red Wines: Relation to So-Called "Reduction Potential". American Journal of Enology and Viticulture 69: 141-147.

De Freitas, V. and N. Mateus. 2011. Formation of Pyranoanthocyanins in Red Wines: A New and Diverse Class of Anthocyanin Derivatives. Analytical and Bioanalytical Chemistry 401: 1463-1473.

Drinkine, J., P. Lopes, J.A. Kennedy, P.L. Teissedre and C. Saucier. 2007. Ethylidene-Bridged Flavan-3-Ols in Red Wine and Correlation with Wine Age. Journal of Agricultural and Food Chemistry 55: 6292-6299.

Elias, R.J. and A.L. Waterhouse. 2010. Controlling the Fenton Reaction in Wine. Journal of Agricultural and Food Chemistry 58: 1699-1707.

Es-Safi, N.E., H. Fulcrand, V. Cheynier and M. Moutounet. 1999. Competition between (+)-Catechin and (-)-Epicatechin in Acetaldehyde-Induced Polymerization of Flavanols. Journal of Agricultural and Food Chemistry 47: 2088-2095.

Es-Safi, N.E., H. Fulcrand, V. Cheynier and M. Moutounet. 1999. Studies on the Acetaldehyde-Induced Condensation of (-)-Epicatechin and Malvidin 3-O-Glucoside in a Model Solution System. Journal of Agricultural and Food Chemistry 47: 2096-2102.

Ferreira, S.L.C., H.S. Ferreira, R.M. De Jesus, J.V.S. Santos, G.C. Brandao and A.S. Souza. 2007. Development of Method for the Speciation of Inorganic Iron in Wine Samples. Analytica Chimica Acta 602: 89-93.

Katalinic, V., M. Milos, D. Modun, I. Music and M. Boban. 2004. Antioxidant Effectiveness of Selected Wines in Comparison with (+)-Catechin. Food Chemistry 86: 593-600.

Kreitman, G.Y., A. Cantu, A.L. Waterhouse and R.J. Elias. 2013. Effect of Metal Chelators on the Oxidative Stability of Model Wine. Journal of Agricultural and Food Chemistry 61: 9480-9487.

Lopez-Lopez, J.A., G. Albendin, M.I. Arufe and M.P. Manuel-Vez. 2015. Simplification of Iron Speciation in Wine Samples: A Spectrophotometric Approach. Journal of Agricultural and Food Chemistry 63: 4545-4550.

Mateus, N., A.M.S. Silva, C. Santos-Buelga, J.C. Rivas-Gonzalo and V. De Freitas. 2002. Identification of Anthocyanin-Flavanol Pigments in Red Wines by Nmr and Mass Spectrometry. Journal of Agricultural and Food Chemistry 50: 2110-2116.

Michaelis, L. and C.V. Smythe. 1931. The Correlation between Rate of Oxidation and Potential in Iron Systems. Journal of Biological Chemistry 94: 329-340.

Nguyen, T.H. and A.L. Waterhouse. 2019. A Production-Accessible Method: Spectrophotometric Iron Speciation in Wine Using Ferrozine and Ethylenediaminetetraacetic Acid. Journal of Agricultural and Food Chemistry 67: 680-687.

Nikolantonaki, M. and A.L. Waterhouse. 2012. A Method to Quantify Quinone Reaction Rates with Wine Relevant Nucleophiles: A Key to the Understanding of Oxidative Loss of Varietal Thiols. Journal of Agricultural and Food Chemistry 60: 8484-8491.

Singleton, V.L. 1987. Oxygen with Phenols and Related Reactions in Musts, Wines, and Model Systems - Observations and Practical Implications. American Journal of Enology and Viticulture 38: 69-77.

Smythe, C.V. 1931. The Mechanism of Iron Catalysis in Certain Oxidations. Journal of Biological Chemistry 90: 251-265.

Timberlake, C.F. and P. Bridle. 1976. Interactions between Anthocyanins, Phenolic Compounds, and Acetaldehyde, and Their Significance in Red Wines. American Journal of Enology and Viticulture 27: 97-105.

Waterhouse, A.L. and V.F. Laurie. 2006. Oxidation of Wine Phenolics: A Critical Evaluation and Hypotheses. American Journal of Enology and Viticulture 57: 306-313.

Waterhouse, A.L. and M. Nikolantonaki. Quinone Reactions in Wine Oxidation, in Advances in Wine Research, ed, Ebeler, S.B., G. Sacks, S. Vidal and P. Winterhalter Eds, 291-301. (2015).

10th International Symposium

Challenges in viticulture and oenology:
Wine Appellations, Authenticity and Innovation.

ŒNOVITI INTERNATIONAL network

Session II. Wine Appellations and Wine Tourism

Volatile markers of wine geographical and varietal origin

Davide Slaghenaufi* and Maurizio Ugliano
Department of Biotechnology, University of Verona, Italy.

Abstract: This work reports different case studies were volatile compounds are markers of the grape variety, or they are markers of the geographical origin both at the very small scale of single vineyard diversity and region of production. In the first case, the volatile compounds profiles of 9 red wine varieties used for the production of Valpolicella DOC were evaluated. Results indicated that terpenes, benzenoids and C-6 compounds were good markers of varietal diversity. To assess the existence of differences in volatile compounds between single vineyard wine, the grapes from eight parcels were vinified separately and with the same protocol. Analyzes of volatile compounds have shown that there are significant differences between vineyards. The main markers of these differences are terpenes, benzenoids and norisoprenoids. Lugana and Verdicchio are two Italian white wines produced with the same grape variety but in different region. Chemical analysis showed that Lugana and Verdicchio were different on cis-3-hexenol, methionol, phenylethyl alcohol, and geraniol content. Moreover, Lugana wines showed generally higher contents of terpenes, esters and methyl salicylate. Sorting task analysis showed that samples were separated into two groups: one characterized by floral note mainly Lugana wines, the second more spicy represented mostly by Verdicchio.

Keywords: Valpolicella grape varieties, Volatile compounds, Single vineyard wines, Lugana, Verdicchio, Methyl salicylate,

1. Introduction

The appellation of origin system plays a central role in the economic success of local wines on the international markets (Dorfmann, 2016) conveying to the final consumer the unique characteristics of a given wine in relationship to the geographical area in which it is produced. Consequently, appellations of origin have been considered of primary importance for sustainable and cultural development of producing areas. Among the specifications of appellations of origin, grape varieties to be employed for winemaking and their area of origin are considered of primary importance.

The connection between these two aspects and the final aroma of a wine has been well described (van Leeuwen & Seguin, 2006; Bokulich *et al.,* 2016; Bramley 2016;). Therefore, identifying the aroma compounds that characterize a variety, or that are attributable to a particular place of cultivation, becomes a key step in order to enhance the distinctive characteristics of a wine.

We report here three cases studies concerning the identification of volatile markers of varietal or geographical

* Corresponding author. E-mail: davide.slaghenaufi@univr.it

identity of Italian wines. One case about the differences in chemical composition of monovarietal wines obtained from different red varieties employed for Valpolicella DOC and DOCG wines. A second study focused on volatile compounds of wines obtained from contiguous vineyards. The last case concerning the chemical markers that discriminate Verdicchio from Lugana, two white wines produced in two different Italian areas (Marche and Veneto/Lombardia respectively) using the same grape variety, locally known as Verdicchio or Trebbiano di Soave.

Materials and methods

1. Wine samples

In the first case study three clones of Corvina, four clones of Corvinone as well as Rondinella, Molinara, Oseleta, Sangiovese, Croatina, Raboso del Piave, and Cabernet Sauvignon have been used. Grapes were harvested from one single experimental plot located in the town of San Pietro in Cariano (VR) and vinified using the same standard protocol. Grapes were destemmed manually, the berries were then pooled together to obtain a homogenous sample. Five kilograms lots were then taken from this larger sample and crushed manually. Musts were inoculated with commercial yeast. Fermentations have been carried out in duplicates.

In the second case study, grapes were harvested from 8 contiguous parcels located in the Maternigo hill (20 km north-east from Verona city; between 2000 and 450 m a.s.l.). From each parcel 20 kg of Corvina grapes were collected by hand at technological maturity, with the entire parcel being harvested. The vinification was carried out using the same protocol described above.
In the third case study 13 wines have been used 6 Lugana and 7 Verdicchio. Wine samples were from the commerce and were included in their respective DOC. Wines were selected from 2016, 2017 and 2018 vintages, and they have not been stored in oak barrels.

2. Wine chemical analysis

Wines were analyzed for a number of volatile compounds. Low molecular weight volatile sulfur-containing compounds were analyzed according to Slaghenaufi *et al.,* (2017). 3-Mercaptohexanol has been analyzed as described by Herbst-Johnstone *et al.,* (2013). The method of Slaghenaufi & Ugliano (2018) were used to analysed terpenes and norisoprenoids. While esters, acids, C6-compounds, alcohols and benzenoids have been determined by SPE extraction followed by GC-MS analysis as reported by Slaghenaufi, *et al.,* (2019).

3. Sorting task analysis

Sorting task was carried out to find wines similitudes or differences as described by Alegre *et al.,* (2017) with slight differences. Twelve milliliters of wine were poured in wine glasses labelled with 3-digit random codes and covered by plastic Petri dishes; all samples were served at room temperature, and glasses were randomized for each panellist. Panellists were asked to sort the wines into groups based on aroma similarities. After forming the groups, the judges were asked to assign to each group aromatic descriptors chosen from a list

4. Statistical analysis

Chemical data have been submitted to Principal Component Analysis (PCA), and Analysis of the Variance ANOVA (α=0.05), both test have been performed using XLSTAT 2017. Hierarchical Cluster Analysis (HCA) of the coordinates obtained from multidimensional scaling (MDS) of sorting task data, as described by Alegre et al. (2017), have been performed using

XLSTAT 2017 (Addinsoft SARL, Paris, France).

Results and discussion

1. Volatile markers of monovareital red wines of the Valpolicella appellation

A total of 46 volatile compounds have been identified and quantified in nine monovarietal red wines from varieties allowed in the Valpolicella appellation, including Corvina, Corvinone, Rondinella, Molinara, Oseleta, Raboso, Croatina, Sangiovese and Cabernet Sauvignon. All grapes were harvested from a single experimental block and vinified using a standard protocol. Statistically significant differences were observed between varietal wines. In particular terpenoids, C_6 compounds and benzenoids appeared to be good varietal markers. Terpenes are responsible for floral notes. Their biosynthesis occurs in grapes and is highly dependent on the variety.

Corvina wines and to lesser extend Corvinone showed significantly higher concentrations of different terpene alcohols, in particular for linalool and α-terpineol, which have been observed in Corvina samples at levels 10 times higher than Cabernet Sauvignon. β-citronellol derived from the yeast conversion of geraniol, and it has been observed to varied significantly, showing higher concentration in Corvina, Corvinone, Rondinella and Molinara samples. Conversely, Oseleta showed the highest concentrations of terpinen-4-ol and cis- and trans- isomers of linalool oxide.

Concentration of C_6 compounds in finished wines could be associated with grape variety (Versini *et al.,* 1994), maturity (Kalua & Boss, 2009), as well as technological factors such as timing of SO_2 addition (Nicolini *et al.,* 1996) and duration of pre-fermentative skin contact. In the present study, differences were observed in all C_6 alcohols across different wines, with wines from Corvinone grape variety generally displaying higher values, while Molinara sample was nearly three times lower.

Oseleta was characterized by higher concentration in ethyl and methyl vanillate. Higher content of vanilic alcohol and benzyl alcohol were found in Oseleta, Rondinella.

2. Volatile compounds differences in single vineyard wines

Corvina grapes from eight contiguous vineyards have been vinified using the same protocol. The obtained wines have been analyzed by mean of GC-MS analysis. Analysis carried out on grape samples showed that differences between parcels were very small. Whereas after fermentation larger differences between wine samples were highlighted (Slaghenaufi *et al*. 2019).

PCA analysis showed the existence of similarities between wine aroma profiles, reflecting to a good extent the geographical location of the corresponding vineyard parcels (Figure 1).

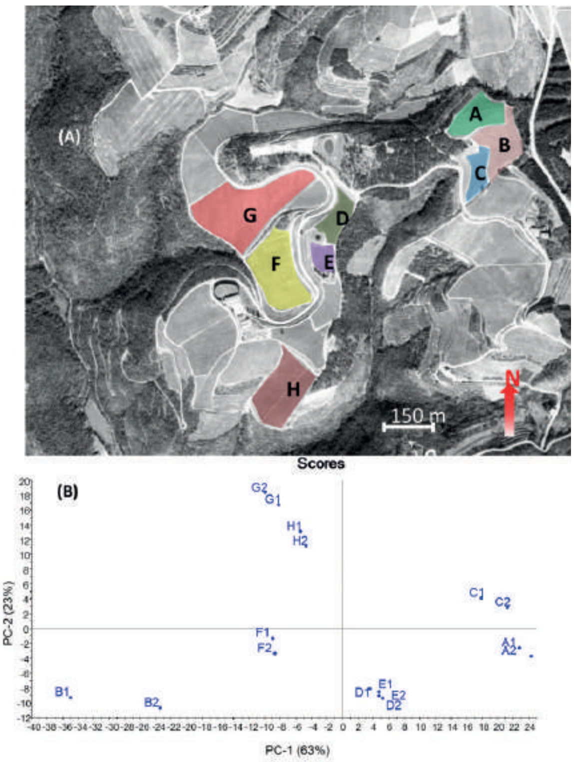


Figure.1. Plan of the studied vineyards location (A) and PCA showing wine samples scores (B). The letters correspond to the different vineyards.

The compounds responsible for single vineyard wines diversity were the monoterpene alcohols linalool, α-terpineol, linalool oxide; the benzenoids vanillin, ethyl vanillate and methyl vanillate; and the norisoprenoid β-damascenone. To assess whether these differences persist even during the aging of the wine, samples have been submitted to accelerated aging. Results indicated that volatiles diversity of wines still reflected the vineyard geographical diversity, even if the separation was less fine. Many reactions occurred during wine aging, as a consequence the marker of parcel diversity changed and they were benzenoids: ethyl vanillate, methyl vanillate and vanillin; the norisoprenoid 3-oxo-α-ionol; the terpenes linalool oxide, linalool, p-methane-1,8-diol, α-terpineol, and the precursors of nerol, geraniol, linalool

3. Volatile markers of Lugana and Verdicchio wines

In order to better understand the existance of chemical and sensorial differences between Verdicchio and Lugana, a samples set of 13 non-barrel-aged commercial wines was carried out, from the 2016, 2017 and 2018 vintages. A sorting task was first carried out on this new set of samples to identify the differences between samples, then on the same samples volatile compounds analysis were performed.

The Hierarchical Clustering Analysis (HCA) of sorting task data identified indeed two groups: one characterized by floral and minty notes, the other characterized by spicy and toasted aromas. The samples in the first group were basically Lugana wines while the second group was formed principally by Verdicchio wines. This good separation indicated that Lugana and Verdicchio are aromatically different with their own distinct aroma character.

The volatile compounds showed that the major differences between Lugana and Verdicchio were observed for *cis*-3-hexenol, methionol, phenylethyl alcohol, and geraniol. Lugana wines showed generally higher contents of terpenes, esters and methyl salicylate. 3-Mercaptohexanol was detected in above-threshold concentrations in both wine types but no statistically-significant difference was observed.

Conclusion

Volatile compounds were found to be good markers of the variety and geographical origin of the grapes used. Specifically, even in non-aromatic red varieties, the volatile compounds that most characterize Valpolicella red wine varieties are terpenes, benzenoids and C_6 compounds.

Terpenes and benzenoids were also found to discriminate Corvina wines obtained from contiguous vineyards.

These results showed that geographical differences at the very small scale of vineyard parcels could induce significant differences in wine aroma composition, highlighting the importance of managing vineyard segmentation at a finer scale.

On a larger scale, wines (Lugana and Verdicchio) obtained from the same grape variety but in two different Italian regions have been compared, showing chemical and sensory differences. These results highlight that environment and viti-enological practices play a fundamental role in the aroma expression of wines in spite of the very similar genetic background of the grape.

References

- Alegre, Y.; Sáenz Navajas, M.P.; Ferreira, V.; García, D.; Razquin, I.; Purificación, H.O. Rapid strategies for the determination of sensory and chemical differences between a wealth of similar wines. Eur. Food Res. Technol. 2017, 243, 1295-1309.

- Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., & Mills,D. A. (2016). Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. mBio, 7, 3.
- Bramley, R. G. V. (2016). Vineyard variability and terroir – Making sense of a sense of place. In K. Beames, E. Robinson, P. Dry, & D. Johnson (Eds.). Proceedings: 16th Australian wine industry technical conference (pp. 45–51). Glen Osmond: Australian Wine Industry Technical Conference Inc.
- Carlin S., Vrhovsek U., Lonardi A., Landi L., Mattivi F. (2019) Aromatic complexity in Verdicchio wines: a case study. OENO One, 4, 597-610.
- Dorfmann H. (2016). The key role of Geographical Indications for the European economy. In: EuropEan WinE: a solid pillar of the European union economy (pp.12). Brussels, Belgium: CEEV publication. https://www.ceev.eu/images/documents/press_releases/2016/Brochure_CEEV_-_High_resolution.pdf
- Herbst-Johnstone, M., Piano, F., Duhamel, N., Barker, D., Fedrizzi, B. (2013) Ethyl propiolate derivatisation for the analysis of varietal thiols in wine. Journal of Chromatography A, 1312, 104-110
- Kalua C.A., & Boss P.K. (2009). Evolution of Volatile Compounds during the Development of Cabernet Sauvignon Grapes (Vitis vinifera L.). Journal of Agricultural and Food Chemistry, 57, 3818-3830.
- van Leeuwen, C., & Seguin, G. (2006). The concept of terroir in viticulture. Journal of Wine Research, 17(1), 1–10.
- Nicolini G., Versini G., Amadei E., & Marchio M. (1996). 3-hexen-1-ol isomers in Muller-Thurgau wines: A "varietal'. characteristic affected by must sulfiting time. Vitis. 35. 3. 147-148.
- Slaghenaufi, D., Guardini, S., Tedeschi, R., Ugliano, M. (2019). Volatile terpenoids, norisoprenoids and benzenoids as markers of fine scale vineyard segmentation for Corvina grapes and wines. Food Research International, 125, 108507.
- Slaghenaufi, D., Ugliano, M. (2018). Norisoprenoids, sesquiterpenes and terpenoids content of Valpolicella wines during aging: Investigating aroma potential in relationship to evolution of tobacco and balsamic aroma in aged wine. Frontiers in Chemistry. 6(Mar), 66.
- Slaghenaufi, D., Tonidandel, L., Moser, S., Román Villegas, T., Larcher, R. (2017). Rapid Analysis of 27 Volatile Sulfur Compounds in Wine by Headspace Solid-Phase Microextraction Gas Chromatography Tandem Mass Spectrometry. Food Analytical Methods, 10, 3706–3715.
- Versini, G., Orriols, I., & Dalla Serra, A. (1994). Aroma components of Galician Albariño, Loureira and Godello wines. Vitis, 33, 165–170

Shaping a wine territory: how is a collective process of strategy making stimulated?

Malida Mooken; Jacques-Olivier Pesme* ; Roger Sugden; Marcela Valania
Wine Research Center, University of British Columbia, Canada
malida.mooken@ubc.ca; jo.pesme@ubc.ca; roger.sugden@ubc.ca; marcela.valania@ubc.ca

Abstract: Geographical indication has always been a fundamental concern in the history of the wine industry. Within a (re)emerging wine territory, in addition to the question of the territory and its borders, there are issues of implementing a collective process of strategy making.
Lessons from experiences tend to demonstrate that successes in the wine industry generally rely on the capacity of the actors to share their knowledge, to exchange information, and to enter and approach markets together. The capacity of a wine territory to collaborate well is a key success factor.
According to the situation and the historical, cultural, geographical nature of the wine region, this collective dynamic can be sometimes hard to find, and to stimulate.
The purpose of this paper is to provide a reflection about the levers of collective dynamics that are applicable in a wine territory. It discusses an application in the British Columbia wine territory to stimulate strategic thinking and strategy making among wineries.

Keywords: Wine territory, collaboration, strategy, cohesion, competitiveness

Introduction

How might a relatively young wine region be shaped in real time? This is the main question that we address in this paper. We do so by focusing on the case of the emerging British Columbia (BC) wine territory in Canada. Bearing in mind the context, it is recognized that the question needs to be explored alongside other concerns:

- How to sensitize actors in the territory to the importance of shaping the wine territory and its development in a *deliberate* manner?
- How to stimulate a *collective* process of strategy-making and actions?

In addition to its natural predispositions for the production of wine in terms of landscape and climate, British Columbia has benefitted from a particularly favourable economic environment, due to two main factors: the growth in wine consumption in Canada; and the increased appeal of the region for Canadians as a summer tourist destination, coupled with the possibility of discovering the vineyards and wines.

Given these advantages, the wine territory was able to organise itself, relatively quickly, around key professional institutions and a few wineries that experienced commercial success on the domestic and international market. Arguably, over the years, the British Columbia wine landscape and wine experience have contributed positively to

*Corresponding author. E-mail: jo.pesme@ubc.ca

the tourism industry and the wider economy. The British Columbia province currently has nearly 300 wineries and over 80% of the total vineyard acreage in BC is located in the Okanagan valley.

1. The British Columbia wine industry

The first vine plantation in British Columbia dates from 1859 when a French Oblate priest decided to make sacramental wine, but it was not until the 1920s that local winemaking started to take off, following removal of a legal prohibition to produce commercial wine in Canada (Hickton 2005). It was the 1980s when an organised wine industry started to take form in the region, and only in the 1990s that the industry underwent a serious transformation, after the conclusion of the North American Free Trade Agreement in 1988, and the related removal of protectionist measures (*inter alia* Ross 1995; Migone and Howlett 2010; Cartier 2014). The BC and Federal governments provided a $28 million aid package to help the grape-wine industry to adjust to changes. Low quality hybrid grapes were replaced with vinifera varieties that would enable the production of premium wines that could compete on the domestic market (Ross 1995; Belliveau et al. 2006).

BC has 3 large wineries producing several million bottles per year (of which a majority are from imported bulk wine), some mid-sized SMEs and many small vineyards. Diverse grape varieties are used, without one predominant variety. The total number of BC wineries grew from about 200 in 2012 to nearly 300 in 2016, and total grape acreage rose from about 3000 in 1999 to about 10000 in 2016. Over 80% of the total BC market is controlled by the 3 large producers, which sell both premium and blended wines. Consider also that in 2010, 92.7% of the BC market was controlled by 19 of the then 209 wineries (Cartier 2014).

The BC wine industry is a significant contributor to the economy. The overall economic impact of the BC wine and grape industry in 2015 was over $2.5 billion; it contributed nearly 12000 direct and indirect jobs, particularly in sectors such as agricultural production, and tourism. The Okanagan is a popular tourist destination in its own right, independent of the wine industry, but there is a strong link to wine. Various large and medium-sized wineries have invested in culinary tourism and opened restaurants at their respective estates. Many wineries have shops, and offer tours of vineyards and facilities. Arguably, over the years, the Okanagan wine landscape and wine experience have contributed positively to the tourism industry and the wider economy.

Although the industry has experienced rapid growth in recent years, and benefitted from a strong domestic market, it still has not managed to establish itself as a recognised wine region on the international market (Migone and Howlett 2010). What appears to be missing is territorial cohesion, in the sense of being a wine territory. Social capital in the Okanagan is considered low, in part due to the high fragmentation of the industry and its weak institutions (Hira and Bwenge 2011). In particular, economic institutions in the industry are fairly undeveloped and there is no strong local governance network linking actors in an effective and sustainable manner (Hickton and Padmore 2004). In their study of industry associations in the Okanagan, Kingsbury and Hayter (2006) report that wineries, especially small-sized ones, do not think that policies put forward by the main industry association represent their interests or that they could impact those policies. Significant mistrust from various wineries in the industry association is still observed. Cartier (2014: 23) also highlights that there is 'little sharing of knowledge and innovation between small and medium wineries, and independent grape growers'. As suggested by Hira and Bwenge (2011: 66), key issues in the industry arise from 'a lack of organization, coordination, and policy foresight'. These suggest that existing institutions have not been able to devise collective strategies nor provide adequate socio-economic support for shaping the wine territory and enabling it to flourish.

The recent development of a highly competitive context for the wine market in Canada, e.g. with the increasing presence of imported wines, alongside the willingness of some local actors to take the region to another level and gain international recognition, imply that there is a need to reorganise current institutional forces, to stimulate cooperation and to build a collective strategy in order to steer the region towards a new cycle of sustainable growth. However, the challenge is to make industry actors critically aware of the significance of international recognition and exports in order for the wine region to maintain development, not least, because the industry has tended to pay little attention to what is going on outside of its context. In parallel, the impetus 'to export and expand markets' can trigger actors to develop a 'coordinated strategy', as observed in other "new world" wine regions (Migone and Howlett 2010: 9).

Our continuing engagement with the industry has enabled us to observe that there is recognition amongst some actors about the need for 'a culture of cooperation, and organizations' so as to enhance the industry's 'collective efforts' (Hira and Bwenge 2011: 67). Borrowing from Storper (1997: 36), we suggest that 'the question is how [individual] actors manage to get themselves into successfully coordinated, forms of collective action', especially under conditions of uncertainty. In Section 2 of this paper, we look at how this culture of cooperation may be fostered, and at the process for developing key strategies from within. We highlight the evolving process that has been put in practice since 2012 — enabling winery owners to interact with each other, so as to identify and deliberate about issues of strategic importance to shaping the British Columbia wine territory. At the core of this process is our approach to the organization of knowledge so as to provide new understanding, connections and resources that actors can use to form and refine collective strategies and actions. Key elements of our approach are presented.

We analyze two distinct literatures to gain insights about issues pertinent to developing the wine region. These focus on: (1) recognized wine territories and the notion of 'terroir' (*inter alia* Hinnewinkel 2007; Van Leeuwen et al. 2006; Patchell 2008; Charters and Michaux 2014; Ditter and Brouard 2014); (2) strategies for shaping territorial competitiveness (*e.g.,* Storper 1997; Karlsen and Larrea 2014; Navarro et al. 2014; Valdiso and Wilson 2015). The purpose of our paper is the application of the strategy formation literature to the wine context. Moreover, we do so in a way that considers a case study in real-time, i.e. by reflecting on our on-going experience as part of a group that is responsible for conceiving, leading and carrying out a project intended to support development of the BC wine industry.

The remainder of the paper is structured around two main sections. The following Section highlights key elements of distinct literatures (as mentioned above) that we draw upon in developing our approach. Section 3 discusses application of that approach in British Columbia.

2. **Theoretical discussion**

2.2. Wine territory and the notion of 'terroir'

Any region seeking to develop as an

internationally recognized wine territory would benefit from demonstrating 'terroir' qualities, i.e. its vineyards and wines need to encapsulate particular characteristics of their origin. Consider the following definition, proposed by a working group at a UNESCO meeting in 2005:

> A terroir is a delimited geographical space, defined from a human community which in the course of its history constructs an assemblage of distinctive cultural traits, knowledge and practices founded on a system of interaction between the natural environment and human factors. The skill set involved reveals originality, conferring a typicity and permitting recognition for the products or services originating from this space and thus for the men who live there. Terroirs are living and innovative spaces which cannot be assimilated into a single tradition (as translated by Unwin 2012: 39).

Indeed, terroir qualities tend to derive from a set of factors, both natural (soil and climatic combinations) and constructed (social, historical, cultural, economic and political) (Gade 2004; Hinnewinkel 2007; Van Leeuwen et al. 2006). Along the same lines, and building on Ditter and Brouard (2014), we consider that a wine territory is a geographical area where local agents share a set of practices, strategies and institutional bodies that contribute to a local identity, and also where players share rules and quality standards, as well as beliefs and representations.

Various wine territories that are internationally recognized owe a major part of their success to the cooperation and collective action amongst local actors on certain issues, albeit they may have other differing interests. In well-developed territories such as St Emilion or Chablis, for instance, winery owners not only ensure the quality and differentiation of their individual products but also, despite the challenges, work towards building and maintaining the collective reputation of the region over time (Castriota and Delmastro 2008; Patchell 2008; Charters and Michaux 2014). For example, in Chablis, winery owners were critically aware of the challenges of entering foreign markets individually — due to the small size of their operations — and undertook collaborative actions to focus on promoting the reputation of the region in order to be able to compete internationally (Ditter and Brouard 2014).

Building on such arguments, we suggest that in order to shape a wine territory and build its position on the international level, a collective process to strategy-making and action is necessary amongst actors that 'cooperate and compete with each other' (Ditter and Brouard 2014: 11). For such a process to be effective and sustainable, it is crucial that the actors are able and willing to interact, and to identify and collaborate on common interests. Moreover, strong institutions that encourage collaboration and trust amongst the actors are also necessary in order to ensure territorial cohesion (Benedetto et al. 2014).

2.2. Shaping strategies for territorial competitiveness and development

We consider Navarro et al. (2014), who argue for a holistic approach that addresses the objectives ("what for"), content ("what") and process ("who and how") in conceptualizing a regional strategy. Following that reasoning, we consider that the *objectives* for a regional strategy include the systematic development of the British Columbia wine region into a mature and cohesive wine territory, and establishing itself internationally over time. We suggest that the *content* of that strategy needs to be organized around three focal points:

cooperation, identity and quality. We also posit that these focal points provide stimuli for industry actors to engage with each other around issues of common interest, and that this may contribute to strengthening individual and collective capabilities, and to building territorial cohesiveness. The *process* is rooted in an understanding of a 'journey of inquiry' — 'an exploratory journey [undertaken] by a group of people where direction, conduct and action are not predetermined but, rather, are chosen through observation, reason and evidence, informed by feeling and sensitivity, as the journey progresses' (Culver et al. 2015: 205-206). That approach also underlies the determination of the objectives and content, and not only the process that follows after these are agreed upon by territorial actors.

Territorial development can be considered as a process in which regional actors are mobilized to interact, identify problems, develop strategies and act collectively (Karlsen and Larrea 2014; Valdiso and Wilson 2015). Drawing on their experience in the Basque Country, Karlsen and Larrea (2014) purport that in the initial stages of a process, territorial actors are mostly concerned with their individual interests and it takes time to develop a collective approach. They also emphasize that the process should have 'a structure (formal or informal) that makes interaction possible and the different actors trust each other' (63). This is also argued by (Asheim 1999), who suggests that the socio-economic development of a region does not necessarily occur spontaneously; it requires a structured process that includes the building up of social capital, involving both private and public actors. For instance, key policy initiatives that 'aimed at promoting and stimulating the collective capacity for cooperation and networking' in order to support the creation of institutional and 'intermediate governance' structures are crucial in shaping the economic competitiveness and development of territories and regions (*ibid*: 351).

Having a structure is particularly important for developing the capability of collective knowing - 'a learned pattern of collective action, where the actors in the agora systematically modify their actions over time, through the learning process in the agora' (*ibid*: 68). Consider also Navarro et al. (2014: 538):

> the analysis of constructing regional strategies should not ignore the actions taken in the region concerning three dimensions: the structure of relations and networks among the actors; the types of personal relations (trust, confidence, friendships etc.) that affect their behaviour; and the shared understandings visions, language and codes (Nahapiet and Ghoshal 1998).

This accords with the literature on 'proximity', which emphasises the significance of 'relational proximity' in a social and cultural sense, as well as 'geographic proximity' for developing localised learning and competitive advantages in a region (*e.g.,* Storper 1997; Amin 1999; Maskell and Malmberg 1999; Asheim 2002).

In shaping a territorial strategy, it is also important to make choices about which socio-economic activities the region will focus upon and develop, now and in the future (Valdiso and Wilson, 2015). In doing so, a territory may develop a "strategy from within" (Culver et al. 2015: 194). This process does not exclude the participation of external actors, who can bring in valuable diverse perspectives, and 'international expertise and experience' (Branston et al. 2003: 283). Rather, a "strategy from within" conveys the significance of a non-prescriptive process, that does not impose a set of actions on people living in the territory, and most likely to be affected by the consequences. Further, consider the following:

> deliberately initiating a regional and economic strategy in a context with little or no experience of a strategy conceived, designed and enacted from within and across the region itself would necessitate as part of the process (1) an initial focal point for developing the strategy and (2) a method for addressing the focal point (Culver et al. 2015: 204).

We suggest that the method could be in the form of an inquiry, where regional actors are enabled to explore possibilities, form collective preferences, make informed choices and find common ground for strategy-making and action through open dialogue and sharing of knowledge and experiences.

3. A process towards stimulating cooperation and collective action in British Columbia

Since fall 2012, the University of British Columbia (UBC) has supported the development of the British Columbia wine territory. Rooted in a critical appreciation of the context, the UBC team was careful and patient in building face-to-face relationships with key actors in the region. Initial steps were taken to engage with the industry, e.g. through visits to various local wineries and meetings with other stakeholders in order to find out more about the context. It was important to have a feel of the wine region, not least in terms of its terroir qualities. Another important aspect of the process, especially at the time of the visits, was to identify the varied interests and needs of the territory from the concerned industry actors themselves, and to begin to lay down the foundations for developing a process that would help the wine region to build territorial cohesiveness. This is in line with the underlying idea of developing a process that deliberately seeks to stimulate a "strategy from within", where actors in the region explore and determine what they consider as valuable for their futures, without the constraints of predetermined agendas.

Following the initial interactions and consultations with members of the British Columbia wine industry, UBC organised a 'Wine Leaders Forum' in April 2014 in response to the region's need for an independent and safe, yet challenging arena where winery owners and principals could engage in thought and deliberation, develop collective knowing and set their priorities and actions for pursuing development. Moreover, the Wine Leaders Forum enabled the sharing of both local and international experiences, perspectives and knowledge. This helped the actors to identify the territory's own unique set of assets, and capacities, and also to determine which capabilities they need to develop. As a result, industry at the Forum identified focal points - cooperation, quality and identity - that should be addressed in order to strengthen the region's competitiveness and development. There was an explicit recognition for the need to have a more organised approach in the industry, and to shape the region's identity along the lines of a "territorial brand" and a terroir strategy.

Since 2014, the Forum, which is a retreat-style program taking place over 3 intensive days, has been hosted continuously, on a yearly basis. In the spirit of a journey of inquiry, the respective Forums allow issues to be explored and discussed by the participants in a continuous process. For example, the notion of shaping the identity of a wine territory was first discussed in the 2014 Forum, and brought up again in each of the Forums that followed. The idea is for the participants to build and sharpen their understanding and actions over time.

In 2016, among other concerns, there was an increased awareness and interest in developing international competitiveness, and in inquiring into the appropriate set of international markets for British Columbia to target. This includes processes for identifying targets, so as to build its reputation internationally; to gain insights from export-ready wineries to define the criteria to determine the target markets, and to identify which local wineries are interested in exporting'.

March 2018 marked the 5th Wine Leaders Forum, and we opened the first full day as a workshop for all interested industry actors to explore the complex notion of identity, following up on our 2014 engagement. Participants in the remaining two days of the Forum then took forward the workshop discussions. They developed an outcomes document, noting challenges and needs for moving forward, and laying out expectations. The dissemination of a report on the work done around identity fulfilled part of those expectations, as did the complementary reports on the identity of the British Columbia wine territory (2018) that describes in detail the richness of the workshops and their outputs.

Each of the Forums features exchange of participants' experiences and approaches, e.g. in international trade fairs; discussion of local needs and characteristics; reference to case studies of wine territories elsewhere in the world, to establish comparisons, use as benchmarks, and stimulate discussion and thought; and deliberation of the current state and future trends of the industry. This is done to help businesses and organizations better understand the region's prospects and growth; and by developing experiential learning opportunities through collective appreciation of both wines and visitor experiences (Mooken et al. 2016). The Forum is carefully designed to enable free expression that is respectful of pluralistic views and feelings, and open discussion through reasoned argument and evidence, without the constraints of pre-conceived outcomes. We also organize and conduct other activities, such as a Wine Industry Collaborative where discussions take place over one day, with industry actors and stakeholders focusing on one topic, and on ways forward to address that topic, in terms of setting the collective work organization. For example, during the 2015 Collaborative the focus was on setting up a taskforce on 'Labelling and Presentation', including the terms of reference, composition and rollout. One of the objectives of the taskforce was to reconsider the content of wine labelling in Canada, and in particular, the sensitive question of the wines labelled 'Cellared in Canada' (http://ubckedgewine.ca/labellingtaskforce report_final.pdf).

The objectives, process and findings of the taskforce were shared with other industry actors through emails and town hall meetings. The town halls were hosted in various sub-regions, in order to make the meetings accessible to diverse participants. Feedback obtained from the town halls enabled the findings to be refined. Both prior to the presentation of the final report on 'Labelling and Presentation' and after its dissemination, members of the taskforce, in collaboration with other industry actors, took actions, without the involvement of UBC, to request members of the Canadian parliament to review policy guidelines in order to address a discrepancy.

In setting up the Forum, the Collaborative and the taskforce, the objective is to provide some structure to the process, so as to stimulate meaningful interactions and enable industry actors to build trust and cooperation, with the university but also amongst themselves. This may trigger actors to start collectively envisaging a valuable future for the wine territory, and to set the foundation for shaping a territorial strategy.

Such a process is in line with the arguments put forward by the literature on regional strategy formation about the significance of formal and informal structure, proximity and collective knowing in the process.

Concluding remarks

Whilst our work in the British Columbia wine industry first started in 2012, we recognise that there is still a long way to go in the process of enabling the region to shape its territorial competitiveness and development. So far, efforts have been concentrated on supporting the wine region to enhance the capabilities of its actors and, by setting its priorities and deliberately focusing on collaboration, identity and quality, to position itself internationally.

We have done so by developing a process where industry actors are able to exchange, deliberate, discover and coalesce around shared knowledge and concerns, in an open, independent and challenging environment. We would argue that this process is effective given the structure that it offers, e.g. through the organisation of the Wine Leaders Forum and the other arenas. The structure is important as it provides significant opportunities for actors to develop the capability of collective knowing, trust and cooperation. Moreover, the continuous interactions, based on sharing experiences, knowledge and concerns, have the potential to help actors to coordinate and modify their actions over time, bearing in mind the interests of the wine region. In our view, these are necessary first steps in stimulating collective strategy-making and actions in any wine region that is (re)emerging and willing to shape a competitive wine territory.

References

Amin, A. (1999), “An institutionalist perspective on regional economic development”, *International journal of urban and regional research,* Vol 23, no. 2, pp.365-378.

Asheim, B.T. (1999), “Interactive learning and localised knowledge in globalising learning economies”, *GeoJournal,* Vol 49, no.4, pp.345-352.

Asheim, B.T. (2002), “Temporary organisations and spatial embeddedness of learning and knowledge creation”, *Geografiska Annaler: Series B, Human Geography* 84, no.2, pp.111-124.

Belliveau, S., Smit, B. and Bradshaw, B., 2006, “Multiple exposures and dynamic vulnerability: evidence from the grape industry in the Okanagan Valley, Canada”, *Global Environmental Change* 16, no.4, pp.364-378.

Benedetto, G., Carboni, D., & Corinto, G. L. (2014), “Humans and viticulture in Sardinia: The history and social relations as signs of identity of the wine-growing area”, In *BIO Web of Conferences* 3, p. 03011. EDP Sciences.

Branston, J.R., Sugden, R., and Wilson, J.R. (2003), “International perspectives on South-eastern Wisconsin’s economic development”, Sugden, R., Cheng, R.H., and Meadows, G.R., (eds.), *Urban and regional prosperity in a globalised new economy*. Edward Elgar Publishing, Cheltenham, UK and Northampton, MA, USA.

Cartier, L. (2014),”The British Columbia Wine Industry: Can It Compete With The Big Guys?”, *American Association of Wine Economists, Business*, Working Paper, no. 147.

Castriota, S. and Delmastro, M. (2008), “Individual and collective reputation: lessons from the wine market”, *American Association of Wine Economists, Economics*, no. 30.

Culver, K. Dhaliwal, N., Mooken, M. and Sugden, R. (2015), “Regional Social and Economic Development in the Okanagan, Canada: Envisioning the Future to Initiate a Strategy”, Valdiso, J.M. and Wilson, J.R. (ed.), *Strategies for Shaping Territorial Competitiveness*, Routledge London & New York, pp.194-217.

Charters, S. and Michaux, V. (2014), “Strategies for wine territories and clusters: why focus on territorial governance and territorial branding?”, *Journal of Wine Research*, Vol 25, no. 1, pp.1-4.

Ditter, J.G. and Brouard, J. (2014), “The competitiveness of French protected designation of origin wines: a theoretical analysis of the role of proximity”, *Journal of wine research*, Vol 25, no.1, pp.5-18.

Gade, D.W. (2004), “Tradition, territory, and terroir in French viniculture: Cassis, France, and Appellation Contrôlée”, *Annals of the Association of American Geographers*, Vol *94*, no.4, pp.848-867.

Hickton, C. and Padmore, T. (2004), “Patterns of Innovation in the Okanagan Wine-making Cluster”. Paper presented at Innovation Systems Research Network Annual Meeting, Vancouver, Canada.

Hickton, C. (2005), *Transformations in the Okanagan wine industry; and Reflections on communication, diffusion of innovation, and social capital in the case of the Okanagan wine cluster*, Doctoral dissertation, School of Communication, Simon Fraser University.

Hinnewinkel, J.C. (2007), «L’avenir du terroir: gérer de la complexité par la gouvernance locale», *Méditerranée*, 2, pp.17-22.

Hira, A. and Bwenge, A. (2011), “The Wine Industry in British Columbia: A Closed Wine But Showing Potential”, Simon Fraser University, Department of Political Science.

Kingsbury, A., and Hayter, R. (2006), “Business associations and local development: The Okanagan wine industry’s response to NAFTA”, *Geoforum,* Vol 37, no.4, pp. 596-609.

Karlsen, J. and Larrea, M. (2014), *Territorial development and action research: Innovation through dialogue*, Routledge London & New York.

Maskell, P. and Malmberg, A. (1999), "Localised learning and industrial competitiveness", *Cambridge Journal of Economics*, Vol 23, No. 2, pp.167-185.

Migone, A. and Howlett, M. (2010), "Comparative networks and clusters in the wine industry", *American Association of Wine Economists*, *Business*, Working Paper, no. 62.

Mooken, M., Sugden, R., and Valania, M. (2016), "University Impact on the Economic Development of Non-Metropolitan Regions: Knowledge Organization, Art and the British Columbia Wine Industry", Working Paper, University of British Columbia.

Navarro, M., Valdiso, J.M., Aranguren, M.J. and Magro, E. (2014), "A holistic approach to regional strategies: The case of the Basque Country", *Science and Public Policy*, Vol 41*,* no.4, pp. 532-547.

Patchell, J. (2008), "Collectivity and differentiation: a tale of two wine territories", *Environment and Planning A,* Vol 40, no.10, pp. 2364-2383.

Rimerman, F. and Company LLP, (2013), *The Economic Impact of the Wine and grape Industry in Canada 2011. Ripe, Robust, Remarkable.*

Rimerman.F. and Company LLP '2017), *The Economic Impact of the Wine and Grape Industry in Canada 2015*. Canada's Wine Economy - *Ripe, Robust, Remarkable*

Ross, K.J., (1995), *An analysis of the effect of the free trade agreement on profitability in the British Columbia wine industry*, Doctoral dissertation, University of British Columbia.

Storper, M. (1997), *The regional world: territorial development in a global economy*, Guilford Press.

Unwin, T. (2012), "Terroir: At the heart of geography", Dougherty P.H. (ed.), *The Geography of Wine: Regions, Terroir and Techniques*, Springer Netherlands, pp. 37-48.

Van Leeuwen, C. and Seguin, G. (2006), "The concept of terroir in viticulture", *Journal of Wine Research,* Vol 17, no.1, pp.1-10.

Valdiso, J.M., and Wilson, J.R. (2015), *Strategies for Shaping Territorial Competitiveness*, Routledge London & New York.

Ukraine, a country with unique *terroir* - opportunities to protect appellations within the EU wine PDO/PGI system

Darko Jaksic [1]*, **Oksana Tkachenko** [2], **Iuliia Bulaieva** [3], **Olha Titlova** [2], **Natalia Kameneva** [2], **Olena Motuzenko** [4], **Federica Bonello** [5]

[1] *Center for Viticulture and Oenology, research office in Belgrade, Bul. kralja Aleksandra 84, 11111 Belgrade, Serbia*

[2] *Institute of Food Industry, Odessa National Academy of Food Technologies, 112, Kanatnaya str., 65039 Odessa, Ukraine*

[3] *National Scientific Center "Tairov Institute of Viticulture and Winemaking", Tairove, 40-let Pobedy street, 27, 65000 Odessa, Ukraine*

[4] *Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska str., 01601 Kyiv, Ukraine*

[5] *CREA-VE Asti-Centro di Ricerca per lla Viticoltura e l'Enologia, via Pietro Micca 35, 14100 Asti, Italy*

Abstract: Ukraine is a country with a long-standing tradition in the production of grapes and wine, and it has significant potentials for increasing surfaces under vineyards and ensuring better recognizability of its wines and wine-growing areas. Changes in the winemaking philosophy of Ukrainian wine producers whose imperative in recent years became the production of high-quality wine, as well as the gradual change of tastes of domestic consumers who are increasingly seeking specific wines and the desire of state institutions and associations for Ukrainian wines to have a better position in the European and global markets were the grounds for reforms in the wine sector and harmonization of domestic legislation with standards and requirements of the European Union. The most challenging activity currently carried out as part of the winemaking reforms in Ukraine is the establishment of the EU system for Protected Designation of Origin/Protected Geographical Indication. Bearing in mind that PDO/PGI appellations for wine will result in better recognizability of Ukraine as a wine country, the EU is financing and implementing the project "Support to the Development of a Geographical Indications Systems in Ukraine", within which work is currently underway on the protection of several geographical indications for wine. Following a detailed analysis of the current situation and potentials, discussions with associations and local producers, and other project activities, work started on drafting product specifications for selected appellations. Wine-growing areas, characterized by specific wines, and specific terroir factors that have a crucial impact on quality and characteristics of wines, were selected as areas for future PDO/PGI appellations. These are areas near large water surfaces and the Black Sea (Odessa oblast) and the area near the Carpathian Mountains (Zakarpattia oblast). For the scientific approach and basis for analysis and characterization of climate, soil, and other environmental factors of terroir in selected wine-growing areas, as well as for analyses of human influence, which are all significant factors for quality and characteristics of wines from the future Ukrainian PDO/PGI appellations, we used the methodology from the resolution of the International Organisation for Vine and Wine: Resolution OIV-VITI 423-2012 REV1 on viticulture zoning methodologies, including the application of the Geographic Information System, OIV Resolution OIV/VITI 333/2010, which defines terroir, and regulations of the European Union which define the quality policy and are the foundations for Ukraine's EU integration process regarding the wine sector. This paper presents some of the characteristics of terroir ecological factors in wine-growing areas that were selected to become the first Ukrainian wine PDO/PGI appellations.

Keywords: Terroir, Ukrainian wine-growing areas, wine PDO/PGIs

*Corresponding author. E-mail: darkojaksic@yahoo.com.au

1. Introduction

One of the greatest challenges in the process aiming to establish geographical indications modelled within the EU PDO/PGI system is to adequately prove the link between the quality, reputation, and other specific characteristics of wine and the wine-growing area in which the wine is produced (for the registration of Protected Geographical Indication), i.e. to demonstrate that there is a significant or crucial influence of special natural (environmental) and anthropogenic factors on the quality and characteristics of wines from the relevant area (for the registration of Protected Designation of Origin). Establishing a causal link between the characteristics of a wine-growing area and the quality and characteristics of wine produced in the area, particularly in case of PDO appellations, requires detailed knowledge of the relevant area's *terroir* (Ninkov et. al, (Ed. Ninkov), 2019). For this reason, it is necessary to undertake a detailed analysis of *terroir* factors. This includes the characterization of climate in the wine-growing area, as well as of soil and other environmental factors, including the anthropogenic impact, all of which have a significant impact on the quality and characteristics of future PDO/PGI appellations.

Conducting research of *terroir* of wine-growing areas with the purpose of establishing PDO/PGI appellations is a challenge for science, and the topic of *terroir* is always relevant for multidisciplinary research. According to Asselin et al., 1996, the term *terroir* is a French word that signifies the overall notion of territory, derived from the Latin word *territorium*. However, the concept of *terroir* is not as easy to explain and it occupied numerous authors, in particular: Morlat and Asselin, 1992; Morlat et al., 2001; van Leeuwen and Seguin., 2006; van Leuwen et al., 2004, 2007, 2010; Gladstones, 1992, 2011; Dougherty et al., 2012 and others, as well as Vaudour, 2002; Jones et al., 2004; van Leeuwen et al., 2004; Unwin, 2012, and other authors particularly underline the interaction between elements of *terroir*.

Burns (2012) states that seven factors can be used as grounds to differentiate between wines: variety, climate, geological substrate (soil), water characteristics of soil, topographic conditions, technology of grape production, and the technology of wine production. The first five factors are particularly important for the concept of *terroir*. *Terroir* is defined as an entity spreading between time and space, consisting of material (soil, climate, vine variety, etc.) and non-material elements (history, culture, tradition, reputation, etc.) (Tomasi et al., 2013).

Various efforts on research of *terroir* mostly focus on specific wine-growing areas, similar to the research conducted in France for Bordeaux and Bourgogne (Bois et al., 2007 and 2008; Lemaire and Kasserman, 2012 and others). In addition, some of the research was directly used to draft or amend production specifications for PDO/PGI appellation for wine, which was the case in Italy (Tomasi and Gaiotti, 2011; Tomasi et al, 2011; Tomasi at al., 2013), Portugal (Guchard, et al., 2004) Serbia (Jakšić et al., 2016, 2018 and 2019; Jakšić (Ed. Ninkov), 2016; Jakšić and Perović, (Ed. Ninkov), 2017); Jakšić and Perović, (Ed. Ninkov), 2019), and other countries.

OIV provisions for viticulture zoning of wine-growing areas and *terroir*, contemporary scientific research and familiarity with Ukrainian viticultural areas and characteristics of Ukrainian wines served as the starting point in the research of *terroir* factors with the aim of establishing PDO/PGI appellations for Ukrainian wines. Scientific and professional experts from Ukraine, Serbia, and Italy conducted the relevant research as part of the project team in the EU funded project "Support to the Development of a Geographical Indications Systems in Ukraine" EuropeAid/138706/DH/SER/UA. The main beneficiary of the project is the Ministry of Economic Development, Trade and Agriculture of Ukraine, and implementation of the project began in 2017 with the scheduled end in April 2021. The project is funded by the European Union and implemented by the consortium composed of DMI Associates (France), GFA Consulting Group (Germany) and ADECIA (France), which hired international experts in various fields. In addition to experts from relevant Ministries, experts from other Ukrainian institutions played an active role in the project. In accordance with the general aim of strengthening the viticultural and wine production sector in Ukraine, Project tasks include, among other, drafting product specifications and protection of PDO and PGI appellations for wines on the national and EU level, and establishing a PDO/PGI system in line with the EU system. In order to make it easier for wine producers and their associations to protect future PDO and PGI appellations in accordance with the EU standards, project activities were focused on joint multidisciplinary scientific and expert work by the project team and representative Ukrainian associations and wine producers.

2. Materials and methods

Study area

Ukraine, being a country with a large territory, has a substantial number of wine-growing areas. Within five administrative regions ("oblast") in the south of the country near the Black Sea and the Zakarpattia oblast, there are 15 wine-growing macro zones and 58 micro zones (Titlova and Thachenko, 2019).

This paper focuses on individual significant ecological elements of *terroir* in wine-growing areas that were selected for future protection of geographical indications and in which there is the highest concentration of vineyards. These are the areas with future PDO/PGI appellations Bilhorod-Dnistrovskyi, Bolhrad, Izmail, and Reny rayon in the Odessa oblast (Southern Ukraine, future PDO Chabag, PDO Yalpuh, PGI Acha-Abag, and PGI Prydunaiska Bessarabia), and several rayons of the Zakarpattia oblast (Western Ukraine, future PGI Zakarpattia) (Figure 1).

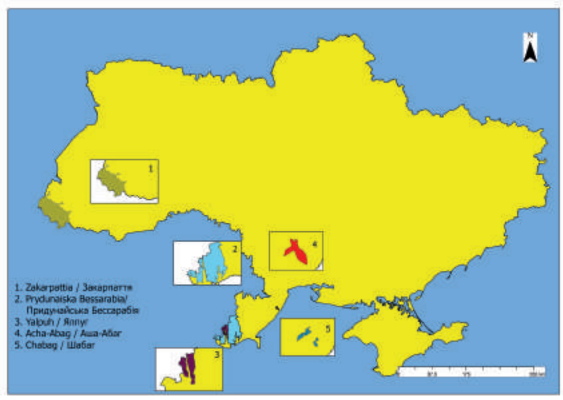


Figure 1. Examined wine-growing areas (future wine PDO/PGIs)

Methodology

Research of climate parameters of *terroir* in wine-growing areas in South Ukraine was carried out on grounds of daily data recorded for the past 30 years (1991-2018) in 15 meteorological stations that function as part of the Ukrainian Hydro-meteorological Institute. Research of climate and other *terroir* factors in Western Ukraine is currently underway, and will not be presented in this paper.

Analysis and characterization of climate *terroir* data for wine-growing areas in Southern Ukraine was carried out through interpolation of observed and previously analysed data from meteorological stations regarding the most important climate parameters and OIV bioclimatic indices in spatial data (maps). Collection and analysis of climate data and interpolation of spatial data was carried out in accordance with

the OIV Resolution OIV-VITI 423-2012 REV1, and with use of operational software which provided remarkably good results in characterization of climate in wine-growing areas in Serbia, Montenegro, Italy and other countries (Vujadinovic et al., 2016; Vukovic at al., 2016, and others). In particular, data on bioclimatic indices used in the Geoviticulture Multicriteria Climatic Classification System (MCC System) (Tonietto and Carbonneau, 1998, 2000, 2004) was analysed and compared.
Data from previous research conducted by the National Scientific Center "Tairov Institute for Viticulture and Winemaking", Odessa, was used for analysis of soil, as one of the basic *terroir* factors. Research in South Ukraine used thematic maps and data for the Black Sea area (Vlasov, 2009).

Research of topographic *terroir* parameters was carried out using the Geographic Information Systems (GIS) technology, including all methods and activities with the aim of collecting, modelling, processing, analysis, and presentation of spatial data. Application of GIS technology was in accordance with the principles of use of GIS as defined in OIV Resolution OIV-VITI 423-2012 REV1. Topographic information derived from the Digital Elevation Model (DEM) was used for spatial analysis and modelling in order to obtain spatial information on elevation, inclination, and exposure of wine-growing areas (future PDO/PGIs). Accuracy of results obtained through the Digital Elevation Model (DEM) depends on the resolution of the source model that, in this case, had 30-meter accuracy.

With the aim of distinguishing environmentally homogenous zones and determining borders for future appellations of geographical indications, relevant maps were scanned, and geo-referencing, vectorisation, establishment of a database and integration of data was carried out, additionally, a large number of thematic layers was prepared and drafted as part of these activities. All activities were carried out using GIS software packages: QGis v2.18., Global Mapper 13 and Google Earth. The standard UTM VGS-84 projection was used for the Odessa oblast: Zone 35 (24ºE - 30ºE - Northern Hemisphere (Zone 35 - Northern Hemisphere), and for the Zakarpattia oblast: Zone 34 (18ºE - 24ºE - Northern Hemisphere (Zone 34 - Northern Hemisphere).

3. Results and discussion

Climate conditions

Climate has a strong influence on the geographic dispersion of cultivated vine varieties, grape production and characteristics and quality of wine (Bois et al., 2012). Van Leeuwen et al. (2004) stress the importance of climate as a *terroir* factor. Climate exhibits spatial variations in different scales: from one area to the other (macroclimatic scale); within a given wine-growing area (mesoclimatic scale); with change in topography (topoclimatic scale); within a given viticultural parcel (microclimatic scale) (van Leeuwen et al., 2007). For this reason, scientific approach in analysis of climatic parameters through application of the most contemporary meteorological and climate methods was to firstly analyse the climate in the larger wine-growing area in the South of Ukraine (Figure 2), followed by a detailed analysis of climate data for every future PDO/PGI appellation.

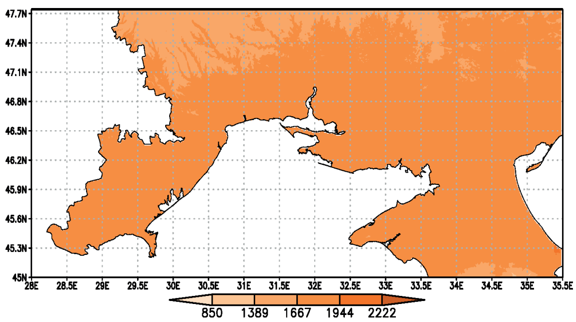


Figure 2. Winkler index (WIN) for southern Ukraine

There is a slight difference between climate factors among the wine-growing areas selected for the procedure for protection of geographical indications. Although 25 climate parameters and OIV bioclimate indices (viticultural climatic indexes) were researched in detail, due to the limited space in this paper, we will present only bioclimate indices included in the Geoviticulture Multicriteria Climatic Classification System (MCC System). These are the Huglin Heliothermal Index (HI) (Huglin, 1978), Cool Night Index (CI) (Tonietto, 1999 and Tonietto and Carbonneau, 2004) and Drought index (DI) (Riou, 1994 and Tonietto, 1999), which have the chief influence on the *terroir* of future of geographical indications in Ukraine. These indices are representative of the variability of the viticultural climate worldwide, related to the requirements of varieties, vintage quality (sugar, colour, aroma), and typeness of the wines.

The HI differs in territories selected for future geographical indications. The highest HI was recorded in the area of the future PGI appellation Prydunaiska Bessarabia, which is the warmest of the examined wine-growing areas. With respect to this index, the examined areas can be classified as Cool (HI-1) (higher value in this class) and Warm (HI+1) climate class (Table 1). This indicates that there are favourable conditions for production of high-quality grapes and wines, and no obstacles for ripening of grapes of vine varieties traditionally grown in this part of Ukraine, in particular in lower parts of the future PGI Prydunaiska Bessarabia (Figure 3).

Table 1. Huglin Heliothermal Index (HI)

Future PDO or PGI	Minimum Value	Maximum Value	Climate classes	Acronym
PDO Chabag	1,988.67	2,018.81	Cool	HI-1
PDO Yaluh	2,088.40	2,275.88	Warm*, Cool	HI+1, HI-1
PGI Acha-Abag	1,990.88	2,020.17	Cool	HI-1
PGI Prydunaiska Bessarabia	2,090.77	2.329.32	Warm*, Cool	HI+1, HI-1

** Dominant climate class*

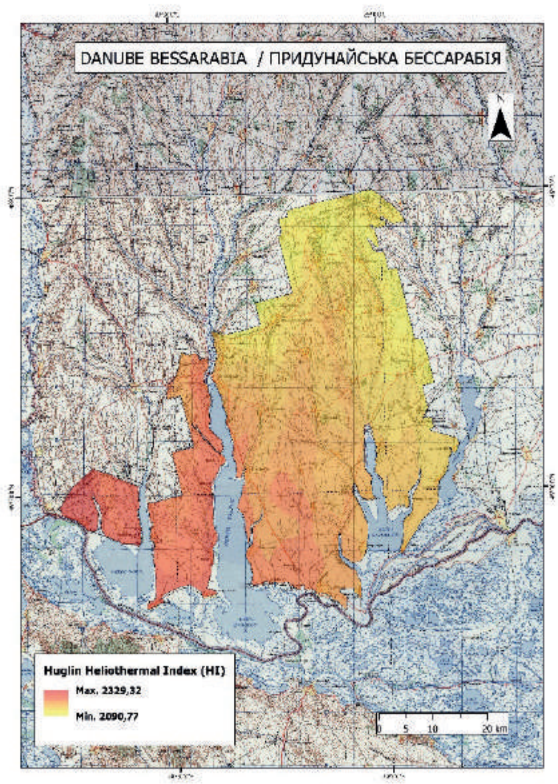


Figure 3. Huglin Heliothermal Index (HI) in the future PGI Prydunaiska Bessarabia

CI, which corresponds to the average minimal temperature during September (in the Northern Hemisphere), has a strong effect on the *terroir* of wine-growing areas, which manifests through the quality and characteristics of wines from the relevant area. CI in the examined wine-growing areas selected for future PDO/PGI appellations ranges from 11.66°C (Cold nights, CI+2) in the future PDO Yalpuh to 14.75 (Warm nights, C-1) in the future PGI Acha-Abag (proposed name for this appellation) (Table 2).

Table 2. Cool Night Index (CI)

Future PDO or PGI	Minimum Value (°C)	Maximum Value (°C)	Climate classes	Acronym
PDO Chabag	14.51	14.70	Warm nights	CI-1
PDO Yaluh	11.66	12.70	Cold nights*, Cool nights	CI+2, CI+1
PGI Acha-Abag	14.54	14.75	Warm nights	CI-1
PGI Prydunaiska Bessarabia	11.85	13.77	Cool nights*, Cold nights	CI+1, CI+2

** Dominant climate class*

Favourable CI in the examined wine-growing areas, in particular in the territory of the future PDO Yalpuh (Figure 4), enables production of intensely coloured wines with rich aromatic complexes and the full expression of newly created Ukrainian varieties grown in the territory of this future appellation.

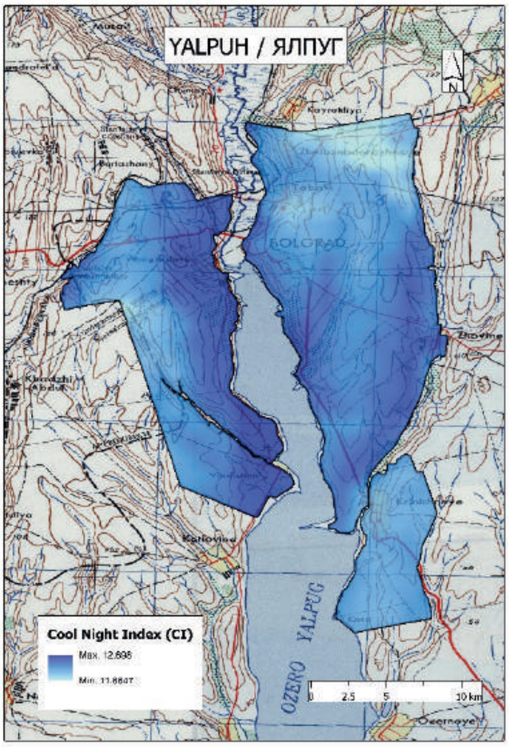


Figure 4. Cool Night Index (CI) within future PDO Yalpuh

Water deficit and stressful conditions caused by this have a negative effect on vine cultivation. However, in the contemporary viticultural practice this issue is commonly resolved with irrigation of vineyards, which is the prevailing practice in Ukrainian wine-growing areas with low precipitation. On the other hand, an unfavourable water balance coupled with high quantities of precipitation has a more significant negative effect on the quality of grapes and therefore wine. DI, as the index that characterizes the water component in a given wine-growing area, is a fairly good indicator of possible quality of grapes and wine, and its application in modern *terroir* research is unavoidable. DI represents the soil humidity value at the end of the vegetative period, with the assumption that the initial soil humidity is 200 mm. The DI was favourable in all examined wine-growing areas (Table 3). This bioclimatic index was lowest in the territory of the future PDO Chabag, which therefore has the least possibility for reduction of grape and wine quality due to high humidity (Figure 5). The highest DI was recorded in the territory of the future PDO Yalpuh (99.62), but this is not a high value, it merely indicates that successful grape and wine production is possible even without irrigation of vineyards.

Table 3. Drought index (DI)

Future PDO or PGI	Minimum Value	Maximum Value	Climate classes	Acronym
PDO Chabag	9.55	17.36	Moderately dry	DI+1
PDO Yaluh	27.35	99.62	Sub-humid*, Moderately dry	DI-1, DI+1
PGI Acha-Abag	10.41	15.87	Moderately dry	DI+1
PGI Prydunaiska Bessarabia	11.97	87.33	Moderately dry*, Sub-humid	DI+1, DI-1

** Dominant climate class*

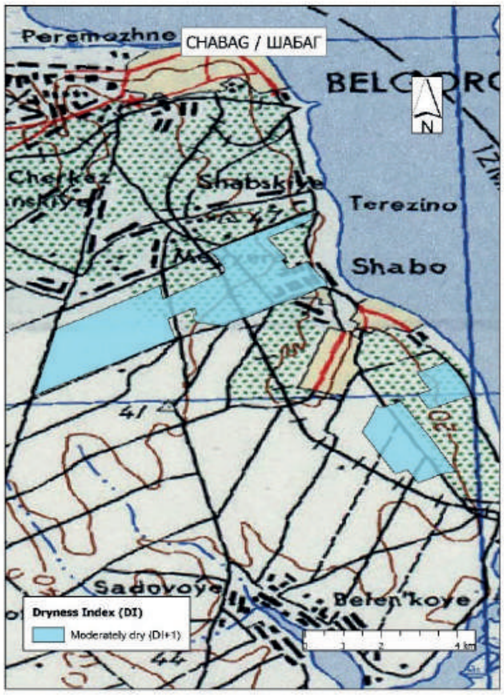


Figure 5. Drought (Dryness) index (DI) within future PDO Chabag

Multi-criteria climate analysis was used to examine wine-growing areas, i.e. to classify future PDO/PGI appellations in the following manner:
- PDO Chabag: HI-1, CI-1, DI+1;
- PDO Yalpuh: HI+1, CI+2, DI-1;
- PGI Acha-Abag: HI-1, CI-1, DI+1;
- PGI Prydunaiska Bessarabia: HI+1, CI+1, DI+1.

Through comparison of combined bioclimatic indices of future PDO/PGI appellations with those indices in wine-growing areas worldwide in 21 countries within the MCC system, it was determined that the future PDO Chabag, PDO Yalpuh and PGI Acha-Abag do not have a classification similar to any other wine-growing area in the MCC system. Climate conditions of the future PGI Prydunaiska Bessarabia are in the same multi-criteria classification as two other known areas - Valladolid (Spain) (PDOs: Ribera del Duero, Rueda and Cigales), and Viseu (Portugal) (PDO Dão). These differences in bioclimatic indices indicate a uniqueness of *terroir* of future Ukrainian PDO/PGI appellations, which represents a great potential in terms of underlining the uniqueness and specificities of wines.

Soil

Soil is a more complex *terroir* factor, significantly influenced by human activities, in both positive and negative sense (Tomasi et al. 2013). Mineral physical characteristics of soil are closely related to the quality of produced wine (Lulli et al., 1989; Costantini et al., 1990), and differences in soil can result in varying quality of grapes (Costantini, 1987). This is the reason why project activities aimed at drafting production specifications for future Ukrainian PDO/PGI appellations were particularly careful regarding soil as a *terroir* factor. According to an earlier research conducted by the “Tairov Institute of Viticulture and Winemaking”, Odessa, chernozem is the main soil type in the Odessa oblast (Vlasov, 2009), while the cambisol soil type is predominant in localities with vineyards in the Zakarpattia oblast. However, there are significant differences among soils in future PDO/PGI appellations in the Odessa oblast. Low humus (1.2-1.7%) and light soil in the future PDO Chabag and PGI Acha-Abag is quite specific and is characterized by mid-carbonate and high carbonate qualities (4-9% $CaCO_3$) and low alkaline pH reaction of soil (7.8-8.2 pH in KCl). Soils that are not rich in combination with low quantity of precipitation have a direct impact on reduced grape yields and production of grapes with smaller berries, all of which influences the *terroir* and results in high quality and unique characteristics of wine from the future PDO appellation Chabag and PGI appellation Acha-Abag. On the other hand, soil in the future PDO Yalpuh has its own specificities. Chernozem with organic matter content from 1.54 to 2.98%, with light loamy to mid-loamy granulometric content is predominantly low alkaline to alkaline. Soil in certain typical wine-growing localities in this future appellation is predominantly mid-carbonated in deeper soil layers, which is favourable for successful grape vine cultivation and production of high-quality grapes.
Characteristics of viticulture soil, which differs among varying wine-growing areas and even between different localities in the examined area certainly affects the characteristics of wine from future PDO/PGI appellations, which is something that Ukraine can be recognized for in the domestic and foreign markets.

Topographic conditions

Topographic conditions of *terroir* play an important role regarding quality and characteristics of wine. They have a direct effect on the development of vine, since they have a significant impact on changes on the mesoclimate and microclimate level, as well as on water, air and chemical characteristics of soil (Tomasi et al., 2013). Topography has an influence on climate parameters, given that for every 100 m of altitude the temperature drops for 0.6 °C (van Leeuwen et al., 2007). Additionally, positive terrain inclination, as an important topographic factor, results in increased exposure to photosynthetic and heat insulation, earlier soil heating, reduced effects of frost and improved soil drainage (Jakšić et al., 2018). Among topographic factors, geographic latitude, altitude, inclination, terrain exposure, and relief, that is, orographic characteristics are the most significant. However, large water surfaces and forests can also have a significant impact on vine cultivation, and therefore on the quality and characteristics of wine from the given wine-growing area, i.e. from the relevant appellation. Today, research into topographic factors of *terroir* in wine-growing areas cannot be imagined without application of GIS technology. For this reason, topographic factors of selected wine-growing areas were analysed through application of GIS technology, and this paper will present only some factors that have the largest impact on characteristics of wines from the future Ukrainian PDO/PGI appellations.

Terrain inclination and altitude

Examined wine-growing areas selected for future PDO/PGI appellations can be classified in three groups with respect to altitude (Table 4). Namely, wine-growing areas in Odessa oblast have a lower altitude, among them future appellations PDO Chabag and PGI Acha-Abag have the lowest altitude ranging between 5 and 48 m. A slightly higher altitude dominates in territories of future appellations PDO Yalpug (up to 168 m) and PGI Prydunaiska Bessarabia (up to 215 m). Finally, since the wine-growing area of the future appellation PGI Zakarpattia is spread along southwestern slopes of the Carpathian Mountains, the altitude is significantly higher here, and goes up to 575 m (Figure 6). These types of differences in altitude of territories of future PDO/PGI appellations certainly have an effect on characteristics of wines, and clear differences and characteristics can be observed among wines from analysed areas. All of this provides great opportunities to present characteristics of wine caused by differences in *terroir* through touristic activities. Insisting on uniqueness and characteristics can result in better valorisation of wines from future PDOs and PGIs.

Table 4. Altitude and Terrain Inclination

Future PDO or PGI	Altitude (m)			Inclination (°)		
	Min.	Max.	Med.	Min.	Max.	Med.
PDO Chabag	6	47	27.43	0	15.64	3.19
PDO Yalpuh	-18	168	71.14	0	40.98	5.61
PGI Acha-Abag	5	48	22.42	0	15.66	2.95
PGI Pryd. Bessarabia	-18	215	66.29	0	62.80	5.90
PGI Zakarpattia	23	575	149.15	0	51.50	5.93

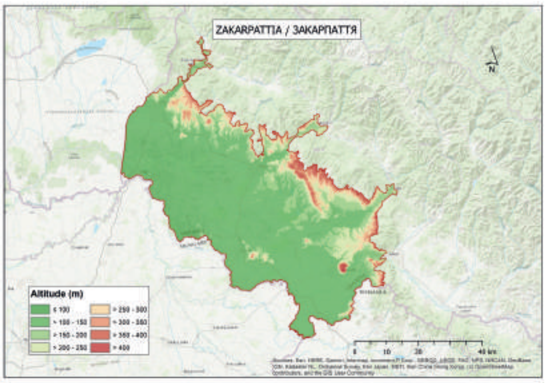


Figure 6. Altitude of Zakarpattia (m)

Since terrain inclination determines temperatures and risk from frost, and thereby affects microclimate conditions for cultivation of vine and ripening of grapes and their quality, examination of terrain inclination in wine-growing areas is necessary when determining the characteristics of *terroir* of those areas. Examined wine-growing areas have different terrain inclinations, the gentlest inclination was recorded in areas of future appellations PGI Acha-Abag (medium value 2.95°) and PDO Chabag and (medium value 3.19°), while slightly steeper inclination was recorded in terrains of the future appellation PDO Yalpuh (medium value of 5.61°). The steepest inclination was recorded in terrains of future appellations PGI Prydunaiska Bessarabia (medium value of 5.90°) and PGI Zakarpattia (medium value of 5.93°). Although vineyards were mostly concentrated in terrains with gentler inclinations, such terrain inclinations values are certainly an important characteristic of the *terroir* of these areas, in particular in the future PGI Zakarpattia (Figure 7).

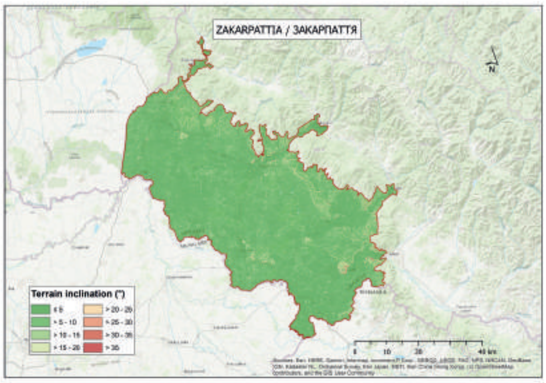


Figure 7. Terrain inclination of Zakarpattia (°)

Terrain exposure and presence of large water surfaces

Exposure of terrain in which vineyards are situated is an important element of topographic *terroir* factors. Differences were recorded in exposure of examined wine-growing areas, i.e. future PDO/PGI appellations, but the majority of vineyards were situated in the so-called warmer exposure (southern, southeastern, eastern, southwestern and western exposure). Additionally, typical vineyards that represent the *terroir* of relevant wine-growing areas are situated in terrain exposures characteristic for the given area. Thus, terrain exposure of vineyards of the future PGI Zakarpattia follows the orographic characteristics of the terrain. They are predominantly spread along southwestern, southern and western slopes of the Carpathian Mountains. However, typical terrain exposures of wine-growing areas in the Odessa oblast are oriented directly depending on location of large water surfaces. Thus, dominant terrain exposures of typical vineyards in the future PDO Yalpuh are south-western and western. Namely, terrains with typical vineyards are mostly facing towards the large water surface of Lake Yalpuh (Figure 8). Typical terrain exposures of representative vineyards in the future PDO Chabag and PGI Acha-Abag (Figure 9) are eastern, northeastern and other exposures with very mild inclinations, facing the large water surface of the Dniester Liman.

Such terrain exposures, facing large water surfaces that additionally improve insulation, temperature conditions in vineyards and reduce temperature extremes, are one of the main characteristics of these wine-growing areas, i.e. of future PDO/PGI appellations, and these characteristics can be underlined as a unique feature of Ukrainian *terroir*.

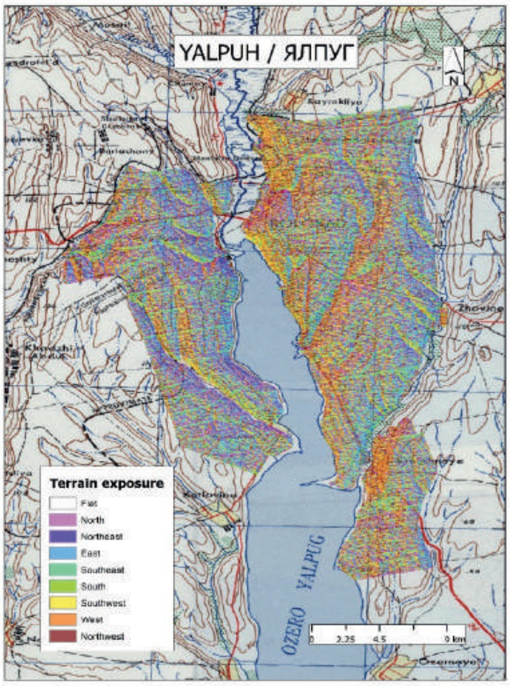


Figure 8. Terrain exposure of Yalpuh

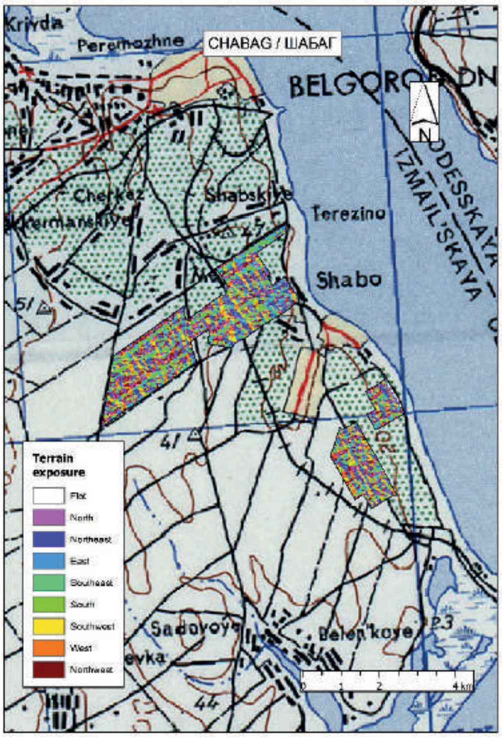


Figure 9. Terrain exposure of Chabag

4. Conclusion

Terroir constantly occupies the attention of wine experts on the global level and it is frequently the subject of multidisciplinary scientific research. With initiation of reforms of the system for geographical indications and harmonization of wine regulations with EU legislation, intense efforts began in Ukraine on examination of *terroir* factors of wine-growing areas with the aim of protecting them as PDO/PGI appellations. During activities within the EU funded project "Support to the Development of a Geographical Indications Systems in Ukraine" and additional research, presumptions that selected wine-growing areas in Ukraine, that is, future PDO/PGI appellations, are distinguished with specific and unique *terroir* have been confirmed on expert and scientific grounds.

Through comparison of classifications of bioclimatic indices within the Geoviticulture Multicriteria Climatic Classification System (MCC System) with data on global wine-growing areas from 21 countries, it was determined that the future PDO Chabag, PDO Yalpuh and PGI Acha-Abag do not have classifications similar to any other wine-growing area in this system, while climate conditions in the future PGI Prydunaiska Bessarabia have the same multi-criteria climate classification as two other areas in Spain and Portugal.

With respect to altitude, future PDO/PGI appellations can be divided in three groups. Localities in the future PGI Zakarpattia are characterized by higher altitude.

Terrain inclinations of selected wine-growing areas, i.e. future PDO/PGI appellations, tend to be different in different wine-growing areas. Inclinations are mostly mild in localities in which vineyards are situated, but vineyard locations of PGI Zakarpattia are especially characterized by steeper terrain inclinations.

Terrain exposure of vineyards facing large water surfaces, which improves insulation, temperature conditions in vineyards and has a favourable effect on temperature extremes, are one of the main characteristics of wine-growing areas of future PDO Chabag, PDO Yalpuh and PGI Acha-Abag.

Characteristics and mutual differences of examined *terroir* factors in selected wine-growing areas indicate high quality and uniqueness of wines from these areas. All of this represents a huge potential for a more active promotion and for emphasising the characteristics of wines, and for better valorisation of production within future PDO/PGI appellations.

References

Asselin C., Morlat R., Cellier P., Bouvet M.H., Jacquet A., Cosneau M., 1996. Aptitude du chenin à l'élaboration de vins liquoreux en relation avec les grandes catégories de terroir de l'A.O.C. Coteaux du Layon. 1er Colloque International "Les Terroirs Viticoles", Centre des Congrès d'Angers.

Bois B., 2007. Cartographie agroclimatique ŕ méso échelle: méthodologie et application ŕ la variabilité spatiale du climat en Gironde viticole. Conséquences pour le développement de la vigne et la maturation du raisin. Thse de Doctorat, Université de Bordeaux II. https://tel.archives-ouvertes.fr/tel-00695507/document.

Bois B., Blais A., Moriondo M., Jones G.V., 2012. High resolution climete spatial analysis of European winegrowing regions. Proceedings of the IX[th] International *Terroir* Congress. 2(1):17-20.

Bois B., van Leeuwen C., Pieri P., Gaudillère J.P., Saur E., Joly D., Wald L., Grimal D., 2008. Viticultural agroclimatic cartography and zoning at mesoscale level using terrain information, remotely sensed data and weather station measurements. Case study of Bordeaux winegrowing area. Proceedins of the VI[th] International *Terroir* Congress, Changins, Switzerland. 2, 455-462.

Burns S., 2012. The importance of Soil and Geology in Tasting *Terrior* with Case History from Willamette Valley, Oregon, in: Dougherty P. (Ed.): The Geography of Wine. Springer Science+Business Media B.V. 978-94-007-0463-3.

Costantini E.A.C., 1987. Cartografia tematica per la valutazione del territorio nell'ambito dei sistemi produttivi. Bacini dei torrenti Vergaia e Borratello: area rappresentativa dell'ambiente di produzione del vino Vernaccia di San Gimignano (Siena). Istituto Sperimentale Per Lo Studio E La Difesa Del Suolo, XVIII:23-74.

Costantini E.A.C., Lulli, L., Pinzauti, S., Cherubini, P., Simoncini, S., 1990. Indagine sui caratteri funzionali del su olo che agiscono sulla qualità del vino. Atti del X incontro su: contributi ed influenza della chimica nella produzione, conservazione e commercializzazione del vino. Istituto di Chimica Organica, University of Siena, 27-40.

Dougherty P.H. (Ed), 2012. The Geography of Wine: Regions, *Terroir* and Techniques. Springer Science+Business Media B.V. 978-94-007-0463-3.

Furet M.I., Christen M., Monteau A.C., Monamy C., Bois B., Guilbault P., 2012: Using multifactorial analysis to evaluate the contribution of *terroir* components to the oenological potential of grapes at harvest. Proceedings of the IXth International Terroirs Congress, Dijon - Rejns, France, 2(1):9-13.

Gladstones J., 1992. Viticulture and Environment. Winetitles, Adelaide, Australia.

Gladstones J., 2011. Wine, *Terroir* and Climate Change. Wakefield Press. ISBN: 1862549249.

Guchard F., Martins Pereira G., Guimaraens D., Peixoto F., Ribeiro de Almeida A., Silva Lopos T., Sandeman G., Carvalho M., 2004. Port Wine. Instituto Dos Vinhos Do Douro E Do Porto. ISBN: 972-8233-14-0.

Huglin P., 1978. Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. C.R. Acad. Agric. Fr., 64, 1117-1126.

Jakšić D., 2016. Some important characteristics of Tri Morave wine-growing region, in: Ninkov J. (Ed.) Pedological and agrochemical characteristics of the Tri Morave wine-growing region. Institute of Field and Vegetable Crops, Novi Sad, ISBN: 978-86-80417-66-0, 41-83.

Jakšić D., La Notte P., Mannini F., Žunić D., Korać N., Todić S., Životić Lj., Perović V., Ivanišević D., Vuković A., Jović S., 2012. New zoning of the viticulture areas in Serbia. IX e Congres International Terroirs Vitivinicoles. 25-29.06.2012. Dijon-Riems, France. 44-45

Jakšić D., La Notte P., Perović V., Ivanišević D., Vujadinović M., Beader M., Vuković A., 2016. Some characteristics of Knjazevac *terroir* – first Serbian modern wine PDO. 5th International Symposium on Agricultural Sciences, Book of Abstracts, 115.

Jakšić D. and Ninkov J., 2016. The concept of the geographical indication system for wines and the importance of soil features in this regard, in: Ninkov J. (Ed.) Pedological and agrochemical characteristics of the Tri Morave wine-growing region. Institute of Field and Vegetable Crops, Novi Sad, ISBN: 978-86-80417-66-0, 15-40.

Jakšić D. and Perović V., 2017. Some important characteristics of Niš wine-growing region, in: Ninkov J. (Ed.) Soil characteristics of the Niš wine-growing region. Institute of Field and Vegetable Crops, Novi Sad, ISBN: 979-86-80417-75-2, 43-91.

Jakšić D. and Perović V., 2019. Important characteristics of *terroir* of Cer-Valjevo wine-growing region, in: Ninkov J. (Ed.) Soil characteristics of the Cer-Valjevo wine-growing region. Institute of Field and Vegetable Crops, Novi Sad, ISBN: 978-86-80417-81-3, 29-78.

Jaksic D., Perovic V., Beader M., Radojevic I., Ivanisevic D., Vukovic A., Vujadinovic M., La Notte P., 2018. Characteristics of Vineyards in Wine-Growing Areas in Serbia. Proceedings of XII[th] International *Terroir* Congress, 1822 June 2018, Zaragoza (Spain), 3A-91.

Jones G.V., Nelson P., Snead N., 2004. Modeling Viticultural Landscapes: A GIS Analysis of the Terroir Potential in the Umpqua Valley of Oregon. Geoscience Canada. 31(4):167-178.

Lemaire D., Kasserman D., 2012. Bordeaux and Burgundy: A Comparison of Two French Wine Regions in Transition, in: Dougherty, P. H. (Ed.), The Geology of Wine. Springer Science+Business Media B.V. 978-94-007-0463-3.

Lulli, L., Costantini, E.A.C., Mirabella, A., Gigliotti, A., Bucelli, P., 1989. Influenza del suolo sulla qualità della Vernaccia di San Gimignano. VigneVini, 1/2:53-62.

Morlat R., Asselin C., 1992. Un terroir de référence pour la qualité et la typicité des vins rouges du Val-de-Loire: La craie tuffeau. Bulletin de l'OIV, 65(34):329-343.

Morlat R., Barbeau G., Asselin C., 2001. Facteurs naturels et humains des terroirs viticoles français methoded´étude et valorization. Etudes et Recherches sur les Systèmes Agraires et le Développement, 32:111-127.

Ninkov J., Jakšić D., Tomić N., Marković S., 2019. *Terroir*, soil and geographical indications of wine, in: Ninkov J. (Ed.) Soil characteristics of the Cer-Valjevo wine-growing region. Institute of Field and Vegetable Crops, Novi Sad, Serbia, ISBN: 978-86-80417-81-3, 13-27.

OIV, 2010. Definition of Vitivinicultural "*Terroir*", Resolution OIV/VITI 333/2010.

OIV, 2012. Guidelines for vitiviniculture zoning methodologies on a soil and climate level, International Organization of Vine and Wine, Resolution OIV-VITI 423-2012 REV1.

Riou C., 1994. Le déterminisme climatique de la maturation du raisin: application au zonage de la teneur en sucre dans la Communauté Européenne (E Commission, Éd.). Publications Office of the European Union, Luxembourg, 322.

Rossello J., Vadell J., Marques M., Cretazzo E., Medrano H., Adrover M., Cifre J., 2012. Characterization of viticultural terroirs in DO Binissalem-Mallorca (Spain). Proceedings of the IXth International Terroirs Congress, Dijon - Rejns, France, 2(1):28-31.

Titlova O., Tkachenko O., 2019. The place of Ukraine in development of the world vine growing and winemaking industry under the changing climate conditions. Proceedings of the 8[th] International Symposium of Oenoviti International network, 89-93.

Tomasi D., Gaiotti F., 2011. I *terroirs* della Denominazione Conegliano Valdobbiadene. CRA-VIT. ISBN: 978-88-97081-07-4.

Tomasi D., Gaiotti F., Jones G., 2013. The Power of the *Terroir*: The Case Study of Prosecco Wine. DOI: 10.1007/978-3-0348-0628-2_1, Springer Basel. ISBN: 978-3-0348-0627-5.

Tomasi D., Marcuzzo P., Giotti F., 2011. Delle terre del Piave. CRA-VIT. ISBN: 978-88-97081-13-5.

Tonietto J., 1999. Les Macroclimats Viticoles Mondiaux et l'Influence du Mésoclimat sur la Typicite de la Syrah et du Muscat de Hambourg dans le Sud de la France - Méthodologie de Caractérisation. Thèse de doctorat, Ecole Nationale Supérieure Agronomique de Montpellier, Montpellier (France), 216.

Tonietto J., Carbonneau A., 1998. Facteurs mésoclimatiques de la typicité du raisin de table de l'A.O.C., ʿMuscat du Ventoux' dans le Département de Vaucluse. Progrès Agricole et Viticole, 12:271-279.

Tonietto J., Carbonneau A., 2000. Système de Classification Climatique Multicritères (C.C.M.) Géoviticole. Proceedings of the 3rd International Symposium. Zonification vitivinicola, Tenerife, Spain, II:1-16.

Tonietto J., Carbonneau A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. Agricultural and Forest Meteorology, 124(1/2):81-97.

Unwin T., 2012. *Terroir*: at the heart of geography, in: Dougherty, P.H. (Ed.), The Geography of wine. Springer Science+Business Media B.V. 978-94-007-0463-3.

Van Leeuwen C., Bois B., De Resseguier L., Pernet D., Roby J.P., 2010. New methods and technologies to describe the environment in *terroir* studies. Proceedings of the VIIIth *Terroir* Congress, Soave, Italy, 2:3-13.

Van Leeuwen C., Bois B., Pieri P., Gaudillère J.P., 2007. Climate as a *terroir* component. Proceedings of the Congress on climate and viticulture, Zaragoza, 90-98.

Van Leeuwen C., Friant P.H., Choné X., Tregoat O., Koundouras S., Dubourdieu D., 2004. The influence of climate, soil and cultivar on *terroir*. American Journal of Enology and Viticulture, 55(3): 207-217.

Van Leeuwen C., Seguin G., 2006. The concept of *terroir* in viticulture. Journal of Wine Research, 17:1-10.

Vaudour E., 2002. The quality of grapes and wine in relation to geography: notions of *terroir* at various scales. Journal of Wine Research, 13(2): 117-141.

Vlasov V.V., 2009. Ekolohiia vynohradu Pivnichnoho Prychornomoria. TOV "LERADRUK". BBK 42.36, UDK 634.83:631.95.

Vlasov V.V., 2013. Ekolohicheskie osnovy formirovaniia vinohradnykh landshaftov. FOP Petrov O.S. ISBN 978-966-2336-12-2.

Vujadinovic M., Vukovic A., Jaksic D., Pavicevic S., Mijanovic T., Pazin N., Drljevic M., Popovic T., Beader M., Perovic V., Ivanisevic D., Maras V., La Notte P., 2016. Bioclimatic Viticultural Indices in Montenegro. Proceedings of the XI International *Terroir* Congress, Oregon, 2016, 111-116.

Vukovic A., Vujadinovic M., Ruml M., Rankovic-Vasic Z., Przic Z., Beslic Z., Matijasevic S., Vujovic D., Todic S., Markovic N., Sivcev B., Zunic D., Zivotic Lj., Jaksic D., 2018. Implementation of Climate Change Science in Viticulture Sustainable Development Planning in Serbia. Proceedings of the XI International *Terroir* Congress, Oregon, 2016, 26-31.

New old world: Danubian Bessarabia. History of wine making.

Alla Plachkova [1]*

[1] *wine expert (WSET Level 3 Award), co-owner of a family winery "Kolonist", 01013, Ukraine, Kyiv, Promyslova street 4.*

Abstract: Many wine experts today divide the whole world into 3 parts: Old World – mother Europe, New World – the US, Argentina, Australia, Chile, New Zealand, South African Republic. New New World – China, with its boom of wine production and buying all the winemaking equipment from Europe for years ahead. But now many wine experts and bloggers define one more part – the 4th part of the wine world – New Old World. I think you already know what it is. The parts of the world, where the wine making had a deep and long-lasting history in the past, but because of certain historical events, wars, changes of rulers and laws, winemaking was lost or forgotten. But now the art of winemaking is revived and the genetic memory of the people and the spirits of the land do their job. We discover now undeservedly forgotten terroir diamonds in the winemaking world. Places, where wine makers have once-in-a-lifetime opportunity to start all over again, and try not to repeat mistakes of the past. Among these New Old World regions, there are the Balkans, The Black sea region, Moldova, Romania, and the South of Ukraine with Bessarabia.

Keywords: Danubian Bessarabia, new old world, colonists, winemaking, viticulture

1. Introduction

Being a local wine producer for over 14 years in Ukraine, every day we ask many questions about our past, but receive no quick answers. Answers appear visible when we learn the history, examine the maps and find out interesting historical facts.

The territory of Ukrainian Bessarabia, which is in fact the territory between two powerful rivers such as the Dniester River and the Danube River, goes along the Black sea. If you look at the climate map of Ukraine, you will see that Ukrainian Bessarabia is located in a temperate continental climate with a strong sea influence. Moreover, the Danube Delta which is a so-called "zero" kilometre location in the South of Ukraine, where the Danube river flows into the Black sea, creates a fantastic microclimate. This place is a nature reserve for fish, pelicans, even flamingos, swans, wild boars, horses, and many other fauna representatives. If you go further to the West towards Romania, you will find even more favourable location for wine growing and winemaking. In a so-called Danubian Bessarabia (a part of Ukrainian Bessarabia which goes along the Danube river) the biggest fresh-water lake in Ukraine named Yalpug with a total area of 149 sq km is located. This lake is officially among the largest lakes in Europe. Now just imagine that you drive about an hour along the lake, which has a huge water surface and has gentle slopes with the vineyards.

I could write a separate article about the lake itself, because this lake is larger than life. It gives everything best to this location, to the flora and fauna and, of course, to the people, who live around the lake. Thanks

*Corresponding author. E-mail: plachkovaan@gmail.com

to the huge water surface, the valley of the lake creates its own microclimate, which resembles the Mediterranean one. But there is a drastic difference and at the same time it is a huge peculiarity of this place, the article will tell you about it later. This climate is most favourable for growing different grape varieties and for making fine wines. Moreover, if you look at the Northern hemisphere of the globe, you will find that Danubian Bessarabia, and especially the valley of this lake, is located at the same latitude with Piedmont in Italy and Bordeaux in France – 45 parallel, most favourable for production of high quality wines in the Northern hemisphere.

When we see the paradise where the vineyards of our family winery "Kolonist" grow, when we see the beauty of the nature, the sun-rises and the sunsets that our vines see every day, we always say: "God was generous to us when he granted this land to us".

2. New Old world

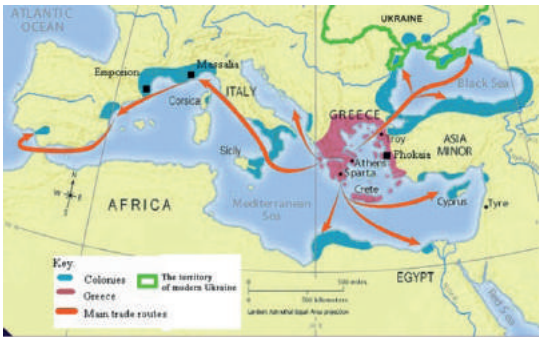
Many wine experts today divide the whole world into 3 peculiar parts: Old World – beloved old mother Europe; New World – the US, Argentina, Australia, Chile, New Zealand, South African Republic (statistically most popular wines today are from the new world, by the way). New New World – China with its boom of wine production and buying all the winemaking equipment from Europe for years ahead, so all the rest of the world is staying in queues to buy French barriques. But, an interesting thing is that China is mostly producing and consuming red wines, even in their hot, wet and seafood regions. The reason is that a Chinese word "wine" means (by one of the meanings) "red alcohol", that is why Chinese psychologically tend to drinking reds.

But the most interesting news is that some wine experts distinguish one more part – the 4th part of the wine world – New Old World. One of the brightest adherents of this theory is Mr. Allan Karl, a global food and wine explorer, a writer, an author of the best-selling book "Forks: A quest for culture, cuisine and connection", award-winning photographer, and a world-known American "WorldRider", who travels the world by motorcycle. He is the first to discover for me a new vision to some parts of the wine globe. New Old World is considered to be the parts of the world, where the wine making had a deep and long-lasting history in the far past, but due or, better to say, because of certain historical events, like wars, changes of rulers and laws, winemaking was lost and forgotten or was very slowly developed. But in course of time, at these regions the art of winemaking is revived and the genetic memory of the people and the spirits of the land do their job. We discover now undeservedly forgotten terroir diamonds in the winemaking world. Places where the wine-makers have once-in-a-lifetime opportunity to start all over again, and try not to repeat mistakes of the past. Among these New Old World regions there are the Balkans (with Albania and former Yugoslavia), Moldova, Romania, the Black sea region, South of Ukraine, including Bassarabia.

3. Danubian Bessarabia

Bessarabia is a region both in Moldova and Ukraine. We used to call a part of Bessarabia in Ukraine – Danubian Bessarabia (along the Danube river).

To prove a winemaking history of this land, we compared an old map of Ancient Greece and a map of the Roman Empire with the map of modern Ukraine (Pic. 1,2).



Picture 1. Ancient Greece colonies and the territory of Ukraine

We found out that Danubian Bessarabia, including the Yalpug lake valley, was a part of these two powerful historical periods. Ancient Greece had a huge period of historical existence. But during VII-VI centuries B.C. the Greek colonies flourished in the Mediterranean and the Northern Black sea regions. About 70 Greek colonies were settled along the Black sea at those times. Greek trade at that period is becoming worldwide. Ancient Greece imported slaves, luxury items, raw materials, and food for increasing population. Of course, the production of wines was a priority of everyday life.

During the Roman Empire, the Trajan Emperor (98-117 A.D.) set the borders of the Roman Empire and built so-called Trajan walls, which still exist! Every time we drive from Odessa along the Black sea towards the Danube, we go across linear earthen fortifications; they look like long even hills, artificially made. These are exactly the borders of the Roman Empire created by the ruler Trajan Emperor. The territory of Bessarabia was called Moesia at those times.

We have many archaeological proofs, in particular remnants of ancient amphorae from Ancient Greece and the Roman Empire, which were found on the banks of the Yalpug lake by archaeologists. At those times wines in amphorae were shipped via the Danube to Europe.

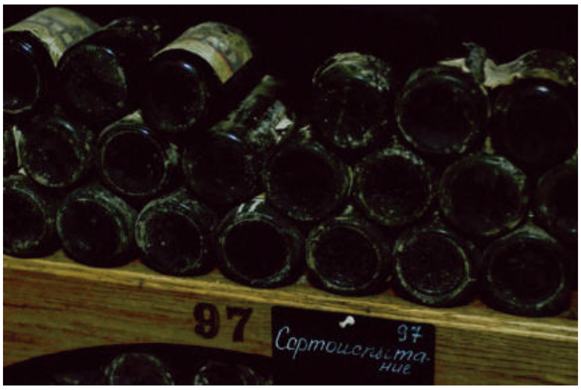
Picture 2. The Roman Empire and the territory of Ukraine

The map and the history prove that Danubian Bessarabia has a long and deep winemaking history. You cannot image Ancient Greece and the Roman Empire without the art of winemaking. So, the wine had been produced here long before the European times. (But French people don’t like this story).
Later on, after many years of domination of the Ottoman Empire, viticulture and winemaking in this area practically stopped.
The revival began after colonial migrants of different nationalities from many European countries came to these lands for cultivation of land and development of the region after the liberation from the Ottoman Empire. Viticulture and winemaking became an important area of agricultural development, especially in the second half of the 19th century.

At the time when the vineyards of European countries were suffering from phylloxera, Danubian Bessarabia compensated wines in Europe, which raised wine-growing and winemaking in the region to a very high level. The Institute of Viticulture and Winemaking named after V.E. Tairov, established in 1910, provided scientific support for development of viticulture and winemaking of high European standards.
During the period prior to World War, Danubian Bessarabia implemented the latest technology with ultra-modern equipment at that time for winemaking. The best European grape varieties of grapes were

planted. Wines from Danubian Bessarabia were already well known in Western Europe, including the Scandinavian countries.
Anyway, after that time many things changed. During the period of the Soviet Union massive wine production was prevailing. In the second half of the 20th century in Soviet times, viticulture and winemaking experienced a tumultuous stage of development. Modern wine-producing enterprises were built at that time, this sector of the agricultural sector gave up to 20 % of revenues to the budget and became a source of economic development of the territory. It should be noted that this industry, for the most part, was developed by industrial methods, which were based primarily on high yields and the quantity of products produced. Absence of foreign competition did not favour the quality either.

But still, during the Soviet Union times we find something positive. One of these positive things was in Krynychne village, located in Danubian Bessarabia. Soviet government settled a special laboratory for variety testing in this village. The laboratory was financed by the government and was dealing with testing various grape varieties, growing them in certain climatic conditions and eventually producing wines from them. The country realized a huge winemaking potential of this very territory with unique climatic conditions and rich soils. Unfortunately, the laboratory was closed and destroyed after the Soviet Union collapsed. But the minds and professionals of that work were saved and still work in this region. At the beginning of 2000s the Plachkov family founded a winery named “Kolonist”. The head of the Soviet laboratory Mrs. Lidiya Zadnipranaya with an over-40-year-experience is now the head of our winemaking laboratory at our Kolonist winery. She managed to save some bottles from those times as a proof of the laboratory existence in the village (Pic. 3).



Picture 3. Bottles dated back to 1970s-1980s from the Soviet laboratory.

In addition to positive things, in 1957, two technical Ukrainian grape varieties of Odessa Black and Sukholymanskyi white were created by the Ukrainian breeders at the Tairov Institute. The breeders brought them out by crossing; parents of Odessa Black are Cabernet Sauvignon and Alicante Bouchet (French), and parents of Sukholymanskyi white are Chardonnay and Plavay (Moldova). The wines from these grape varieties were mainly used for blending.

The Kolonist winery was one of the first to launch Odessa Black and Sukholymanske wines on a commercial basis; and the birth of the “Ukrainian wine” brand began from these two grape varieties. In a short period of time, Odessa Black wine has become very popular and has been estimated not only by experts in Ukraine, but also by experts abroad. These wines are recognized as wines of their own character, recognizable and as wines-individuality. Recently, these wines have become the hallmark of Ukraine on the international stage.

4. Colonists come to the lands of Bessarabia. Bulgarians.

More than 200 years ago, many colonists of various nationalities from different countries came to the lands of Ukrainian Bessarabia, rushing from the Ottoman Empire pressure.

Colonists were scattered across the big territory of Bessarabia, beginning from Odesa city, along the Moldavian and Romanian borders. Bulgarians, in particular, settled in the Yalpug lake valley. Bulgarians, as many other colonists, during the Russian Empire times were granted liberty; they received a free colonial status. The word "kolonist" at those times meant a free person. Every colonist was dreaming to become a "kolonist". It gave them free education, free medicine, no taxes for many years, granted parcels of land. But instead, settlers had to turn an abandoned sunburnt land into a flourishing region.

A characteristic feature of Bessarabia is multi-nationality. Each nationality was trying to preserve peculiarities of their culture, traditions, language, and enogastronomic heritage. Bulgarians thus settled several villages. One of the biggest villages is Krynychne village, with a population over 4000 people.

Bulgarians brought with them their traditions of viticulture and winemaking, preserved since the Thracian times. Today, in the Krynychne village, which is one of the strongest Bulgarian communities, since the establishment of the village more than 200 years ago, the production and consumption of wine has been a must in the gastronomic culture of each family. Given that the region has a large shortage of quality drinking water and high annual air temperatures, wine consumption has played an important role in daily nutrition as well as in preventing diseases. Now every Bulgarian in Bessarabia knows how to produce wine. My husband comes from the Bulgarian family. In honour of Bulgarian ancestors our family winery was called "Kolonist".

It should be noted that culture of wine consumption in Ukraine is growing very fast, which increases the demand for quality wines, including the Ukrainian producers. Luckily, we see an increase of number of producers in the Yalpug Valley and in Ukraine in general.

5. Terroir of Danubian Bessarabia

Danubian Bessarabia is situated in the Black Sea region, on the south of Ukraine in Odesa region, near the Danube Delta. The most favourable terroir here for growing grapes is microclimate in the Yalpug lake valley. In the valley Krynychne village is located at an altitude of 30 meters above sea level. In addition to its close proximity to the Black Sea, Krynychne has one of its greatest advantages - its immediate location on the shore of Yalpug lake.

Sloping shores of the lake allow the vineyards to receive an equal amount of sun, and even double sunrays: direct from the sun and reflected ones from the surface of the lake.

One feature of terroir is the aerodynamics of the slopes of the vineyards. There is intense aeration due to winds, which greatly reduces the likelihood of grapevine diseases. Due to the existence of a large reservoir, the risk of freezing of the vineyards in winter is reduced, and at low average annual rainfall of 300-400 mm, moist air thanks to the large evaporation area saves the situation.
A distinctive feature of this area is a drastic diurnal temperature fluctuation during ripening of the grapes, which allows maintaining the required acidity level and Ph during the accumulation of sugar in the berries. There are more than 300 sunny days a year in the Yalpug Valley. Soils are loamy with deep limestone deposits.

Due to these climatic conditions, almonds, pomegranates, persimmon, Himalayan Ziziphus jujube, olive trees are grown in the valley of Lake Yalpug, and of course grapes are grown.

No wonder, this place was considered to be unique and most favourable for the cultivation of grapes and the production of high quality wines at any historical time.

Making Inroads into Grape Production and Winemaking and Preservation of Agricultural Resources in Japan

Izumi SAWADA[1], **Shigeaki ODA**[2]*, **Shigenaga YOKOTA**[2], **Noriaki KAWASAKI**[2], **Tetsuji SENDA**[2], **Masaki ODA**[2], **Hisao FUNADA**[3], **Toru KONISHI**[4], **Yuma HOSHINO**[5], **Teruya INAGAKI**[6]

[1] *Louis Pasteur Center for Medical Research, 103-5, Kyoto 606-8225, Japan*

[2] *Division of Natural Resource Economics, Graduate School of Agriculture, Kyoto Univ., Kyoto 606-8502*

[3] *Shinshu Ueda Farm, 1049-1 Netsu, Tomi-shi, Nagano Prefecture*

[4] *Arc-en-Vigne, 6667 kano, Tomi-shi, Nagano Prefecture*

[5] *Stardust Vineyard,2682-2 Shigenootu, Tomi-shi, Nagano Prefecture*

[6] *National Chamber of Agriculture, 9-8 Nibancho, Chiyoda-ku, Tokyo*

Abstract: The increasing number of abandoned and non-cultivated farmland has become a critical issue for Japanese agriculture in recent years. It is vital for Japanese agriculture, as an economic sector, to preserve and pass on agricultural resources, such as farmland, to future generations. In the meantime, recently, more and more people who are unrelated to the agriculture sector have started private wineries in Japan. This has prompted us to undertake this study to empirically reveal the mechanism by which abandoned and non-cultivated farmland is revived, preserved, and passed on by private wineries newly established by people who do not belong to the Japanese agricultural sector. To clarify the mechanism, we focused on a winemaking area in Toumi region in Shinshu Ueda area, which is famous for a growing number of new private wineries and is now called the wine valley of Chikuma River.

Keywords: wine-tourism, agricultural resources, innovation, custom crush, Japan

1. Introduction

Farmland abandonment has become widespread in Japan, adversely affecting agriculture, which has mainly relied on land for produce. It is critical for Japanese agriculture to strike a balance between sustainability of agricultural resources for the next generation and farmers' health while respecting farmers' will, which is the foundation of this country that has adopted liberalism and democracy, as well as of its industry. Whereas this balance has been seriously disturbed these days, starting a wine business has become a major trend in Japan. Wine entrepreneurs have had various careers, such as doctor, etc., and more and more people have shown interest in this field. Most of them plan to make their own wine by cultivating their own wine grapes for fermentation.

In this report, we present evidence from a case study of the processes involved in farmland revival, conservation, and inheritance by entrepreneurs who cultivate wine grapes and ferment wine by themselves.

2. Methodology

We focused on the Shinshu-Ueda Tomi region as the study area. This region is

*Corresponding author. E-mail: oda@kais.kyoto-u.ac.jp

called Chikuma River Wine Valley and one of the most popular starting points for entrepreneurs. We analyzed this region from five aspects by using hearing and survey to reveal the relationships among them for contribution to the process. The five aspects are as follows. The first aspect is the background of the entrepreneur. The second aspect is the historical background of this region and the incidental events that have contributed to the current situation of this region. The third aspect is the support system for entrepreneurs conducted by the local government, the agricultural cooperative, etc. The fourth aspect is a case study of a pioneer winery in this region. The fifth and final aspect is the support extended by other institutions involved in wine business to entrepreneurs.

3. Process revealed through case study

The Shinshu-Ueda Tomi region was chosen for a major winery project that planned to cultivate grapes on a 30-ha scale. The present condition of this region was determined by three key persons who had gathered for this project. The first one was Tomio Tamamura, who worked at the research institution of this winery as director and is a famous writer. The second person was Shogo Asai, one of the leading winemakers in Japan who worked at Mercian. After retirement, Asai was invited by Tamamura to work as an advisor at the research institution. The third member was Cho Konishi, who worked at the research institution after graduating from the Division of Agriculture, Kyoto University. Unfortunately, this project ended abruptly after three years of all-out effort. The reason was that the 30 ha of farmland to be purchased for growing wine grapes was owned by more than 100 farmers and negotiations failed because the farmers had various expectations. Furthermore, because profits diminished, the company gave up its wine business. Tamamura started to grow wine grapes in this region after recovering from a serious illness and organized Villa D'est Winery, the flagship winery in this region. Konishi gave his utmost effort to help organize this winery. Subsequently, other pioneer wineries were established in this region while Tamamura wrote a book about the history and development of his winery for popularization and wisdom. With these events, this region started to attract people who are looking into entering the wine business. These pioneer wineries contributed to the development of this region in many ways, such as by supporting newcomers, and these contributions consequently led to the transformation of the 30 ha of farmland into wine grape fields in Tomi region, which was farmland that the wine company tried to buy in the past for growing wine grapes. This transformation happened because they were able to negotiate with more than 100 farmers using knowledge gained from the last failed negotiation.

Figure 1- The Vinyard in Shinshu-Ueda Tomi region

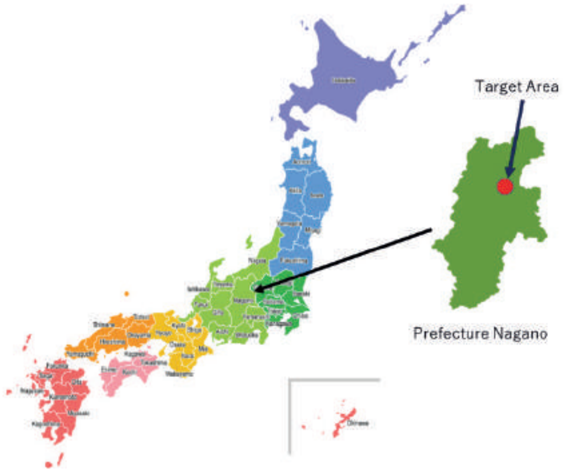


Figure 2 – The map of case study area (in Japan)

Figure 3 – the Training Center to New comers

Newcomers who had dreams of entering the wine business and coming to this region found this region on the internet and chose to stay after comparing with other pioneer regions of wine business. Tamamura's book about his winery had played a significant role in motivating newcomers to enter the wine business in this region.

Meanwhile, the Shinshu-Ueda farm, which was funded by JA Shinshu-Ueda, also supported newcomers in the following ways. First, it interviewed newcomers, signed them up for work at its institution as trainees, and gave them instructions on agricultural skills. Second, when the newcomers became independent farmers, they were allowed to arrange and revive farmland for cultivating wine grapes by themselves, and the grapevines were prepared by the institution. In an instruction program, not only wine grape cultivation skills but also vegetable and other fruit tree cultivation skills were taught, so that they could earn money from growing vegetables when grapevines had not yet reached maturity for producing grapes.

Tomi City also had its own support system for newcomers. It leased homes to newcomers, introduced highly skilled farmers who could teach them farming skills, arranged farmland and homes for them when they became independent, guided them even after they had become independent, and helped them find farmland suitable for grape cultivation. It even supported newcomers who could not work at the institution managed by Shinshu-Ueda farm. Tomi City had played a major role in supporting newcomers in this region. Newcomers who did not have their own fermentation facilities commissioned "Arcanvigne" or other pioneer wineries to ferment their wine grapes. However, instead of completely letting the commissioned winery make their wines, they learned fermentation skills from the commissioned winery while renting and using the winery's de-infraction crusher, juice extraction machine, and fermentation tank.

Because of these efforts, more and more newcomers who were attracted to the wine business gathered in this region. Table 1 shows the survey results of trainees and farmer graduates of the institution managed by Shinshu-Ueda farm. At first, the reason why they came to this region for winemaking was that they assumed that the wine business has a big frontier and is promising. All of them had strong feelings for wines and most of them believed that wines would sell well if you made them. As the cultivation scale was mostly 3 ha, their farmland was larger than the average of approximately 1 ha in

Japan. Finally, most of them dreamt of having their own wine facilities someday.

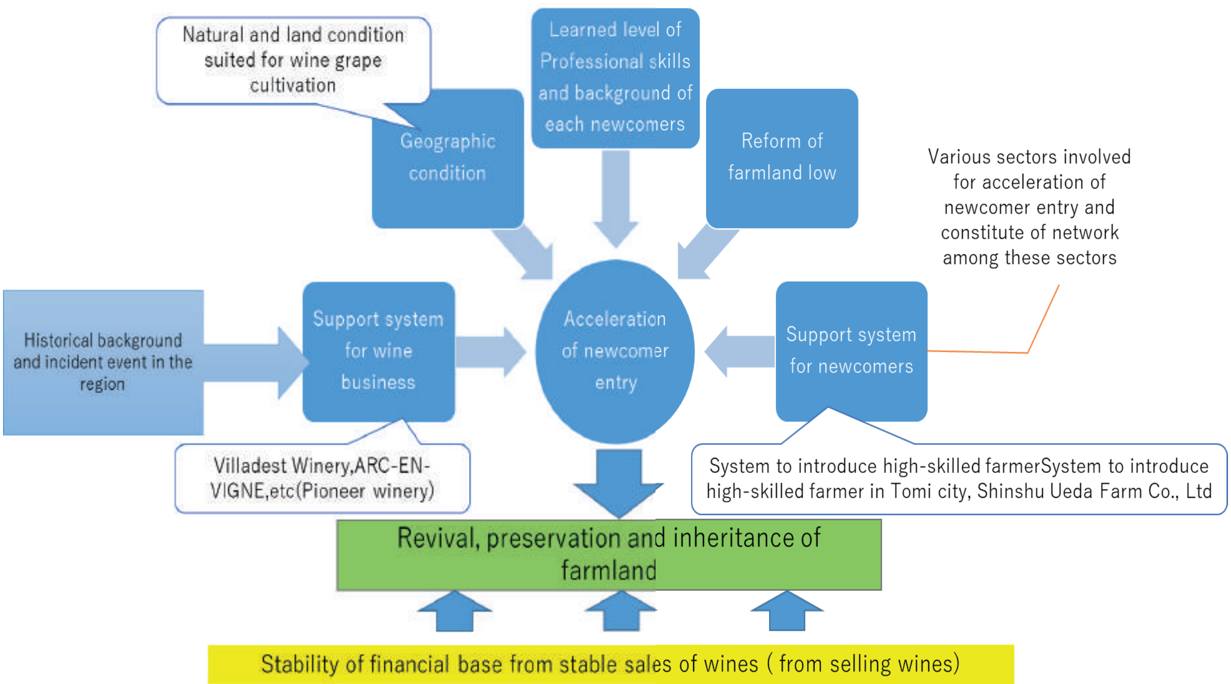
Table 1 – The Overvies of the Wine Business

Current Situation	Training	Viticulture Started	Wine Making Started	□
	30%	40%	30%	
Age	30s	40s	50s	60s
	30%	10%	50%	10%
Vineyard Scale	□1ha	1□3ha	3□5ha	5ha□
	10%	40%	40%	10%
Sales Amount	No Sales	□500,000□1,000,000	□1,000,000 □3,000,000	□3,000,000□5,000,000
	50%	10%	30%	10%
About Winery	Already Owned	Owned in the future	Hope for Jointed	No Need

Table 2 – The Reasons to Make Wine and Grow Grapes

	Exactly	Think so	Don't know	Don't think so	Not at all
1. I like wine	80%	20%	0%	0%	0%
2. Able to sell a lot	60%	20%	10%	10%	0%
3.Popular area in grapes.	30%	40%	0%	20%	10%
4.Recommendation by the winery	0%	10%	30%	10%	50%

Figure 4 – The Revival Mechanism of Agricultural Resource(eps. Farmland)



4. Academic implications

Through the case study, we found that the newcomers had different backgrounds and entered the wine business through various ways. For instance, one entered the institution managed by Shinshu-Ueda farm and become a trainee first and another one worked at a pioneer winery as an intern or as a worker and then depended on the winery's help to start a wine business after learning the adequate skills for running the business by oneself. These newcomers were willing to leas in and revival abandoned farmland that was under unfavorable conditions to obtain their own land for growing grapevines, except the trainee at the institution of Shinshu-Ueda farm, whose farmland was arranged by the institution. To reveal the farmland revival process, we show a revival mechanism in Figure 2. Farmland revival in this region took place basically because of historical and incidental events. In addition, five factors had contributed to the farmland revival process. First, the natural conditions of this region were suitable for winemaking and wine grape cultivation. Second, the newcomers had various backgrounds as well as winemaking and grape cultivation skills. Third, the requirements of the farmland leasing system were eased for wine districts. Fourth, the local government and the agricultural cooperative created a support system for newcomers. Finally, pioneer wineries supported newcomers by accepting them as interns or trainees and consigning winemaking. These support systems had enticed newcomers to this region for wine business and their settlement and winery had resulted in farmland revival. The issue of farmland abandonment in Japan is difficult to resolve because farmland is distributed around a region in small sizes, and an adequate number of small-farm farmers are needed. Therefore, attracting a sufficient number of small-farm farmers who have unique characteristics and skills and distributing them around a region are vital. The success of pioneer wineries in this region is due to not only their effort but also the unique attraction wine has as an agricultural product.

Furthermore, to preserve and inherit revived farmland, the wines had to attract consumers and be valued by them so that farmers could earn sufficient basic income from wine sales. In this regard, it was vital to have other business projects while attempting to sell wines at high prices. On the other hand, as most of the newcomers have just started their wine business, we need to look forward to their future management. In the case of pioneer wineries, using the characteristics of this region, which enable fruit tree cultivation, they made cider and sparkling wine using local apples and Kyoho grapes bought at low price from farmers around the region, because these products did not satisfy the requirements for sales in markets. They were able to gain financial stability by deriving income from these side businesses.

5. Conclusion

In the target region that started with pioneer wineries, several wineries have been established and various kinds of wines have been made by them. By using the region as a model case for winemaking districts in Japan, revealing the development process gave us not only important insight into how the region that is suitable for wine grape cultivation can develop but also critical ideas to solve the problem of how to revive, preserve, and inherit agricultural resources such as farmland for passing on to the next generation, by cultivating other agricultural products.

We discuss three remaining problems faced by small wineries in Japan and give a rough sketch of solutions for them. First, it is difficult for small winery owners to learn professional skills for wine grape cultivation and winemaking techniques systematically in Japan. Therefore, most of them rely on internship or training in pioneer wineries or institutions to learn those skills. In addition, the skills they learn mostly come from watching and imitation. On the other hand, famous winemaking regions around the world have colleges or professional schools for winemaking and those educational institutions have special classes or divisions for wine grape cultivation and winemaking. As students in those classes or divisions learn professional knowledge and skills systematically, wineries in those regions hire them for maintaining and improving the quality of their wines. Second, the cost of wine grape cultivation in Japan is higher than that in other winemaking countries because the unfavorable weather conditions in Japan, including high temperature and humidity, heavy rainfall throughout the year, and high nighttime temperature, hinder the cultivation of high-quality wine grapes. To overcome these problems, wineries need to exert a lot of effort by introducing canopy cultivation and deep furrow cultivation. Therefore, the price of wine made by small wineries is higher than that of imported wine, leading to loss of competitive power. Indeed, small wineries face the challenge of competing with low-priced imported wines. Third, because newcomers with various backgrounds enter the wine business and attempt to make their own brand by self-reliance, it is difficult for them to cooperate with other wineries.

Considering these facts, winery owners should understand that the fundamentals of winemaking are the science and they should learn and apply new knowledge every day. However, because winery owners have limited access to new professional knowledge, we present three solutions to this problem. First, local colleges as well as government and private institutions should offer systematic support to small wineries so that they can learn professional skills, by introducing new professional knowledge and implementation methods to them. Moreover, it is desirable that winery owners attend international wine conferences, seminars, and extension lectures. Second, setting up a new marketing system that would enable local residents and supporters to support a specific winery is needed. Specifically, it is vital to establish a marketing system where a small winery is able to sell 10,000 bottles of wine at 3,000 yen per bottle. Third, winery owners throughout Japan should cooperate with each other and establish a cooperative institution so that they can compete in one area but cooperate in other areas, thereby gaining maximum benefit from cooperation. For instance, they should, together with others, invest resources in order to send a representative to international wine conferences, seminars, and extension lectures, and have the representative share new professional knowledge.

Although we have placed high expectations on the development of small wineries in Japan, we are anxious about how it would turn out as well. We hope research on the development of small wineries and continuous outreach will offer clues to resolve the issues surrounding farmland revival, preservation, and inheritance.

10th International Symposium

Challenges in viticulture and oenology:
Wine Appellations, Authenticity and Innovation.

ŒNOVITI INTERNATIONAL network

Session III. Authenticity

Strontium isotopic signatures for authenticity and wine geographical assessment

Sofia Catarino *

Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

Center of Physics and Engineering of Advanced Materials (CeFEMA), Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal

Abstract: The assessment of wine authenticity is of utmost importance in the current context of a growing market globalization. In the last years several studies were developed on the application of 87Sr/86Sr isotopic ratio for the evaluation of wine geographical origin, involving wine producing regions worldwide, evidencing its reliability as a provenance marker. Aspects such as 87Sr/86Sr relation with the vineyard substratum, analytical methodologies, and effect of technological processes have been addressed. Data has been obtained for different wine regions/PDO. Nevertheless, some important issues remain, such as the interpretation of the data from the soil (it is crucial to know the soil geochemistry), and the need for better understanding of the impacts of anthropogenic factors. Precise and accurate 87Sr/86Sr data is required for origin discrimination and analytical methods used should be officially recognized or validated in order to support comparison. Sr isotopic data can be used to build an authentic wine reference database (e.g. official or wine organization, PDO consortium), or to integrate a global database (e.g. EU wine databank). 87Sr/86Sr data combined with other discriminating parameters, namely elemental composition, can provide increasingly robust results for the identification of wine provenance. This work reviews the main aspects of the topic and includes recent research results obtained by the author's team.

Keywords: Wine, authenticity, geographic origin, 87Sr/86Sr isotopic ratio.

1. Introduction

Wine traceability and the assessment of its authenticity are of utmost importance in the current context of a growing market globalization (OIV, 2007). To boost wine quality, promote good practices and minimise fraud, wine authenticity is commonly addressed by a regulatory "wine of origin" system in many countries. In European Union (EU), traceability systems are applied to promote and protect certain denominations, such as Protected Geographical Indication (PGI) and Protected Designation of Origin (PDO). These designations of origin are awarded to high quality wines, strictly linked to their geographic provenance and specific viticulture and enological practices.

The main issues related to wine authenticity concern adulterations (e.g. sugaring, and watering, in EU), geographic origin, grape varieties, and vintage year. The high value of some specific PDO wines make economically interesting the use of not certified grapes

*Corresponding author. E-mail: sofiacatarino@isa.ulisboa.pt

and wines to mislead consumers, producing wines under counterfeit mentions. This type of fraudulent actions is not rare, being known several cases of relabelling of imported wines under a prestigious PDO. Moreover, fraudulent practices are not only on prestige wines but also on medium quality wines.
The place of origin of food products is regarded as value-added information and as a guarantee of quality and authenticity. For wine in particular, geographical origin has a direct effect on its quality and commercial value, being one of the most studied products in terms of food authentication. In fact, information of wine's origin on the labels provides dignity to the wine in the eyes of consumers.

The development of analytical methodologies which can positively identify the geographical origin of a given product is one of the most challenging issues for scientific community. Stable isotope analyses ($^{2}H/^{1}H$, also referred as D/H, $^{13}C/^{12}C$ and $^{18}O/^{16}O$) recognized by the International Organisation of Vine and Wine (OIV) for detecting adulterations, inclosed in the european Wine DataBank (since 1990, updated every year), are limited in terms of interpreting the data and relating them to the wines provenance. Regional differences in sensory characteristics of wines have commonly been attributed to, among other factors (e.g. grape variety, technological processes, and vintage), local variations in soil composition.

Soil-related fingerprints justify special attention, given that there is a relationship between the chemical composition of wine and the composition of the provenance soil. In fact, the most explored fingerprinting techniques combine chemical analysis, namely element and isotope ratio analysis, and multivariate statistical analysis of the chemical data to classify wines according to the geographical origin. However the successful application of the techniques based on multi-element composition of a wine strongly depends on the selection of suitable elements that would reflect the relationship with soil geochemistry and therefore have discriminating potential (Catarino *et al*., 2011; Catarino *et al*., 2018), requiring deep knowledge of wine chemistry and technology. The data on mineral elements in wine as a probe for origin determination has to be carefully interpreted since there are many environmental, agricultural and oenological factors that can easily mask vital elemental information (Catarino *et al*., 2008a,b).

2. The isotopic ratio $^{87}Sr/^{86}Sr$

The strontium isotopic ratio $^{87}Sr/^{86}Sr$ is a well-established tool for dating and tracing the origin of rocks and minerals (Faure, 1986), with special interest for wine traceability. Strontium occurs in nature as four isotopes. Isotopes ^{84}Sr, ^{86}Sr and ^{88}Sr, non-radiogenic, occur in constant relative proportions, whereas ^{87}Sr, radiogenic, gradually increases in minerals due to the radioactive β-decay of the ^{87}Rb isotope (half-life: 48.8×10^{9} years). The $^{87}Sr/^{86}Sr$ isotope abundance variations given by IUPAC fall in a range of 82.29–82.75 % for ^{88}Sr, 6.94–7.14 % for ^{87}Sr, 9.75–9.99 % for ^{86}Sr and 0.55–0.58 % for ^{84}Sr, respectively (Berglund and Wieser, 2011). Observed range in the nature for ^{87}Sr is 0.0694–0.0714 mole fractions while ^{86}Sr is 0.0975–0.0999 which gives the ratio range from 0.695 to 0.732.

The strontium isotopic ratio $^{87}Sr/^{86}Sr$, depending on the initial $^{87}Sr/^{86}Sr$, initial Rb/Sr ratio, and age of the rock (time), gives particular data in different geological regions. Differences in the relative abundance of ^{87}Sr vary with geological age, and consequently with geographic locations, providing a fingerprint for different rock types (Capo *et al*., 1998). Horn *et al*. (1993) stated that soils of respective vineyard regions have different $^{87}Sr/^{86}Sr$ ratios.

Weathering of the underlying rock and/or sediments is a significant source of strontium for the soil. Pre-Cambrian granitic bedrock and alluvial sands derived from felsic rocks show high $^{87}Sr/^{86}Sr$ (0.710-0.716) reflecting the age of the continental crust and high Rb/Sr from which these materials originated. Limestones have intermediated $^{87}Sr/^{86}Sr$ ratio values (0.706-0.709) and young oceanic basalts and their sediments show the lowest values (0.702-0.705) (Faure, 1986; Capo *et al.,* 1998).

As a provenance tracer, a crucial feature of Sr is that this element is assimilated by the vine plant roots in the same isotopic proportions in which they occur, under available forms (labile Sr); biological processes involved in vine metabolism do not significantly fractionate Sr isotopes (Horn *et al*., 1993; Capo *et al*., 1998; Song *et al*., 2015). According to the literature, vine plants reflect the environment of growth: bedrock, soil and soil water (Horn *et al*., 1993; Capo *et al*., 1998). Nevertheless, studies on the hypothetical influence of rootstock and grapevine variety are limited.

3. Sr isotopic ratio analysis

The radioactive decay of ^{87}Rb induces extremely low differences in Sr isotopic composition. For samples with very close $^{87}Sr/^{86}Sr$ values, the possibility of discriminating often lies on the fourth or fifth decimal places of the isotopic ratio. Thus, precision of the measured $^{87}Sr/^{86}Sr$ values represents a quality parameter of utmost importance to ascertain the goodness of the experimental results (Sighinolfi *et al*., 2018). Thermal ionization mass spectrometry (TIMS), the reference technique in the field of isotopic analysis, and more recently multicollection inductively coupled plasma mass spectrometry (MC-ICP-MS) are capable to provide precise (0.002 %, RSD) and accurate isotopic measurements (Barbaste *et al*., 2002; Rosner, 2010; Durante *et al*., 2015). Several studies have been published with these techniques for assessing $^{87}Sr/^{86}Sr$ ratio in soils and wines (Barbaste *et al*., 2002; Marchionni *et al*., 2013; Durante *et al*., 2015; Petrini *et al*., 2015; Epova *et al*., 2019).

The lower precision of quadrupole ICP-MS (Q-ICP-MS), typically below 0.1 % (RSD), can be a limiting factor, especially in studies involving samples with very close $^{87}Sr/^{86}Sr$ values. On the other hand, Q-ICP-MS is robust and less time consuming, and its precision allowed distinguishing the Sr isotopic composition of wines in several studies (Vanhaecke *et al.,* 1999; Almeida and Vasconcelos, 2001; Vorster *et al*., 2010; Martins *et al*., 2014; Catarino *et al*., 2019). Due to the isobaric overlap of ^{87}Sr and ^{87}Rb, an effective Rb/Sr separation is a pre-requisite for the accurate determination of Sr isotope ratios by Q-ICP-MS.

A sample preparation procedure for $^{87}Sr/^{86}Sr$ determination by Q-ICP-MS was optimized by the author's team (Martins *et al*., 2014), comprising three main analytical steps in sequence: 1) sample digestion by high pressure microwave digestion (HPMW), 2) chromatographic separation of ^{87}Sr and ^{87}Rb, and 3) determination of Sr and Rb content by Q-ICP-MS. For elimination of organic substances in order to prevent any interference during chromatographic separation, soil and wine samples were digested by HPMW. Separation of Sr was performed by using Dowex 50W-X8/400 (Sigma-Aldrich) mesh resin and EDTA as eluent. Separation consists of four phases which are resin activation/pre-treatment; resin conditioning; sample preparation/dilution and elution. Sr and Rb total content and $^{87}Sr/^{86}Sr$ isotopic ratio were measured by a Q-ICP-MS equipment.

Determination of Sr and Rb total contents in Sr-containing fractions previously to the isotopic measurement is important in order to keep Rb concentration less than 1% of the Sr content in Sr-fraction. The SRM 987 ($SrCO_3$) from National Institute of Standards and Technology (NIST) was used as an isotopic reference material for correction of mass bias phenomenon.

4. Application of $^{87}Sr/^{86}Sr$ isotopic ratio for wine geographical assessment

Literature on the progress made since the first application of $^{87}Sr/^{86}Sr$ isotopic ratio for wine traceability purposes in the 1990's is available, with a significant increase number of studies developed in the last decade, and involving wine producing regions worldwide (Horn *et al*., 1997; Almeida and Vasconcelos, 2001; Barbaste *et al*., 2002; Almeida and Vasconcelos, 2003; Vorster *et al.*, 2010; Di Paola-Naranjo *et al*., 2011; Durante *et al*., 2013; Marchionni *et al.*, 2013; Martins *et al*., 2014; Mercurio *et al*., 2014; Durante *et al*., 2015; Marchionni *et al*., 2016; Vinciguerra *et al*., 2016; Durante *et al*., 2016; Geanã *et al*., 2017; Durante *et al*., 2018; Epova *et al*., 2019; Catarino *et al*., 2019).

Pioneer studies by Horn *et al.* (1997) demonstrated that the $^{87}Sr/^{86}Sr$ values of several wines were within the respective ranges for rocks and soils. A few years later Almeida *et al.* (2003) investigated the potentialities of both multi-element composition and Sr isotopic ratio as tracers, by studying the influence of the provenance soil. Wine and soil samples from Douro wine region (Portugal) were analysed by Q-ICP-MS. This combined strategy showed to be suitable to act as fingerprint of the origin of a wine. The $^{87}Sr/^{86}Sr$ isotope ratios in soils and wines of four South African wine-producing regions were determined by Q-ICP-MS (Vorster *et al.*, 2010). Only one producing region could be distinguished from the rest.

In the last years a series of significant studies were developed on Italian wines and PDO, namely Lambrusco, evidencing the matching of $^{87}Sr/^{86}Sr$ ratios in wine and those from substratum of vineyards (Durante *et al.*, 2013; Marchionni *et al.*, 2013; Mercurio *et al.*, 2014; Petrini *et al.*, 2015; Durante *et al.*, 2018). Based on a representative number of authentic samples and Sr isotopic measurements acquired with high precision and accuracy, additional information of interest was obtained: the bio-available (labile) fraction of soil is the one of interest for Sr isotopic analysis (Durante *et al.*, 2013); vine-branches $^{87}Sr/^{86}Sr$ values show traceability power, overcoming the problems associated with soil sampling as well as those related to the evaluation of the element bioavailable fraction (Durante *et al*., 2016); $^{87}Sr/^{86}Sr$ isotopic ratio seems not to be affected by vintage year (Marchionni *et al*., 2013). Data on $^{87}Sr/^{86}Sr$ ratios of soils, vine branches and wines sampled in Modena, were used to build maps to objectively support the Lambrusco PDO wines (Durante *et al*., 2018).

Two studies from Vinciguerra *et al*. (2015) and Geanã *et al*. (2017) announced very promising results by attributing a strong relationship between $^{87}Sr/^{86}Sr$ on grape and wine and soils from different vineyards in Quebec and in Romania, respectively. Geanã *et al*. (2017) classified with a 100% success, the geographical origin of twenty one red wines with denomination PDO or PGI, by combining the Sr isotopic ratio together with Ca/Rb value and other elements concentration (Ga, Sr, Al).
One study in Australia also justifies the robustness of the strontium isotopic ratio method to differentiate between Australian and non-Australian wines (Wilkes *et al*., 2016). Nevertheless, overlap of the strontium isotopic ratio from different countries was observed, suggesting that the

use of only one fingerprinting method may not be sufficient.

Recently, a study concerned the Sr isotopic and elemental compositions of a large selection of genuine Bordeaux wines from four regional wineries (Epova *et al.*, 2019). Results demonstrated a moderate variability of $^{87}Sr/^{86}Sr$ ratio and Sr concentrations with a strong possibility to assigned Sr specifications for individual winemaking estates. Furthermore, the $^{87}Sr/^{86}Sr$ ratio found to be relatively stable in in wine from different vintages.

Within a research program on strategies for wine fingerprinting carried out by the author's team, the transference of $^{87}Sr/^{86}Sr$ signature through soil-wine system was examined (Martins *et al.*, 2014). The $^{87}Sr/^{86}Sr$ ratios of soils from four vineyards located in three Portuguese PDO (Dão, Óbidos and Palmela), established on distinct soil types, were determined, by Q-ICP-MS. Significant differences were found between soils of different PDO. As expected, the soil developed on granites, showed a higher $^{87}Sr/^{86}Sr$ ratio than the other soils, which were developed on sedimentary formations. The results show clearly that $^{87}Sr/^{86}Sr$ may represent a suitable fingerprint for these Portuguese PDO (Martins *et al.*, 2014).

More recently, the variation of $^{87}Sr/^{86}Sr$ in wines from Douro Valley, taking into account the effects of vineyard location and grape variety, was examined (Catarino *et al.*, 2019). A total of twenty-two varietal wines, from relevant white and red grapevine varieties for the Portuguese Douro region, and respective soils from six vineyards were analysed. The range of $^{87}Sr/^{86}Sr$ observed in soils and wines was of 0.708-0.725 and 0.711-0.717. No significant difference was observed between vineyards soils and wines (Catarino *et al.*, 2019). This study represents a development background for building an authentic wine reference database (e.g. official or wine organisation, PDO consortium) to evaluate the provenance of

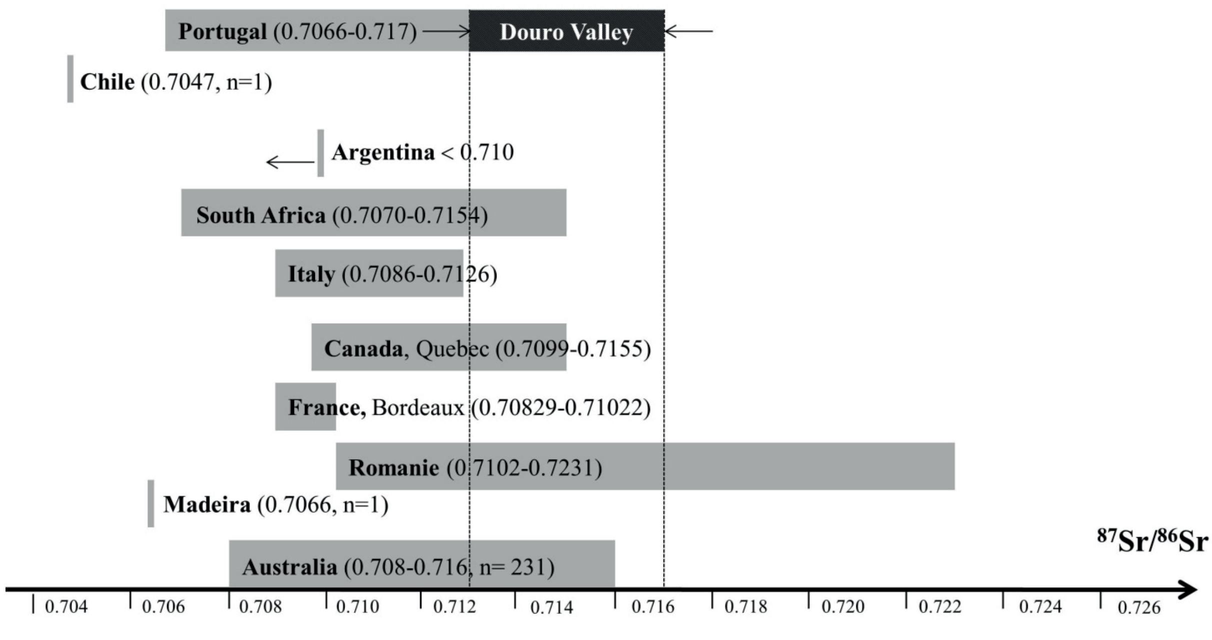


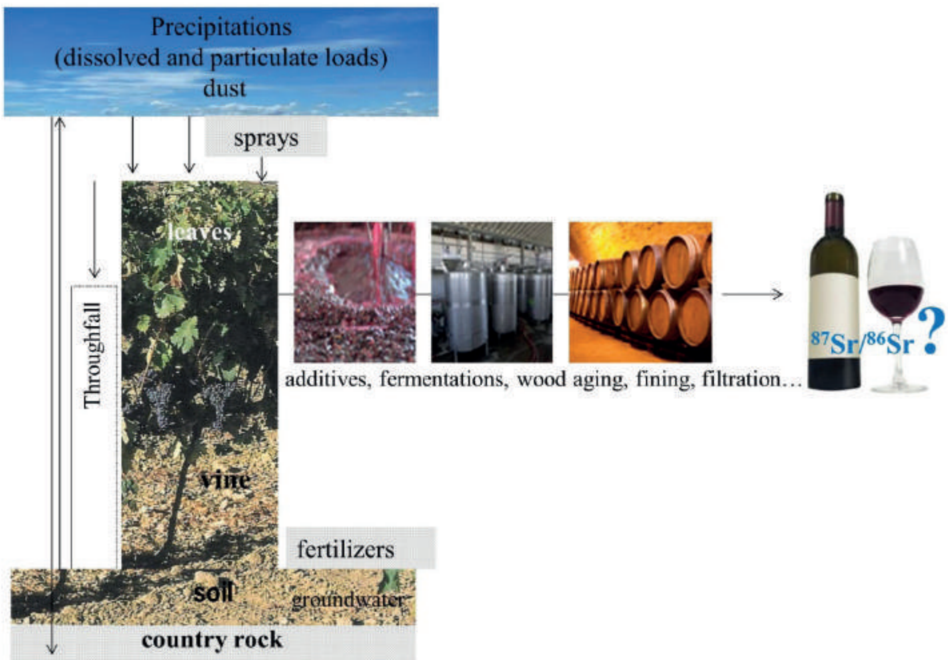
Figure 1. $^{87}Sr/^{86}Sr$ in wines on a global scale.

Data from Almeida and Vasconcelos, 2001; Barbaste *et al*., 2001; Vorster *et al*., 2010; Di Paola-Naranjo *et al*., 2011; Marchionni *et al*., 2013; Mercurio *et al*., 2014; Petrini *et al*., 2015; Durante *et al*., 2015; Durante *et al*., 2016; Marchionni *et al*., 2016; Vinciguerra *et al*., 2016; Wilkes *et al*., 2016; Geană *et al*., 2017; Moreira *et al*., 2017; Kaya *et al*., 2017; Catarino *et al*., 2019; Epova *et al*., 2019.

wine labelled as Douro, or to be integrated in a global wine database (e.g. EU wine databank) of great usefulness for industry. Nevertheless, despite the potential of $^{87}Sr/^{86}Sr$ for determining the provenance of wines, it seems it can be difficult to differentiate them both at the country and regional level only through $^{87}Sr/^{86}Sr$, as shown in Figure 1, suggesting that it should be used together with other discriminating parameters, e.g. elemental composition.

5. Influence of technological processes

The use of $^{87}Sr/^{86}Sr$ as a geographic origin marker is based on the assumption that there is a relationship between soil, plants and wine. Therefore, the $^{87}Sr/^{86}Sr$ ratio should not be modified significantly by agricultural and winemaking practices through the chain from vineyard to bottle. It is also important to take into consideration anthropogenic factors such as irrigation water, pollution and fertilizers that can contribute as mineral sources to the vine plants (Figure 2).
According to Horn *et al*. (1997), fining with bentonites, deacidification with carbonates and storage in glass showed little effects on wine $^{87}Sr/^{86}Sr$ ratio. Nevertheless, it is well known that the use of some technological aids, namely bentonite, can result in significantly concentrations of Sr (Catarino *et al*., 2008b). Almeida and Vasconcelos (2004) stated that although winemaking processes, and chemical applications in the vineyard change the element composition of must and wine, a strong correlation in terms of Sr isotopic ratio between wine and grape juice is still found. No influence of the production year or the winemaking process was observed in the $^{87}Sr/^{86}Sr$ ratio (Marchionni *et al*., 2013). Sr isotopic ratios of red and white wines were not affected by addition of fining agents during winemaking proving the close relation with the vineyard (Tescione *et al*., 2015). Durante *et al*. (2016) investigated the cellar practices that used different additives, such as clarification and deacidification agents as well concentrated musts. The hypothesis was that Sr concentration could be modified; hence the $^{87}Sr/^{86}Sr$ ratio of wine may be affected by these practices. Once again, the $^{87}Sr/^{86}Sr$ ratio was found to be a powerful



re 2. Reservoir scheme for Sr in wines, with possible transfer routes (adapted from Horn *et al.*, 1997).

tool discriminate the wines based on their region.
More recently the effect of two enological practices, namely nanofiltration for dehalcoholisation and wood aging, on the Sr isotopic ratio were investigated by the author's team. No significant differences in the $^{87}Sr/^{86}Sr$ of wines and corresponding permeate fractions were observed, suggesting that nanofiltration does not preclude the use of this isotopic ratio for wine traceability purposes (Moreira *et al.,* 2017). After three months of aging with oak staves in stainless steel vats, it was found that wood aging did not alter $^{87}Sr/^{86}Sr$ ratio of wine (Kaya *et al.*, 2017).

6. Principal current issues and future perspectives

The reliability of $^{87}Sr/^{86}Sr$ for wine fingerprinting is evidenced by the studies. Nevertheless, applied solely, this analytical approach shows some limitations to definitely identify the geographical origin. Soils from different wine regions and countries have been originated from similar geological formations, which can be a constraint in terms of interpreting the data and relating them to the wines provenance. Also, the heterogeneity of some wine regions and PDO in terms of soils and geological materials is well known, making it difficult to match wines with their substrata data. Further studies should be developed, on diverse lithological situations and other world regions, to confirm the feasibility of $^{87}Sr/^{86}Sr$ fingerprinting and enlarge data. A correct and representative soil sampling procedure is crucial in order to develop robust models.
Another issue concerns the interpretation of the data from the soil substratum. It is crucial to know the soil geochemistry background so a reliable wine origin relationship can be established. In particular, the discrepancy between the bioavailable fraction and the total amount of strontium in a soil may represent a limiting factor for the building of a reliable geographical traceability system.
The influence of the vintage year, in direct relationship especially with climate changes, requires further research. Also, a better understanding of the impacts of anthropogenic factors and technological processes on this marker is essential.
Analytical precision is a pivotal requirement for discriminating between samples with very close $^{87}Sr/^{86}Sr$ values. Furthermore, analytical methods should be officially recognized or validated (through interlaboratorial trials, proficiency tests) in order to support comparison.
For a statistical approach to the geographic origin of wine, a large database of precise and accurate values is needed in order to evaluate the indicator variability range of both the wine and the soils and to build robust classification models. Sr isotopic data can be used to build an authentic wine reference database (e.g. official or wine organization, PDO consortium), or to integrate a global database (e.g. EU wine databank).
At last, increasingly robust results for the identification of geographical origin can be achieved by combining Sr isotopic ratio and elemental signatures of wines. This approach supplies the distinguishing terroir-inherent and winemaking-related tracer for authenticity and provenance assignment.

Funding and acknowledgments

This work was funded through European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 872394, and by FCT - Foundation for Science and Technology under the project UID/AGR/04129/2020 (LEAF) and thourgh DL 57/2016/CP1382/CT0025. We gratefully acknowledge INIAV – Dois Portos for the laboratorial facilities.

References

Almeida C.M., Vasconcelos M.T.S.D., 2001. ICP-MS determination of strontium isotope ratio in wine in order to be used as fingerprint of its regional origin. J. Anal. At. Spectrom., 16, 607-611.

Almeida C.M.R., Vasconcelos M.T.S.D., 2003. Multi-element composition and $^{87}Sr/^{86}Sr$ of wines and their potentialities as fingerprints of wine provenance. Ciência Téc. Vitiv., 18 (1), 15-27.

Almeida C.M.R., Vasconcelos M.T.S.D., 2004. Does the winemaking process influence the wine $^{87}Sr/^{86}Sr$? A case study. Food Chem., 85, 7–12.

Barbaste M., Robinson K., Guilfoyle S., Medina B., Lobinski R., 2002. Precise determination of the strontium isotope ratios in wine by inductively coupled plasma sector field multicollector mass spectrometry (ICP-SF-MC-MC). J. Anal. Atom. Spectrom., 17, 135–137.

Berglund M., Wieser M., 2011. Isotopic compositions of the elements 2009 (IUPAC Technical Report). Pure Appl. Chem., 83 (2), 397-410.

Camin F., Boner M., Bontempo L., Fauhl-Hassek C., Kelly S.D., Riedl J., Rossmann A., 2017. Stable isotope techniques for verifying the declared geographical origin of food in legal cases. Trends Food Sci. Tech., 61, 176-187.

Capo R.C., Stewart B.W., Cadwick O.A., 1998. Strontium isotopes tracers of ecosystems processes: theory and methods. Geoderma, 82,197-225.

Catarino S., Curvelo-Garcia A.S., Bruno de Sousa R., 2008a. Revisão: Elementos contaminantes nos vinhos. Ciência Téc. Vitiv., 23 (1), 3-19.

Catarino S., Madeira M., Monteiro F., Rocha F., Curvelo-Garcia A.S., Bruno de Sousa R., 2008b. Effect of bentonite characteristics on the elemental composition of wine. J. Agric. Food Chem., 56, 158-165. http://dx.doi.org/10.1021/jf0720180.

Catarino S., Trancoso I.M, Madeira M., Monteiro F., Bruno de Sousa R., Curvelo-Garcia A.S., 2011. Rare earths data for geographical origin assignment of wine: a Portuguese case study. Bulletin de l'OIV, 84 (965-967), 223-246.

Catarino S., Madeira M., Monteiro F., Caldeira I., Bruno de Sousa R., Curvelo-Garcia A.S., 2018. Mineral composition through soil-wine system of Portuguese vineyards and its potential for wine traceability. Beverages, 4, 85. https://doi.org/10.3390/beverages4040085

Catarino S., Castro F.P., Brazão J., Moreira L., Pereira L., Fernandes J.R., Eiras-Dias J.E., Graça A., Martins-Lopes P., 2019. $^{87}Sr/^{86}Sr$ isotopic ratios in vineyard soils and varietal wines from Douro Valley. Bio Web of Conferences, 12, 02031. https://doi.org/10.1051/bioconf/20191202031

Coelho I., Castanheira I., Bordado J.M., Donard O., Silva J.A.L., 2017. Recent developments and trends in the application of strontium and its isotopes in biological related fields. Trend Anal. Chem., 90, 45-61.

Di Paola-Naranjo R.D., Baroni M.V., Podio N.S., Rubinstein H.R., Fabani M.P., Badini R.G., Inga M., Ostera H.A., Cagnoni M., Gallegos E., Gautier E., Peral-García P., Hoogewerff J., Wunderlin D.A., 2011. Fingerprints of main varieties of Argentinean wines: Terroir differentiation by inorganic, organic, and stable isotopic analyses coupled to chemometrics. J. Agric. Food Chem., 59, 7854-7865.

Durante C., Baschieri C., Bertacchini L., Cocchi M., Sighinolfi S., Silvestri M., Marchetti A., 2013. Geographical traceability based on $^{87}Sr/^{86}Sr$ indicator. A first approach for PDO Lambrusco wines from Modena. Food Chem., 141, 2279-2787.

Durante C., Baschieri C., Bertacchini L., Bertelli D., Cocchi M., Marchetti A., Manzini D., Papotti G., Sighinolfi S., 2015. An analytical approach to Sr isotope ratio determination in Lambrusco wines for geographical traceability purposes. Food Chem., 173, 557–563.

Durante C., Bertachinni L., Bontempo L., Camin F., Manzini D., Lambertini P., Marchetti A., Paolini M., 2016. From soil to grape and wine: Variation of light and heavy elements isotope ratios. Food Chem., 210, 648-659.

Durante C., Bertachinni L., Cocchi M., Manzini D., Marchetti A., Rossi M.C., Sighinolfi S., Tassi L., 2018. Development of $^{87}Sr/^{86}Sr$ maps as targeted strategy to support wine quality. Food Chem., 255, 139-146.

Epova E.N., Bérail S., Séby F., Vacchina V., Bareille G., Médina B., Sarthou L., Donard O.F.X., 2019. Strontium elemental and isotopic signatures of bordeaux wines for authenticity and geographical origin assessment. Food Chem., 294, 35-45.

Faure G., 1986. *Principles of Isotope Geology*. 2nd edition; John Wiley & Sons, New York.

Geanã E.-I., Sandru C., Stanciu V., Ionete R.E., 2017. Elemental profile and $^{87}Sr/^{86}Sr$ isotope ratio as fingerprints for geographical tracebility of wines: an approach on Romanian wines. Food Anal. Methods, 10 (1), 63-73.

Horn P., Shaaf P., Holbach B., Hölz S., Eschnauer H., 1993. $^{87}Sr/^{86}Sr$ from rock and soil and vine and wine. Z Lebenesm Unters Forsh., 196, 407-409.

Horn P., Hölz S., Todt W., Matthies D., 1997. Isotope abundance ratios of Sr in wine provenance determination, in a tree-root activity study, and of Pb in a pollution study on tree-rings. Isot. Environ. Health Stud., 34, 31-42.

Kaya A., Bruno de Sousa R., Curvelo-Garcia A.S., Ricardo-da-Silva J., Catarino S., 2017. Effect of wood aging on mineral composition and wine $^{87}Sr/^{86}Sr$ isotopic ratio. J. Agric. Food Chem., 65, 4766-4776. https://dx.doi.org/10.1021/acs.jafc.7b01510

Marchionni S., Braschi E., Tommasini S., Bollati A., Cifelli F., Mulinacci N., Mattei M., Conticelli S., 2013. High-precision $^{87}Sr/^{86}Sr$ analysis in wines and their use as geological fingerprint for tracing geographic provenance. J. Agric. Food Chem., 61, 6822–6831.

Marchionni S., Buccianti A., Bollati A., Braschi E., Cifelli F., Molin P., Parotto M., Mattei M., Tommasini S., Conticelli S., 2016. Conservation of $^{87}Sr/^{86}Sr$ isotopic ratios during the winemaking processes of "Red" wines to validate their use as geographic tracer. Food Chem., 190, 777-785.

Martins P., Madeira M., Monteiro F., Bruno de Sousa R., Curvelo-Garcia A.S., Catarino S., 2014. $^{87}Sr/^{86}Sr$ ratio in vineyard soils from Portuguese denominations of origin and its potential for origin authentication. J. Int. Sci. Vigne Vin, 48 (1), 21-29.

Mercurio M., Grilli E., Odierna P., Morra V., Prohaska T., Coppola E., Grifa C., Buondonno A., Langella A., 2014. A "Geo-Pedo-Fingerprint" (GPF) as a tracer to detect univocal parent material-to-wine production chain in high quality vineyard districts, Campi Flegrei (Southern Italy). Geoderma, 230-231, 64–78.

Moreira C., de Pinho M., Curvelo-Garcia A.S., Bruno de Sousa R., Ricardo-da-Silva J.M., Catarino S., 2017. Evaluating nanofiltration effect on wine $^{87}Sr/^{86}Sr$ isotopic ratio and the robustness of this geographical fingerprint. S. Afr. J. Enol. Vitic., 38 (1), 82-93. http://dx.doi.org/10.21548/38-1-942

OIV. *Traceability guidelines in the vitiviniculture sector*. Resolution OIV CST 1/2007 (International Organisation of Vine and Wine, Paris, France, 2007).

Petrini R., Sansone L., Slejko F.F., Buccianti A., Marcuzzo P., Tomasi D., 2015. The $^{87}Sr/^{86}Sr$ strontium isotopic systematics applied to Glera vineyards: A tracer for the geographical origin of the Prosecco. Food Chem., 170, 138-144.

Rosner M., 2010. Geochemical and instrumental fundaments for accurate and precise strontium isotope data of food samples: Comment on "Determination of the strontium isotope ratio by ICP-MS gingeng as a tracer of regional origin" (Choi et al, 2008). Food Chem., 121, 918-921.

Sighinolfi S., Durante C., Lisa L., Tassi L., Marchetti A., 2018. Influence of chemical and physical variables on $^{87}Sr/^{86}Sr$ isotope ratios determination for geographical traceability studies in the oenological food chain. Beverages, 4, 55.

Song B.-Y., Gautam M.K., Ryu J.-S., Lee D., Lee K.-S., 2015. Effects of bedrock on the chemical and Sr isotopic compositions of plants. Environ. Earth Sci., 74, 829-837.

Tescione I., Marchionni S., Mattei M., Tassi F., Romano C., Conticelli S., 2015. A comparative $^{87}Sr/^{86}Sr$ study in red and white wines to validate its use as geochemical tracer for the geographical origin of wine. Procedia Earth and Planetary Science, 13, 169-172.

Vanhaecke F., Wannemacker G., Moens L., Hertogen J., 1999. The determination of isotope ratios by means of quadropole-based ICP-mass spectrometry: a geochronological case study. J. Anal. At. Spectrom., 14, 1691-1696.

Vinciguerra V., Stevenson R., Pedneault K., Poirier A., Hélie J.-F., Widory D., 2016. Strontium isotope characterization of wines from Quebec, Canada. Food Chem., 210, 121-128.

Vorster C., Greeff L., Coetzee P.P., 2010. The determination of $^{11}B/^{10}B$ and $^{87}Sr/^{86}Sr$ isotope ratios by quadrupole-based ICP-MS for the fingerprinting of South African wine. S. Afr. J. Chem., 63, 207-214.

Wilkes E., Day M., Herderich M., Johnson M., 2016. In vino veritas – investigating technologies to fight wine fraud. Wine & Viticulture Journal, March/April (2016).

Bordeaux wines authenticity experience

Tristan Richard[1]*, **Inès Le Mao** [1], **Eric Pedrot** [1], **François Guyon** [2], **and Grégory Da Costa** [1]

[1] *Unité de Recherche Œnologie, EA 4577, USC 1366 INRA, MIB Lab, Univ. Bordeaux, ISVV, 210 chemin de Leysotte, F-33882 Villenave d'Ornon, France*

[2] *Service Commun des Laboratoires, 146 Traverse Charles Susini, 13388 Marseille, France*

Abstract: In response to the increase in fraud cases in the wine world, many analytical approaches have been developed. Among the most promising is quantitative Nuclear Magnetic Resonance (q-NMR). This technique allows the rapid and simultaneous quantification of nearly fifty wine constituents from different chemical families. Numerous studies have shown that NMR analyses allow discriminating wines according to different parameters such as geographical origin, grape variety, vintage or wine-making practices. This study presents the used of 1H RMN spectrometry combined with chemometrics on Bordeaux wines classification. Our results show that q-NMR discriminate wines based on their geographical origin from region to winery. Wines from the same appellation have their own chemical signature. Thus, q-NMR is proving to be a fast and robust technique that requires a relatively small volume of sample for distinguishing wines, even those that are geographically close. These data show the interest of building up an analytical NMR fingerprinting database to guarantee the authenticity of wines and to fight against fraud.

Keywords: wine, NMR, metabolomics, chemometrics, Bordeaux

1. Introduction

International exchanges have increased the cases of fraud in the wine world. Some experts estimate that fraud can account for up to 20% of the global wine market. The European Union Intellectual Property Office (EUIPO) estimated the direct impact of counterfeiting in spirit and wine sectors at €1.3 billion/year in Europe (Wajsman *et al.*, 2016). In this context, the control of wine traceability is a major objective of the vine-growing sector (OIV Strategic Plan 2015-2019).

To fight against counterfeiting of wines, various analytical approaches have been developed, ranging from classic oenological analyzes to cutting-edge methods (Médina *et al.*, 2013; Gougeon *et al.*, 2019a). Nowadays, technological advances in analytical instrumentation together with the implementation of powerful data processing tools have allowed the development of metabolomics approaches which give access to different levels of quantitative, discriminant, and/or predictive data (Geana *et al.*, 2016; Valls Fonayet *et al.*, 2020).

Among the approaches recently developed, quantitative NMR spectrometry (q-NMR) is a promising technique. This high resolution technic allows rapid and simultaneous dosing of a large number of wine constituents without initial pre-treatment from a relatively small volume of wine (Godelmann *et al.*, 2016; Gougeon *et al.*, 2018). This technique combined with robust chemometrics tools made it possible to discriminate different wine parameters

*Corresponding author. E-mail: tristan.richard@u-bordeaux.fr

including geographic origin, grape varieties, vintage or winemaking techniques (López-Rituerto *et al.*, 2009; Godelmann *et al.*, 2013; Gougeon *et al.*, 2019b).

Based on an original q-NMR metabolomics approach, a database was developed for Bordeaux wines. This database has made it possible to distinguish Bordeaux wines from other French Protected Designation of Origin (PDO) and between Bordeaux appellations (Gougeon *et al.*, 2019b). The main results obtained are summarized below.

2. Material and methods

2.1. Sample preparation

Wine samples were mixed with a phosphate buffer and a deuterated water solution (ratio 7:2:1). Trimethylsilylpropanoic acid sodium salt (TMSP) and calcium formate were used as frequency and quantification references, respectively. The pH was fixed to 3.1 using a BTpH unit (Bruker BioSpin, Germany).

2.2. ^{1}H NMR spectra analysis

^{1}H NMR spectra were acquired on a 600 MHz spectrometer (Bruker BioSpin, Germany). Two specific sequences were used for the quantification of wine constituents. The ZGPR pulse program was used for water signal suppression. NOESYGPPS1D sequence was performed for the suppression of water and ethanol signals. Spectra were processed using Topspin 3.2 (Bruker Biospin, Germany) and analyzed using MestReNova 12 (Mestrelab Research, Spain). Spectra were summited to phase and baseline corrections before data extraction. Finally, compounds were semi-automatically quantified using the plugin Simple Mixture Analysis (SMA) of MestReNova software.

2.3. Chemometrics

For direct comparison of suspicious and authentic wines, an adapted z-score value approach was used, and a similarity score (s-score) was evaluated, for each wine constituent (Gougeon *et al.*, 2019a).

For unsupervised principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) the SIMCA 16 software (Umetrics, Sweden) was used. PCA was carried out in first stage to visualize the data. In a second stage, PLS-DA was completed to refine the discrimination between sets.

3. Results and discution

3.1. ^{1}H NMR Spectroscopy

The representative spectrum of a wine sample after water and ethanol suppression was presented in Figure 1. Compounds were identified by using pure chemical standard, 2D-NMR experiments and literature data (Fotakis et al., 2013). Nearly fifty wine constituents were identified and quantified including organic acids, alcohols, amino acids, phenolic compounds and sugars (Table 1).

Table 1. List of the wine constituents identified in the ^{1}H NMR spectra of wines. The chemical shifts, multiplicities and assignments are reported.

Compound	δ_H (multiplicity, assignment)
Acids	
acetic acid	2,08 (*s*, CH_3)
citric acid	2.79 (*d*, 15.6, CH_2) ; 2.94 (*d*, 15.6, CH_2)
formic acid	8.36 (s, CH)
fumaric acid	6,78 (*s*, 2CH)
galacturonic acid	5,32(*d*, 3.8, CH)

glucuronic acid	3.29 (*t*, 8.6, CH), 3.51 (*m*, 2CH), 3.58 (*dd*, 9.7, 3.7, CH), 3.73 (*m*, 2CH), 4.08 (*d*, 10.8, CH), 4.64 (*d*, 7.9, CH), 5.25 (*d*, 3.7, CH), 5,55(*d*, 4, CH)
lactic acid	1.40 (*d*, 7.0, CH_3), 4,31 (*q*, 7.0, CH)
malic acid	2,78 (*dd*, 16.3 and 7.0, CH), 2.89 (*dd*, 16.3 and 4.5, CH), 4,53 (*dd*, CH)
pyruvic acid	2,35 (*s*, CH_3)
sorbic acid	5.83 (*d*, 15.3, CH)
succinic acid	2,65 (*s*, $2CH_2$)
tartaric acid	4,60 (*s*, 2CH)
Alcohols	
butane-2,3-diol	1.13(*d*, 6.2, $2CH_3$) ; 3.61 (*m*, 2CH)
ethanol	1,17 (*t*, 7.2, CH_3), 3,65 (*q*, CH_2)
glycerol	3,55(*dd*, 11.8 and 6.5, CH_2), 3.64 (*dd*, CH_2), 3.77 (*m*, CH)
isobutanol	0,87 (*d*, 6.7,$2CH_3$), 1,73 (*m*, CH), 3,36 (*d*, 6.7, CH_2)
isopentanol	0,88 (*d*, 6.7, $2CH_3$), 1,44 (*q*, CH_2); 1,66 (*m*, CH); 3,61 (*t*, 6.7, CH_2)
mannitol	3.65 (dd, 11.7, 6.2 CH_2), 3,73 (*m*, CH), 3,77 (*d*, 9.0, CH), 3.84 (*dd*, 11.9, 2.8, CH_2)
methanol	3,35 (*s*, CH_3)
myo-inositol	3,27(*t*, 9.7, CH); 3,52 (*dd*, 10.0 and 2.8, 2CH); 3,61 (*t*, 2.8, 2CH); 4,05 (*t*, 2.8, CH)
phenethyl alcohol	2.85 (t, 6.62, CH_2), 3.74 (t, CH_2), 7.33 (*m*, 5CH)
Amino acids	
alanine	1.50(*d*, 7.2, CH_3) ; 3,76 (*q*, CH))
arginine	1.70 (*m*, CH_2), 1.89 (*m*, CH_2), 3.23 (*t*, CH_2), 3.75 (*t*, 6.5, CH)
γ-aminobutyric acid	1.96 (*m*, CH_2), 2,50 (*t*, 7.3, CH_2), 3.05 (*m*, CH_2)
histidine	3.16 (*dd*, 15.6, 7.7, CH), 3.23 (*dd*, 16.0, 5.0, CH), 3.98 (*dd*, 7.7, 5.0, CH), 7.09 (*d*, 5.0, CH), 7.90 (*d*, 1.1, CH)
isoleucine	0.93 (*t*, 7.4, CH_3), 0.99 (*d*, 7.0, CH_3), 1.24 (*m*, CH_2), 1.45 (*m*, CH_2), 1.97 (*m*, CH), 3.66 (*d*, 3.9, CH)
leucine	0,96(*d*, 6.2, 2CH3), 1.71 (*m*, $CHCH_2$), 3.74 (*m*, CH)
proline	1,99(*m*, CH_2), 2,06 (*m*, CH), 2,33 (*m*, CH); 3,32 (*dt*, 14.0, 7.1, CH), 3,42 (*dt*, 11.6 and 7.0, CH); 4,11 (*dd*, 8.6 and 6.4, CH)
threonine	1.32 (*d*, 6.7, CH_3), 2.58 (*d*, 4.9, CH), 4.24 (*m*, CH)
tyrosine	3.02 (*dd*, CH_2), 3.17 (*dd*, CH_2), 3.92 (*dd*, CH), 6,86(*m*, 8.4, 2CH), 7,17 (*m*, 8.6, 2CH)
valine	0,99 (d, 7.3, CH_3), 1,04 (*d*, 7.3, CH_3), 2.28 (*m*, CH), 3.66 (*d*, 4.3, CH)
Phenolic compounds	
caffeic acid	6.33 (*d*, 16.0, CH), 6.92 (*d*, 8.0, CH), 7.07 (*dd*, 8.2, 2.0, CH), 7.14 (*d*, 2.0, CH) 7.29 (*d*, CH)
catechin	2.53 (*dd*, CH_2), 2.85 (*m*, CH_2), 4.15 (*m*, CH), 4.41 (*d*, 7.0, CH), 5.99 (d, 2.0, CH), 6.08 (*d*, 2.3, CH), 6.84 (*d*, 8.6, CH), 6.92 (*m*, 2CH)
epicatechin	2.76 (*m*, CH_2), 2.90 (*m*, CH_2), 4.32 (*m*, CH), 4.95 (*m*, CH), 6.09 (*d*, 2.0, CH), 6.12 (*d*, 2.0, CH), 6.93 (*m*, CH_2), 7.03 (*d*, 2.0, CH)
gallic acid	7,16 (*s*, 2CH)

shikimic acid	2.21 (*dd*, 18.2,7.0, CH_2), 2.75 (*dd*, 18.0, 5.3, CH_2), 3.74 (*dd*, 8.6, 4.3, CH), 4.01 (*m*, CH), 4.42 (*t*, 4.1, CH), 6.82 (*dt*, CH)
syringic acid	3.84 (*s*, $2CH_3$), 7.36 (s, 2CH)
tyrosol	2.77 (*t,* CH_2), 3.77 (*t*, CH_2), 6.84(*m*, 8.4, 2CH), 7.17 (*m*, 8.4,2CH)
Sugars	
arabinose	3.51 (*dd*,CH), 3.68 (*m*, $CHCH_2$), 3.83 (*dd*, CH), 3.90 (*m*, $CHCH_2$), 3.95(*m*, CH), 4.02 (*m*, $CHCH_2$), 4.50(*d*, 7.7, CH), 5.25 (*d*, CH)
fructose	3.57 (*m*, CH_2), 3.69 (*m*, $2CH_2$), 3.82 (*m*, $CHCHCH_2$), 3.90 (*dd,* 9.9, 3.4, CH) 4,00 (*m*, CH), 4.02 (*dd*, 12.8, 1.0 CH_2), 4.11 (*m,*2CH)
glucose	3.23 (*dd,* 9.2, 8.0, CH), 3.39 (*m*, CH), 3.45 (*dd*, 9.8, 3.7, CH) 3.72 (m, $CHCH_2$), 3.82 (*m,* $CHCH_2$), 3.88 (*dd,* 12.2, 2.1, CH_2), 4.63 (*d*, 7.9, CH), 5.22 (*d*, 3.6, CH)
xylose	3.21 (*dd*, 9.3, 7.9, CH), 3.31 (t, 11.4, CH_2), 3.42 (t, 9.25, CH), 3.51 (*dd*, 9.3, 3.7, CH), 3.63 (m, $CHCHCH_2$), 3.91 (*dd*, 11.5, 5.5, CH_2), 4.57 (*d*, 7.9, CH), 5.19 (*d*, 3.7, CH)
Others	
acetoin	1.37(*d*, 7.0, CH_3) ; 2.21 (*s*, CH3) ; 4.42 (*q*, CH)
choline	3.19 (*s*, $3CH_3$) ; 3.51 (*dd,* CH_2) ; 4.05 (*m*, CH_2)
ethanal	2,23(*d*, 3.0, CH_3), 9,67 (*q*, CH)
ethyl acetate	1,26(*t*, 7.2, CH_3), 4,12 (*q*, CH_2), 2.07 (s, CH_3)
ethyl lactate	1.28 (*t*, CH_3), 1.42 (*d*, 7.0, CH_3), 4.22 (*q*, 7.06, CH), 4.39 (*q*, 7.0, CH)
trigonelline	4.42 (s, CH_3), 8,07 (*m*, CH), 8,82 (*m*, 2CH), 9,11(*s*, CH)

To perform an accurate quantification, ^{1}H NMR spectra were exported into MestReNova and treated using the plugin SMA (Gougeon *et al.*, 2018). Peak deconvolution was realized using global spectral deconvolution method (Cobas *et al.*, 2011). The precision of the method was validated by comparison with reference samples and by comparison with official OIV methods (Gougeon *et al.*, 2018; Gougeon *et al.*, 2019a).

3.2. *Comparison between bottles*

The first objective is to be able to compare two wines with each other, a suspicious wine and the original one coming from the winery. One of the main problems is to take into account the wine aging in bottle. To avoid this drawback, an adapted z-score value approach was developed to compare suspicious and authentic wines (Gougeon *et al.*, 2019a). To evaluate the wine aging effect in bottle, the uncertainties on each constituent content was controlled using different sets of old wines provided by the Chateau Mouton Rothschild (Médoc, France).

To illustrate the process, twelve commercial Bordeaux red wines from Medoc and Saint-Emilion DPO were compared using q-NMR analyses and total s-score approach (Figure 2). As observed in Figure 2, all the wines presented a total similarity score (s-score) upper than five except two Médoc and two Saint-Emilion red wines. These results indicate that the method allows discriminating wines, even from close wineries.

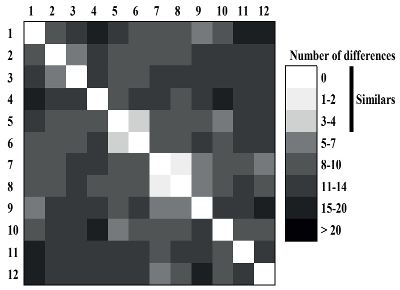


Figure 2. Cross comparison of twelve Bordeaux wines from Médoc (1-6) and Saint-Emilion (7-12) based on total s-score.

3.3. *Classification of French red wines*

To compare wines, a database containing today more than a thousand references was built including red wines from different French PDO. These wines were compared using multivariate analysis to discriminated geographical origins and vintages (Gougeon *et al.*, 2019b).

Commercial red wines from six different wine-growing regions of France were compared: Beaujolais, Bordeaux, Burgundy, Côtes du Rhône, Languedoc and Loire Valley. The classification was performed using Principal components analysis (PCA) followed by partial least squares discriminant analysis (PLS-DA). The models were evaluated by cross validation using internal leave one-out cross validation (LOOCV). First at all, Bordeaux red wines were generally discriminated from other French wine-growing regions (Gougeon *et al.*, 2019b). On the one hand, Bordeaux red wines presented larger amounts of isopropanol, gallic acid, methanol; phenethyl alcohol, proline and succinic acid. On the other hand, these wines presented smaller amount of 2-3-butanediol, caffeic acid, ethyl lactate and lactic acid. These differences could be associated to grape varieties such as proline content. But other factors may also contribute to the phenomenon such as climate and cultural practices.

To illustrate the ability of q-NMR to discriminate between wines from different French regions, 66 white wines from Bordeaux and Burgundy (37 Bordeaux and 29 Burgundy wines), belonging ten different vintages, were analyzed by q-NMR spectrometry followed by PLS-DA

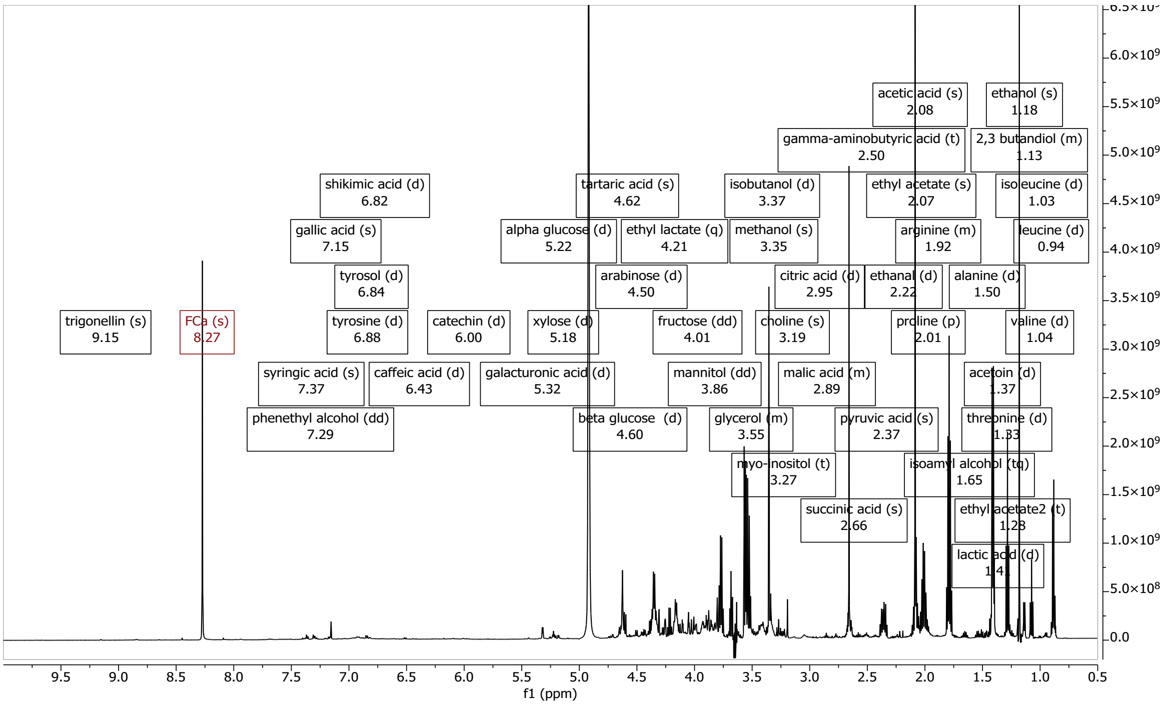


Figure 1. Representative [1]H NMR spectrum of a wine sample after water and ethanol signals suppression.

treatment using SIMCA 16 (Figure 3). A clear discrimination was observed between the two sets of wines despite the wide range of vintages analyzed.

Similarly, red wines from different Bordeaux appellations were compared. These works show that it is possible to classify PDO wines from the same area by q-NMR analysis (Gougeon *et al.*, 2019b).

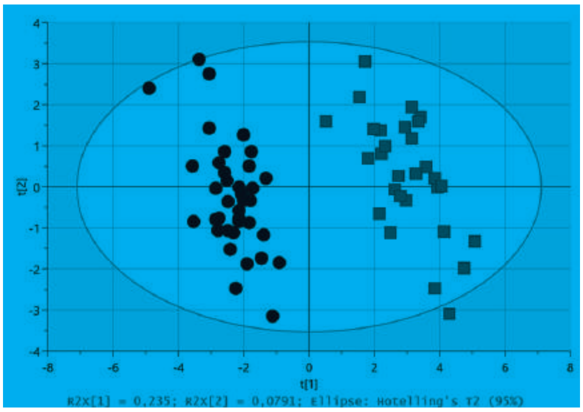


Figure 3. PLS-DA score scatter plot of 66 white wines from Bordeaux (37 wines, black circles) and Burgundy (29 wines, grey squares) analyzed by [1]H NMR spectroscopy.

To illustrate these results, 60 red wines from Médoc and Libournais areas (24 Médoc and 36 Libournais red wines), from twelve different vintages, were analyzed by [1]H NMR spectroscopy. NMR data were processed by PLS-DA as shown in Figure 4A. The unsupervised statistical analysis shows a clear separation of the two areas. More detailed analysis of discriminant factors seems to indicate an influence of the grape variety. Indeed, even if Bordeaux wines are blends of different grape varieties, Cabernet Sauvignon is dominant in the Médoc and Merlot is the main cultivated variety in the Libournais. However, as for the wines of the different French regions, other parameters seem to influence the classification such specific climate and winemaking processes.

Finally, [1]H NMR spectra of wines from close wineries were recorded. Twenty four wines from two close wineries of Médoc PDO were analyzed from twelve different vintages distributed between 1990 and 2010 (twelve wines for each vineyard). The results, processed by PLS-DA, were presented in Figure 4B. A clear classification of the two domains is observed. The [1]H NMR spectrometry allowed the discrimination of these two vineyards. This result is remarkable because these two wineries use the same grape varieties and are subject to the same environmental conditions. This observation suggests that the classification is linked to the oenological practices.

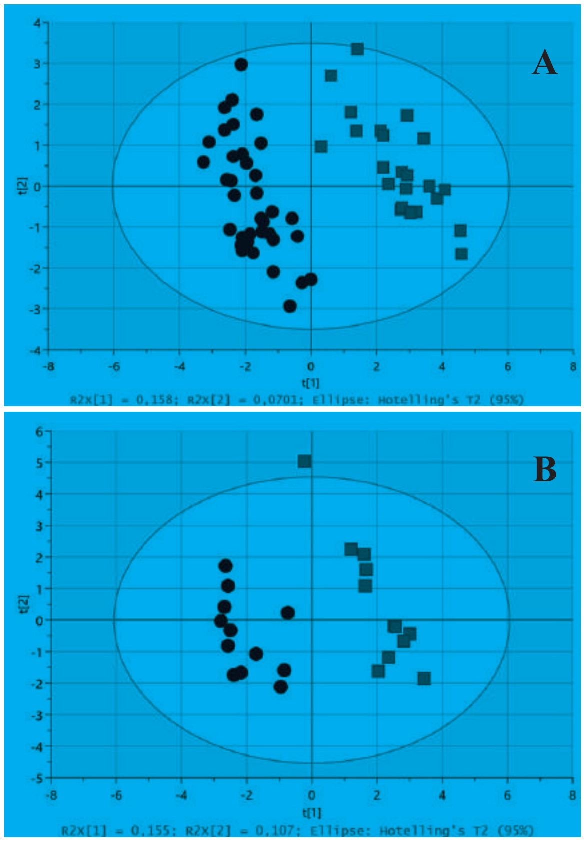


Figure 4. PLS-DA score scatter plot of Bordeaux red wines analyzed by [1]H NMR spectroscopy. A: comparison between Libournais (36 wines, black circles) and Médoc (24 wines, grey squares). B: comparison between two wineries of Médoc PDO.

All these results suggest that NMR can discriminate wines between wine-growing regions but also on the scale of a wine-growing estate.

4. Acknowledgments

This work was supported by Conseil Interprofessionnel du Vin de Bordeaux (CIVB), Conseil Régional d'Aquitaine, FranceAgriMer program (grant number 2014–0785), Château Mouton Rothschild and Fondation of Bordeaux (donors: Baron Philippe de Rothschild SA, Chateau Cheval Blanc, Château Lafite Rothschild, Le Domaine Clarence Dillon, Château Petrus). The experiments were performed thanks to the Bordeaux Metabolome Facility and MetaboHUB program (ANR-11-INBS-0010 project).

5. References

Cobas C., Seoane F., Domínguez S., Sykora S., Davies A.N., 2011. A new approach to improving automated analysis of proton NMR spectra through Global Spectral Deconvolution (GSD). *Spectrosc. Eur.* **23**, 26-30.

Geana E.I., Popescu R., Costinel D., Dinca O.R., Ionete R.E., Stefanescu I., Artem V., Bala C., 2016. Classification of red wines using suitable markers coupled with multivariate statistic analysis. *Food Chem.* **192**, 1015-1024.

Godelmann R., Fang F., Humpfer E., Schütz B., Bansbach M., Schäfer H., Spraul M., 2013. Targeted and Nontargeted Wine Analysis by 1H NMR Spectroscopy Combined with Multivariate Statistical Analysis. Differentiation of Important Parameters: Grape Variety, Geographical Origin, Year of Vintage. *J. Agric. Food. Chem.* **61**, 5610-5619.

Godelmann R., Kost C., Patz C.-D., Ristow R., Wachter H., 2016. Quantitation of Compounds in Wine Using 1H NMR Spectroscopy: Description of the Method and Collaborative Study. *J. AOAC Int.* **99**, 1295-1304.

Gougeon L., Da Costa G., Le Mao I., Ma W., Teissedre P.L., Guyon F., Richard T., 2018. Wine Analysis and Authenticity Using 1H-NMR Metabolomics Data: Application to Chinese Wines. *Food Anal. Met.* **11**, 3425-3434.

Gougeon L., da Costa G., Richard T., Guyon F., 2019a. Wine Authenticity by Quantitative 1H NMR Versus Multitechnique Analysis: a Case Study. *Food Analytical Methods* **12**, 956–965.

Gougeon L., da Costa G., Guyon F., Richard T., 2019b. 1H NMR metabolomics applied to Bordeaux red wines. *Food Chem.* **301**, 125257.

López-Rituerto E., Cabredo S., López M., Avenoza A., Busto J.H., Peregrina J.M., 2009. A thorough study on the use of quantitative 1H NMR in Rioja red wine fermentation processes. *J. Agric. Food. Chem.* **57**, 2112-2118.

Médina B., Salagoïty M.H., Guyon F., Gaye J., Hubert P., Guillaume F., 2013. 8 - Using new analytical approaches to verify the origin of wine, in: Brereton, P. (Ed.), New Analytical Approaches for Verifying the Origin of Food. Woodhead Publishing, pp. 149-188.

Valls Fonayet J., Loupit G., Richard T., 2020. MS- and NMR-metabolomic tools for the discrimination of wines: Applications for authenticity, *Adv. Bot. Res.*, In Press, doi: 10.1016/bs.abr.2020.11.003.

Wajsman N., Arias Burgos C., Davies C., 2016. The economic cost of IPR infringement in spirits and wine, in: EUIPO (Ed.), Alicante, Spain, p. 31.

10th International Symposium

**Challenges in viticulture and oenology:
Wine Appellations, Authenticity and Innovation.**

ŒNOVITI INTERNATIONAL network

Session IV. Winemaking and Winegrowing Innovations and Novelties

Grape diversity mining with a high-throughput phenotyping tool

Yongjian Wang [1], **Michael Henke** [1], **Junhua Kong** [1], **Peige Fan** [1], **Zhenchang Liang** [1], **and Zhanwu Dai** [1]*

[1] *Beijing Key Laboratory of Grape Sciences and Enology, CAS Key Laboratory of Plant Resources, LIA Innogrape, Institute of Botany, Chinese Academy of Sciences, Beijing, China*

Abstract: Grape possesses a large diversity in various traits, such as size, shape, and color etc. These diversities make grape berries more attractive to consumers and offers possibility for variety innovation by breeding. Traditionally, berry exterior traits are described by categories based on visual evaluation, which may introduce annotator bias. For example, OIV defined 10 categories of grape berry shapes and 6 categories of berry colors. However, these traits frequently vary continuously and quantitatively and are under the regulation of genotype x environment interactions. Therefore, innovations are needed to develop high-throughput tools for assessing grape exterior traits standardized and therefore unbiased, in a speedy and affordable manner. To this end, we developed an automate image acquisition and analysis tool which is able to extract berry traits including size, shape, texture, and color features. The tool was used to mine the berry diversity in a cross progeny with 160 genotypes. The results showed that the tool can precisely capture berry length and width by recognizing pedicel as a reference. It can also extract various color information with an analysis rate of roughly 1s/berry. A total of 10,010 berries were analyzed at maturity for the progeny, revealing comprehensive information on the berry diversity. In conclusion, this innovative tool is able to provide high quality of quantitative phenotyping data of grape berries and paves the way for bridging the gap between grape berries and phenotypes.

Keywords: grape, phenotyping, feature extraction, berry size, berry shape, color

1. Introduction

Grape is one of the most important fruit crops, which possesses large diversity in both exterior quality traits, such as size, shape and color, as well as intrinsic quality traits, such as sugars, organic acids, tannins, aromas etc (Houel et al., 2013; This et al., 2006; Wolkovich et al., 2018). Knowledge about these traits are essential for grape growers and consumers, and are under the regulation of genotype, environment, and management interactions (Poni et al., 2018). Exploring grape diversity is important for breeding new varieties, which can not only diversify the products for consumers but also serves as an important way to mitigate the influence of the ongoing climate change (Wolkovich et al., 2018).

With the fast development of technologies in genome sequencing, molecular-assisted breeding may have strong powers to speed up breeding for all kinds of plants, as well as for grapes, a perennial fruit trees with long juvenile periods. However, the identification of pertinent molecular markers has been hampered by the strong

*Corresponding author. E-mail: zhanwu.dai@ibcas.ac.cn

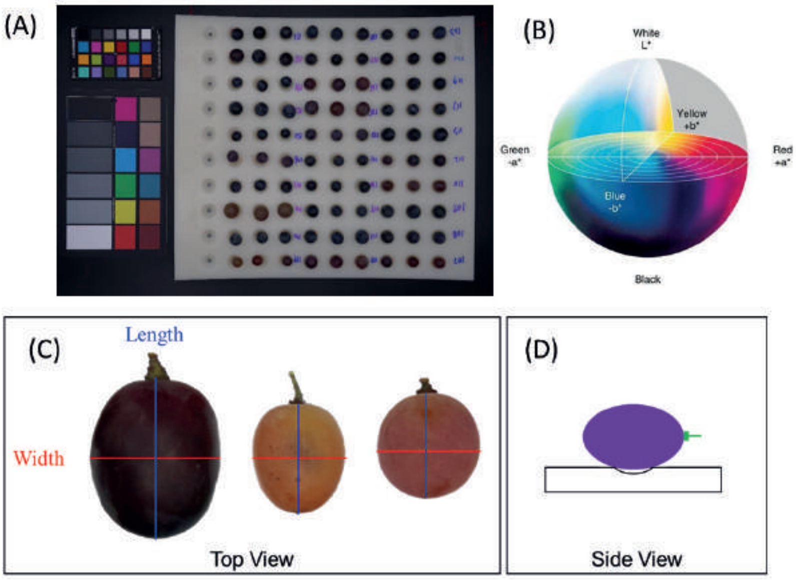


Figure 1. Outline of the grape phenotyping tool. (A) raw image with berries distributed in the 10 ×10 grids of a plastic plate with white background in accompanied with a standard 24-color chart ; (B) Lab color space; (C) Top view of berries with different size, shape, and color. The pedicel is retained serving as a reference point for length and width determination; (D) Side view of the berry on the plastic plate and it is important to keep the pedicel parallel to the plate.

interaction between genotype, environments and managements, which lead to significant phenotype variations. Therefore, efficient high-throughput phenotyping pipelines turn out to be a bottleneck (Araus and Cairns, 2014; Fiorani and Schurr, 2013). Recently, such tools have been developed based on image analysis for model plants, including Arabidopsis, and provide very promising results (Huther et al., 2021). There are also a few developments for grapes, such as for bunch architecture and compactness (Rist et al., 2018; Tello et al., 2016; Underhill et al., 2020a; Xin et al., 2020), bunch color (Underhill et al., 2020b), berry numbers and size (Kicherer et al., 2013) etc. However, these existing tools for grape are not able to capture the berry size, shape and color features simultaneously. Therefore, we aimed to develop a tool to fill this gap and apply the new tool to cross progenies to explore berry diversity in an automate, high-throughput, and precision manner.

2. Materials and Methods

A grape cross progeny between 'Beifeng'(*V. bryoniifolia* x *V. vinefera*) x 'Yan73' (*V. vinefera*) growing in the grape germplasm vineyard of the Institute of Botany was used as plant materials. This progeny is 13-year-old with 160 genotypes and 3-5 vines for each genotype. Vines were managed with local viticulture standards, and all of them produce fruits during the experiment.

One hundred berries were harvested at maturity from 5-10 clusters for each genotype. Berries were brought back to the lab and distributed into a custom plastic plate with a 10 x 10 grid of grooves (Figure 1A), keeping the pedicel parallel to the plate (Figure 1C and D). A fixed camera (Sony, Tokyo, Japan) was used to capture images within a custom image acquisition facility equipped with a LED lighting system to obtain a homogeneous illumination all over the berry grid. A 24-color standard color chart (C-Rite ColorChecker classic mini) was placed aside (Figure 1A) to allow a color correction during image processing.

After image acquisitions, 10 berries from 10 representative genotypes were further manually measured for berry length, width, fresh weight. Moreover, berry colors were measured with a 'Lab' colorimeter as described in Liang et al. (2011) with outputs of the Lab color space (Figure 1B). These data served as ground-truth data for assessing the validation of the developed tool.

The image processing and analysis pipeline as well as the feature extraction was developed within MATLAB 2020b (MathWorks Inc.).

3. Results and Discussions

After several trials of lighting improvement and image acquisition, the tool succeeded to identify berries, correct colors, anchor pedicels, and finally extract about 100 phenotypic parameters, including berry morphological features and color features (Figure 1). A comparison between manually measured results with those estimated from the tool showed a very high precision, with R^2 of 0.98 for berry length and width, and R^2 of 0.80-86 for the parameters of L, a, b of the 'Lab' color space (Figure 1B). These results indicate that the accuracy of our tool is assured. Then the tool was applied to 10010 berries imaged at maturity, and the tool was able to segment and analyze 1 berry per second, and finish all the berries within 3 hours on a standard computer.

There are several characteristics that distinct the current tool to those previously developed, such as the BAT tool developed by Kicherer et al. (2013). Firstly, our tool can differentiate berry length and width. In fact, these two traits seem very easy to separate for humans, it is rather challenging for image analysis tools. Without a reference point for determining grape orientation, one may consider the width of a berry with oblate form as its length, as did in the BAT tool (Kicherer et al., 2013). Secondly, machine learning algorithms that are frequently used in image analysis are highly dependent on rather large image sets for training for a given background, berry color range, and light conditions. Most machine learning approaches tend to overfitting, which would further require to increase the training data set to compensate. They may work very well for certain berries, e.g. green berries with a black background, but most

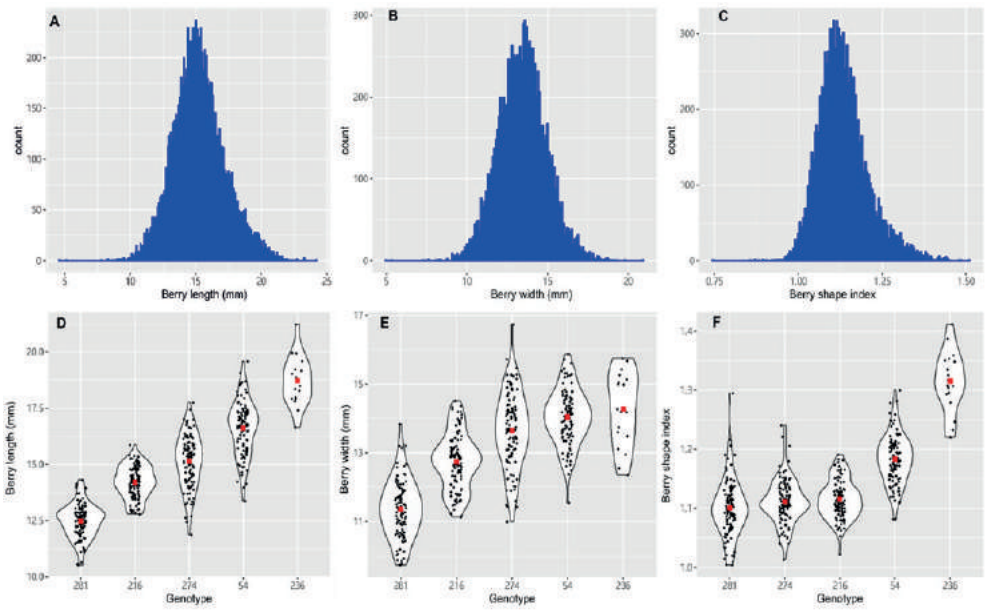


Figure 2. The diversity of berry size and shape in a grape cross progeny with 160 genotypes at maturity. Top panels (A, B, C) for the population distribution of berry length, width and berry shape index (length:width ratio); bottom panels (D, E, F), the variations of berry length, width and berry shape index for five representative genotypes.

probably loss its accuracy for dark-purple-colored berries with the same configuration. Our tool applied well established image processing techniques that enables the tool to work very robust for a large diversity of berry colors and shapes. Thirdly, the current tool integrates the capacity to extract color and texture features for berries, which has not been incorporated with existing tools.

Applying the tool to the progeny highlighted interesting diversity in berry size, shape and colors (Figure 2 and 3). Berry length varied from 5 mm to 25 mm with an average at 15 mm at the population level (Figure 2A). Moreover, the distribution of berry length followed a normal distribution. Five representative genotypes, including the genotype IDs of 54, 216, 236, 274, and 281, were further explored at genotype scale (Figure 2D). The variation in berry length also followed normal distribution for each genotype, and the genotypes were not only different at their mean berry length but also at the variability within a given genotype. Berry width showed a similar trend as berry length (Figure 2B and 2E). Interestingly, we also observed significant segregation in the berry shape index, defined as the ratio between berry length and width. It varied from 0.75 to 1.5, which indicate that there are oblate formed berries and ellipsoid berries (Figure 2C). It seems that the smaller berries (281, 274, 216) tended to have a shape index at about 1, which represent a globose form; while those bigger genotypes (236) had a shape index at 1.3, representing an ellipsoid shape (Figure 2F).

For berry color analysis, we projected berry images of each genotype to the space of L and b of the Lab color space (Liang et al., 2011). Most genotypes of this progeny had a deep dark color, with few genotypes showing pink-green colors (Figure 3). These results indicate that the Lab color space can make well discrimination of different genotypes and may aid in identifying novel molecular markers for color in combination with quantitative genetic approaches. Similarly, a color quantification tool has been developed for dissection color patterns of Coleus leaves (Li et al., 2021). However, the latter tool does not incorporate shape and size analysis.

In future, the tool will be applied to the same progeny for another growing season and its phenotyping data will be combined with quantitative genetic approaches, such as QLT or SNP identifications, for exploring novel stable and pertinent molecular markers for grape berry (Underhill et al., 2020a,b).

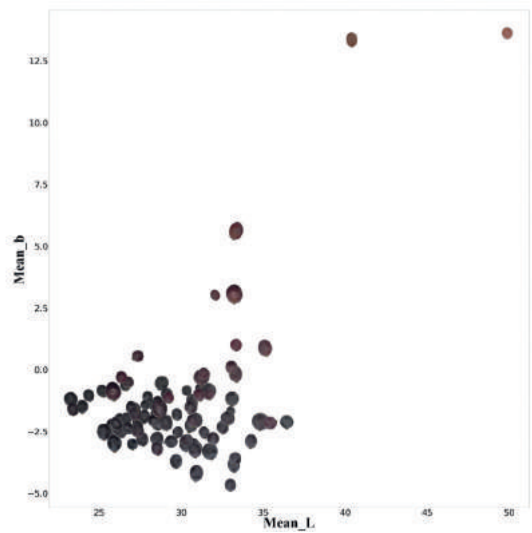


Figure 3. The diversity of berry color in a grape cross progeny with 160 genotypes. The figure design was inspired by Li et al. (2019, arXiv:1903.01652)

4. Acknowledgements

This work is partially supported by the National Key R&D Program of China (2019YFD1000100).

References

1. Araus JL, Cairns JE, 2014. Field high-throughput phenotyping: the new crop breeding frontier. Trends Plant Sci, 19(1):52-61.
2. Fiorani F, Schurr U, 2013. Future scenarios for plant phenotyping. Annu Rev Plant Biol, 64:267-291.

3. Houel C, Martin-Magniette M, Nicolas S, Lacombe T, Le Cunff L, Franck D, Torregrosa L, Conejero G, Lalet S, This P et al, 2013. Genetic variability of berry size in the grapevine (Vitis vinifera L.). Aust J Grape Wine Res, 19:208 - 220.
4. Huther P, Schandry N, Jandrasits K, Bezrukov I, Becker C, 2021. ARADEEPOPSIS, an automated workflow for top-view plant phenomics using semantic segmentation of leaf states. Plant Cell in press, doi: 10.1105/tpc.1120.00318.
5. Kicherer A, Roscher R, Herzog K, Šimon S, Förstner W, Töpfer R, 2013. BAT (Berry Analysis Tool): A high-throughput image interpretation tool to acquire the number, diameter, and volume of grapevine berries. Vitis, 52(3):129-135.
6. Li M, Coneva V, Clark D, Chitwood D, Frank M, 2021. Quantitative dissection of color patterning in the foliar ornamental Coleus reveals underlying features driving aesthetic value. bioRxiv:2021.2001.2011.426252.
7. Li M, Frank MH, Migicovsky Z, 2019. ColourQuant: a high-throughput technique to extract and quantify colour phenotypes from plant images. arXiv: arXiv:1903.01652.
8. Liang Z, Sang M, Fan P, Wu B, Wang L, Yang S, Li S, 2011. CIELAB coordinates in response to berry skin anthocyanins and their composition in Vitis. J Food Sci, 76(3):C490-497.
9. Poni S, Gatti M, Palliotti A, Dai Z, Duchene E, Thuy-Thanh T, Ferrara G, Matarrese AMS, Gallotta A, Bellincontro A et al, 2018. Grapevine quality: A multiple choice issue. Sci Hortic, 234:445-462.
10. Rist F, Herzog K, Mack J, Richter R, Steinhage V, Töpfer R, 2018. High-precision phenotyping of grape bunch architecture using fast 3D sensor and automation. Sensors, 18(3):763.
11. Tello J, Torres-Pérez R, Grimplet J, Ibáñez J, 2016. Association analysis of grapevine bunch traits using a comprehensive approach. Theor Appl Genet, 129(2):227-242.
12. This P, Lacombe T, Thomas MR, 2006. Historical origins and genetic diversity of wine grapes. Trends Genet, 22(9):511-519.
13. Underhill A, Hirsch C, Clark M, 2020a. Image-based phenotyping identifies quantitative trait loci for cluster compactness in grape. J Am Soc Hortic Sci, 145(6):363.
14. Underhill AN, Hirsch CD, Clark MD, 2020b. Evaluating and mapping grape color using image-based phenotyping. Plant Phenomics, 2020:8086309.
15. Wolkovich EM, García de Cortázar-Atauri I, Morales-Castilla I, Nicholas KA, Lacombe T, 2018. From Pinot to Xinomavro in the world's future wine-growing regions. Nature Climate Change, 8(1):29-37.
16. Xin B, Liu S, Whitty M, 2020. Three-dimensional reconstruction of Vitis vinifera (L.) cvs Pinot Noir and Merlot grape bunch frameworks using a restricted reconstruction grammar based on the stochastic L-system. Aust J Grape Wine Res, 26(3):207-219.

Innovation in traditional vine cultivars based on somatic variation: A case study in Tempranillo

Carolina Royo, Pablo Carbonell-Bejerano, Maite Rodríguez-Lorenzo, Yolanda Ferradás, Javier Ibáñez, Elisa Baroja, Juana Martínez, Enrique García-Escudero y José Miguel Martínez-Zapater *

Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, CSIC, Gobierno de La Rioja). Finca La Grajera, Ctra. de Burgos km 6, 26007 Logroño, Spain.

Corresponding author: J.M. Martínez Zapater, Phone: +34-941894981, email: zapater@icvv.es

Abstract: Evolution of plant material in viticulture has traditionally relied on the phenotypic variation generated by spontaneous somatic mutations. This variation has long contributed to cultivar adaptation under changing environmental conditions and has also been a source of novel traits and cultivars. New phenotypic and genomic technologies offer now possibilities to increase the efficiency of this selection process that can have an impact on classical cultivar innovation. In this report we describe the value of somatic variation in the innovation of traditional cultivars. Specifically, we focus on a successful example in Tempranillo that ended in the new white-berried variety Tempranillo Blanco. Here we describe the history of this new variety, its particular features, its genetic origin, as well as all the information generated by its genome sequence that helps understanding possible varietal viticultural issues and improvement strategies. In addition, we shortly mention other grape color Tempranillo variants under study that could be the basis to generate additional new clones and cultivars.

Keywords: Somatic variation, Cultivar innovation, Genomic strategies, Fruit color variation, Tempranillo.

Introduction

Current wine market is conservative for varietal change. World elite varieties such as Cabernet Sauvignon, Merlot, Tempranillo or Chardonnay are highly appreciated and have been cultivated for centuries and vegetatively propagated from a single original plant derived from spontaneous hybridization among grapevine plants (This et al., 2006). Although nowadays new varieties resistant to fungal diseases and more adapted to climate change can be generated through classical or marker-assisted breeding, current elite varieties will continue to be highly relevant in the coming decades. Their sustainability will depend in part on the study, characterization and use of the genetic variation that is somatically generated during vine growth and propagation. Traditional viticulture has relied on the phenotypic variation generated by spontaneous somatic mutations for improvement, diversification and adaptation of the varieties to new growing areas or to consumer and market demands. Somatic variation has thus been selected by farmers along the history of viticulture to improve cultivars and adapt production to changing conditions through the mass selection practiced in the multiplication of their best vineyards and vines. From the 20th century on, this selection was rationalized and developed into clonal selection (Reynolds, 2015). Somatic variation allowed the development of highly productive clones in the 70s and 80s decades of the last century. The same source of variation is now the basis for the selection of less productive and higher quality clones, with loose bunches and small grapes, with more homogeneous ripening and less prone to diseases. Somatic variation also enables wine diversification by selecting color or flavor variants that are sometimes the origin of new varieties (Carbonell-Bejerano et al., 2019). This innovation in traditional varieties, also comes with some production and commercial advantages. Selected clones keep the original cultivar name and are already adapted to vineyard management practices, wine making processes as well as market demands, allowing their immediate incorporation into the stock of a cultivar for a more sustainable and higher quality viticulture (Ibáñez et al., 2015). When clones of the same variety have different enough phenotypes as to be phenotypically distinguished and can be used to elaborate different wines, they can be considered as derived varieties that could keep the name of the progenitor variety, as it is the case of Pinot Blanc or Pinot Meunier derived from Pinot Noir (Pelsy, 2010).

Because plants lack a fully segregated germline, somatic variation is an important source of genetic variation that can be transmitted through generations in these organisms. Somatic variation is particularly relevant in vegetatively multiplied as well as in woody plant species with long life cycles. Some of these species may have genotypes that survive thousands of years almost invariable and occupy large areas through vegetative reproduction. In a similar way, some grapevine cultivars, such as Tempranillo, have been multiplied over centuries and occupy areas corresponding to hundreds of thousands of hectares (Anderson and Nelgen, 2020).

The availability of the first grapevine reference genome sequence since 2007 (Jaillon et al., 2007) and the development of derived tools and new phenotypic techniques enables the identification of the origin and features of somatic mutations and the associated phenotypic variation. Through a combination of genetic and genomic strategies it is possible to identify mutations and genes responsible for somatic variation. In this way, transposable element insertions in gene regulatory regions were identified as causing unstable phenotypes in clusters and berries (Fernandez et al., 2010; 2013). Point mutations, causing amino acid changes with dominant deleterious effects on the encoded proteins were found at the origin the variant phenotypes in Pinot Meunier, the particular grape flavor in Muscat cultivars or Sultanina-derived seedlessness (Boss and Thomas, 2002; Emanuelli et al., 2010; Royo et al., 2018). As expected, most of the causal somatic mutations identified are heterozygous and have a dominant phenotype expressed in the sporophyte generation itself (Carbonell et al., 2019). Thus, in most cases somatic variants represent the emergence of new gain-of-functions that cannot be predicted from the original function assigned to the affected genes. These dominant traits are of particular scientific interest in breeding and allow the identification of new gene functions. Somatic mutations with recessive effects can also be at the origin of phenotype variation when mutations affect the functional allele of heterozygous loci for a null allele. Examples of the later are deletions of the functional allele in the color locus causing loss of berry color, as analyzed in different cultivars (Carbonell-Bejerano et al., 2017).

More recently, the development of long-read sequencing technologies such as PacBio is facilitating the release of haplotype-resolved genome assemblies, which now are available for grapevine cultivars as Cabernet Sauvignon, Chardonnay and Zinfandel (Chin et al., 2016; Roach et al., 2018; Vondras et al., 2019). This information can be extremely useful for the identification of the nucleotide diversity underlying variation for relevant phenotypic traits. However, it is not yet clear the amount of genetic variation present in those specific cultivars given the different analyses used in variant calling, as well as the number of accessions analyzed and their different genetic divergence.

In order to describe the value of somatic variation in the innovation of traditional cultivars we will focus on the presentation of a successful case in Tempranillo (TT) cultivar, giving rise to the new variety Tempranillo Blanco (TB). We describe the history of this variety, its particular features, its genetic origin, as well as all the information generated by its genome sequence that also helps understanding possible variety related viticultural issues. Finally, we will shortly mention other Tempranillo variants under study that could be the base to generate new Tempranillo Gris or Tempranillo Blanco clones and cultivars.

History of Tempranillo Blanco and its success factors

In traditional viticulture it is not frequent to witness the birth of a new variety. Getting into production a new wine variety requires many years of selection, multiplication and viticultural and oenological assays that must culminate with its varietal registration and with the authorization for cultivation by the corresponding Regulatory Councils in areas with designation of origin. Once in the market, still many years will pass before its cultivation spreads and its production reaches a sufficient level that permit its wines to be visible and accepted and create a market demand. From this perspective, the Tempranillo Blanco variety is a newcomer to the world of wine. It is now more than thirty years since in a vineyard, about to be uprooted, the owner detected a Tempranillo vine in which one of the canes carried a cluster of white grapes (Figure 1). This phenomenon did not go unnoticed to the vine grower and to researchers of the former Centre for Agricultural Research and Development (CIDA) of the Government of La Rioja with whom he contacted, and who sensed the opportunity to expand the spectrum of white varieties of the DOCa Rioja.

Figure 1. Original Tempranillo vine in which variation for berry color was observed.

Still, it would take twenty years of sanitary tests, multiplication of the material, cultivation trials in different locations, vinifications and comparisons with other white varieties, for TB to be registered as a commercial variety in 2005 and to be authorized in the DOCa Rioja as a permitted variety in 2009. Eleven years later, TB has become the second white variety of the DOCa Rioja in terms of cultivation area, with more than 700 ha.

The rapid increase in the surface cultivated with TB shows the wide acceptance that the variety has had among vine growers and Rioja wineries. This could be related with the fact that it carries a varietal name associated with quality what makes it easily recognizable in the wine market. In addition, as a variety derived from TT, it shares many morphological and development characteristics, responses to pests and diseases, phenological behavior and adaptation to the cultivation conditions of the DOCa Rioja, which altogether facilitate its cultivation in conditions similar to those used for TT. In addition, TB appeared at a time when the international wine markets demanded more white wines, a segment of wines in which DOCa Rioja needed to increase its offer, with differentiating and typical elements.

Growth and quality features of Tempranillo Blanco

Apart from berry color, TB shows some significant morphological differences with respect to its red parent, which are worth to mention, particularly regarding to its reproductive development (Martínez et al., 2017). TB bunches are smaller, looser and shorter and with less presence of shoulders than TT bunches; its berries are smaller on average and with fewer seeds, although seeds can be larger (Figure 2). These characteristics usually result in lower bunch and berry weight values in the order of 180 g and 1.7 g respectively, reaching average yield levels of around 3.3 kg / plant.

TB ripens early, which allows it to properly complete the reproductive cycle even in cold areas, showing potential adaptation to a wide range of production areas. On the other hand, this feature makes necessary to carefully monitor the ripening process in hot years or in warmer areas, in order to properly choose the harvest date, avoiding an excessive concentration of sugars. Regardless a high alcohol degree, it is generally balanced by the high acidity of this variety. In certain seasons, TB may present cluster millerandage, limiting the expected yield. This response has been associated with low pollen fertility that leads to poor fruit set in interaction with unfavorable climatic conditions for pollination and fertilization (generous rainfall, high humidity and low temperatures) around the flowering period (Carbonell-Bejerano et al., in preparation).

An important feature of TB is its great oenological potential for the production of white wines. It is a balanced variety in alcoholic strength, acidity and polyphenolic content (Martínez et al. 2017). The sensory analysis of its wines shows high quality organoleptic characteristics. Its color is straw yellow-greenish of medium intensity, and it presents a high aromatic potential, with fruity notes of apple, pear, banana, pineapple and citrus, and with intense and characteristic floral aromas. Its palate is balanced, structured and persistent, offering many possibilities in terms of diversification of wines

Figure 2. **Clusters of Tempranillo and Tempranillo Blanco.**

Genetic origin of Tempranillo Blanco and phenotypic consequences

White berry variants have been described in several traditional red varieties and in some cases they have led to the development of interesting white varieties. Well known examples are Pinot Blanc and Pinot Gris derived from Pinot Noir or Garnacha Blanca and Carignan Blanc respectively derived from Garnacha Tinta and Carignan Noir. While it is not known when color variation first appeared, white-berried somatic variants got attention in the 19th century and Garnacha Blanca, Pinot Blanc and Carignan Blanc derived cultivars were first mentioned, respectively, in the years 1865, 1895 and 1900 (Castellet, 1865; Galet, 2000).

Grapevine berry color is determined by a major locus on linkage group 2 (Doligez et al., 2006) co-localizing with a cluster of tandemly repeated *VviMybA* genes encoding MYBA transcription factors (Wong et al., 2016). Among them, factors encoded by two genes, *VviMybA1* and *VviMybA2*, are required to positively regulate anthocyanin biosynthesis (Walker et al., 2007). Red-berried cultivars carry at least one functional copy of both *VviMybA1* and *VviMybA2* linked into a functional allele of the color locus while white-berried cultivars lack functional copies of those *MybA* genes at the color locus on linkage group 2. White-berried cultivars are mostly homozygous for the canonical null allele of the locus, which carries a *Gret1* retrotransposon insertion in the promoter of *VviMybA1*, preventing its expression, along with a small INDEL, causing a frame-shift, in *VviMybA2* (Kobayashi et al., 2004; Walker et al., 2007). Most of the diversity in berry color observed among grapevine varieties and their somatic variants has been related to nucleotide sequence variation in this locus (Fournier-Level et al., 2009).

As mentioned above, red-berried varieties heterozygous for the null color allele can occasionally generate grape color somatic variation with bunches bearing either grey or white berries. Molecular characterization of grey and white berry somatic variants of Cabernet Sauvignon and Pinot Noir varieties through Southern blot analyses showed that loss of berry anthocyanin accumulation capacity was associated to the deletion of the functional allele of the color locus (Walker et al., 2006; Yakushiji et al., 2006). The grey or white berry color depends on whether deletions are only present in the L2 or in both L1 and L2 meristem cell layers, respectively (Vezzulli et al., 2012; Walker et al., 2006; Furiya et al., 2009; Migliaro et al., 2014; Pelsy et al., 2015). Given the effect of these deletions on genotypic marks, the loss of heterozygosity for SSR and SNP molecular markers along the colour locus has been used to detect them and size their length in different berry colour variants from different varieties (Vezzulli et al., 2012; Migliaro et al., 2014; Pelsy et al., 2015).

In the case of Tempranillo Blanco, we used a whole genome sequencing (WGS) strategy comparing the genomes of Tempranillo and Tempranillo Blanco to characterize the process that gave rise to the loss of anthocyanins in Tempranillo. Loss of heterozygosity directly detected at the color locus sequence was related to spontaneous deletions involving the functional color locus allele and resulting in hemizygosity for the null allele at the grape color locus (Carbonell-Bejerano et al., 2017). However, in this case, WGS uncovered a catastrophic genome rearrangement in Tempranillo Blanco involving linkage groups 2 and 5. This event caused the hemizygous deletion of 313 genes that are present in the region of the *MybA* genes mentioned above as well as in other interspersed regions within the right arm of both linkage groups. In addition, this event generated a heterozygous translocation between both linkage groups as well as an inversion on linkage group 2.

This information has been useful to better understand the phenotype of Tempranillo Blanco and to propose strategies for its improvement. In this way, from an applied point of view, the reorganization of the genetic material observed in Tempranillo Blanco could cause a reduction in gamete viability and affect the ability to set fruit of this variety. In fact, the study of the viability of pollen grains in Tempranillo and in Tempranillo Blanco indicates that it decreases from 80-90% in the red variety to 25% in the white one (Figure 3).

In those seasons and areas in which flowering occurs under favorable meteorological conditions such as low relative humidity and high temperatures, this percentage of viable gametes is sufficient to ensure a normal fruit set, although still producing a low number of seeds per berry. Appearance of millerandage and fruit set problems is directly related to relative humidity during the flower opening week and inversely related to the temperature, indicating that the lower viability of the gametes limits fruit set under adverse weather conditions.

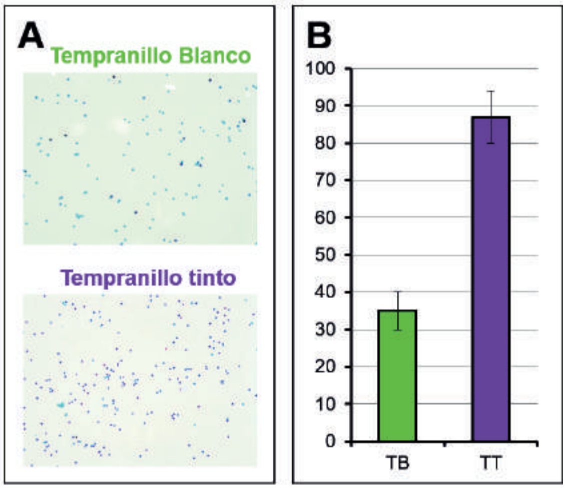


Figure 3. Pollen viability in Tempranillo Blanco. A: Alexander staining of pollen grains. Viable pollen grains are stained dark blue and non-viable ones light blue. A higher proportion of unviable pollen grains is observed in TB compared to the original TT. B: Quantification of pollen viability in samples collected at La Grajera experimental farm in 2014.

Further metabolomic and transcriptomic studies also indicated that the absence of skin anthocyanins changes the microclimate of the berry in terms of light irradiation and diurnal temperature. The new conditions favor the accumulation of light alternative protectant molecules such as flavonols, carotenoids and their derived metabolites as well as free and glycosylated terpenes, many of them are important aroma and aromatic precursor compounds that would be responsible for the new floral and fruity features of TB (Rodriguez-Lorenzo et al., in preparation). These profiles have also been shown to be characteristic of white varieties (Rambla et al., 2016; Massonnet et al., 2017) and could be more related to the new features of white berries than to the genetic constitution of white cultivars.

Further improvement of color variants in Tempranillo

Altogether studies on TB have shown its great oenological potential to contribute to the differentiation and personality of DOCa Rioja white wines. Nevertheless, its productive potential could be optimized by reducing its susceptibility to millerandage. With this goal, we have screened Tempranillo vineyards in Rioja for additional white or grey variants that could carry independent deletion events to the one characterized and without reduction of pollen viability. None of the three putative Tempranillo vines with white berries detected were found to be real TB plants. Their molecular markers genotype was not coincident to that of Tempranillo variety, either because they were segregating white berry plants from self-pollination of Tempranillo or because they corresponded to other varietal genotypes. However, we collected more than 12 Tempranillo grey variants that appeared spontaneously in vineyards. Using a SNP genotyping chip specifically designed for the detection of deletions along linkage group 2 in Tempranillo we found that they correspond to at least four independent deletion events that in all cases overlapped with the *VviMybA2-VviMybA1* chromosomal region. Selected Tempranillo grey lines carrying shorter deletions are currently being used for the regeneration of white-berried plants from the L2 cell layer through somatic embryogenesis (Acanda et al., 2013). These genotyping datasets can further be used for the clonal selection of Tempranillo Gris lines and varieties. In the course of those screens we also identified a somatic variant named as "black" Tempranillo displaying an altered anthocyanin profile in the berry skin and anthocyanin accumulation in the seeds, which can contribute to increased color intensity and phenolic content of Tempranillo wines.

In conclusion, these results exemplify the potential contribution of somatic variation to the improvement and innovation of traditional cultivars. Understanding how this somatic variation originates also provides tools for genetics-assisted tracking of selected variants and breeding and could help to direct genome editing approaches to improve those cultivars. Finally, molecular characterization of vine somatic variants also generates basic information useful to understand gene biological function in grapevine.

References

Acanda, Y, Prado, MJ, González, MV, Rey, M. 2013. Somatic embryogenesis from stamen filaments in grapevine (*Vitis vinifera* L. cv. Mencía): changes in ploidy level and nuclear DNA content. *In Vitro Cell Developmental Biology-Plant* **49**: 276-284.

Anderson, K., Nelgen, S. 2020. Which Winegrape Varieties Are Grown Where? Adelaide University Press, Adelaide, Australia. 794 p.

Boss, P.K, Thomas, M.R. 2002. Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* 2002; **416**, 6863, 847–850.

Castellet, B. 1865. Enología española. Imprenta de Gómez e Inglada, Barcelona.

Carbonell-Bejerano, P., Royo, C. Torres-Perez, R., Grimplet, J., Fernandez, L., Franco-Zorrilla, J.M., Lijavetzky, D., Baroja, E., Martinez, J., Garcia-Escudero, E., Ibáñez, J. Martinez-Zapater, J.M. 2017. Catastrophic Unbalanced Genome Rearrangements Cause Somatic Loss of Berry Color in Grapevine. *Plant Physiology* **175**, 2, 786-801.

Carbonell-Bejerano, P., Royo, C., Mauri, N., Ibáñez, J., Martínez Zapater, J.M. 2019. Somatic variation and cultivar innovation in grapevine. In Advances in Grape and Wine Biotechnology; IntechOpen, pp 1-22.

Chin, C.S., Peluso, P., Sedlazeck, F.J., Nattestad, M., Concepcion, G.T., Clum, A., Dunn, C., O'Malley, R., Figueroa-Balderas, R., Morales-Cruz, A., Cramer, G.R., Delledonne, M., Luo, C., Ecker, J.R., Cantu, D., Rank, D.R., Schatz, M. C. 2016. Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*, **1,** 1050-1054.

Doligez, A., Adam-Blondon, A.F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglu, D., Meredith, C.P., Riaz, S., Roux, C., This, P. 2006. An integrated SSR map of grapevine based on five mapping populations. *Theoretical and Applied Genetics*, **113**, 369–382.

Emanuelli, F., Battilana, J., Costantini, L., Le Cunff, L., This, P., Grando. M.S. 2010. A candidate gene association study for Muscat flavor in grapevine *Vitis vinifera* L. *BMC Plant Biology*, **10**, 241.

Fernandez, L., Torregrosa, L., Segura, V., Bouquet, A., Martinez-Zapater, J.M. 2010. Transposon-induced gene activation as a mechanism generating cluster shape somatic variation in grapevine. *The Plant Journal*, **61**, 4, 545-557.

Fernandez, L., Chaib, J., Martinez-Zapater, J.M., Thomas, M.R., Torregrosa, L. 2013. Mis-expression of a *PISTILLATA*-like MADS box gene prevents fruit development in grapevine. *The Plant Journal*, **73**, 918-928.

Fournier-Level, A., Le Cunff, L., Gomez, C., Doligez, A., Ageorges, A., Roux, C., Bertrand, Y., Souquet, J.M., Cheynier, V., This, P. 2009. Quantitative genetic bases of anthocyanin variation in grape (*Vitis vinifera* L. ssp. *sativa*) berry: A quantitative trait locus to quantitative trait nucleotide integrated study. *Genetics*, **183**, 3, 1127-1139.

Furiya, T., Suzuki, S., Sueta, T., Takayanagi, T. 2009. Molecular characterization of a bud sport of Pinot gris bearing white berries. *American Journal of Enology and Viticulture*, **60**, 66-73.

Galet, P. 2000. Dictionnaire encyclopédique des cépages. Hachette Livre. 935 pp.

Ibañez, J.., Carreño, J., Yuste, J., Martínez-Zapater, J.M. 2015. Grapevine breeding and clonal selection programs in Spain. In: Reynolds, AG, (Ed). Grapevine Breeding Programs for the Wine Industry. Elsevier, pp.183-209.

Jaillon, O, Aury, JM, Noel, B, Policriti, A, Clepet, C, et al. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, **449**, 463-467.

Kobayashi, S., Goto-Yamamoto, N., Hirochika, H. 2004. Retrotransposon-induced mutations in grape skin color. *Science*, **304**, 5673, 982.

Martínez, J., Gonzalo-Diago, A., Baroja, E., García-Escudero, E. 2017. Características agronómicas y potencial enológico de las variedades de vid blancas autorizadas en la D.O.Ca. Rioja. *Zubía*, **29**, 63-78.

Massonnet, M., Fasoli, M., Tornielli, G.B., Altieri, M., Sandri, M., Zuccolotto, P., Paci, P., Gardiman, M., Zenoni, S., Pezzotti, M. 2017. Ripening Transcriptomic Program in Red and White Grapevine Varieties Correlates with Berry Skin Anthocyanin Accumulation. *Plant Physiology*, **174**, 4, 2376–2396.

Migliaro, D., Crespan, M., Muñoz-Organero, G., Velasco, R., Moser, C., Vezzulli, S. 2014. Structural dynamics at the berry colour locus in *Vitis vinifera* L. somatic variants. *Australian Journal of Grape and Wine Research,* **20**, 3, 485-495.

Pelsy, F. 2010. Molecular and cellular mechanisms of diversity within grapevine varieties. *Heredity*, **104**, 4, 331–340.

Pelsy, F., Dumas, V., Bevilacqua, L., Hocquigny, S., Merdinoglu, D. 2015. Chromosome replacement and deletion lead to clonal polymorphism of berry color in grapevine. *PLoS Genetics*, 11, e1005081.

Rambla, J.L., Trapero-Mozos, A., Diretto, G., Rubio-Moraga, A., Granell, A., Gómez-Gómez, L., Ahrazem, O. 2016. Gene-Metabolite Networks of Volatile Metabolism in Airen and Tempranillo Grape Cultivars Revealed a Distinct Mechanism of Aroma Bouquet Production. *Frontiers in Plant Science*, **7**, 1519, 1-23.

Reynolds, A.G. (ed). 2015. Grapevine Breeding Programs for the Wine Industry. Woodhead Publishing, 446 pp.

Roach, M.J., Johnson, D.L., Bohlmann, J., van Vuuren, H.J.J., Jones, S.J.M., Pretorius, I.S., Schmidt, S.A., Borneman, A.R. 2018. Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar Chardonnay. *PLoS Genetics*, **14**, e1007807.

Royo, C., Torres-Pérez, R., Mauri, N., Diestro, N., Cabezas, J.A., Marchal, C., Lacombe, T., Ibáñez, J., Tornel, M., Carreño, J., Martínez-Zapater, J.M., Carbonell-Bejerano, P. 2018. The major origin of seedless grapes is associated with a missense mutation in the MADS-box gene *VviAGL11*. *Plant Physiology*, **177**, 1234-1253.

This, P., Lacombe, T., Thomas, M.R. 2006. Historical origins and genetic diversity of wine grapes. *Trends in Genetics*, **22**, 9, 511–519.

Vezzulli, S., Leonardelli, L., Malossini, U., Stefanini, M., Velasco, R., Moser, C. 2012. Pinot blanc and Pinot gris arose as independent somatic mutations of Pinot noir. *Journal of Experimental Botany*, **63**, 18, 6359-6369.

Vondras A.M., Minio A., Blanco-Ulate B., Figueroa-Balderas R., Penn M.A., Zhou Y., Seymour D., Ye Z., Liang D., Espinoza L.K., Anderson M.M., Walker M.A., Gaut B., Cantu D. 2019. The genomic diversification of grapevine clones. *BMC Genomics*, **20**, 1, 972.

Walker, AR, Lee, E, Robinson, SP. Two new grape cultivars, bud sports of Cabernet Sauvignon bearing pale-coloured berries, are the result of deletion of two regulatory genes of the berry colour locus. Plant Molecular Biology; 2006; 62: 623-635.

Walker, A.R., Lee, E., Bogs, J., McDavid, D.A., Thomas, M.R., Robinson, S.P. 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. *The Plant Journal*, **49**, 5, 772–785.

Wong, D.C., Schlechter, R., Vannozzi, A., Höll, J., Hmmam, I., Bogs, J., Tornielli, G.B., Castellarin, S.D., Matus, J.T. 2016. A systems-oriented analysis of the grapevine R2R3-MYB transcription factor family uncovers new insights into the regulation of stilbene accumulation. *DNA Research*, **23**, 5, 451–466.

Yakushiji, H., Kobayashi, S., Goto-Yamamoto, N., Tae Jeong, S., Sueta, T., Mitani, N., Azuma, A. 2006. A skin color mutation of grapevine, from black-skinned Pinot Noir to white-skinned Pinot Blanc, is caused by deletion of the functional *VvmybA1* allele. *Biosciences Biotechnology Biochemistry*, **70**, 6, 1506-1508.

Innovation in the wine supply chain: an integrated approach from vineyards to consumers

Alejandro Gennari [a], **Liliana Martínez** [b], **Jimena Estrella Orrego** [a], **Marcos Maza** [c]

[a] *Department of Economics, Policy and Rural Management,* [b] *Department of Biological Science,* [c] *Department of Enology and Food Science, Faculty of Agrarian Science, National University of Cuyo, Almirante Brown 500, Lujan de Cuyo, Mendoza, Argentina*

Abstract: The wine industry is a highly competitive sector with world demands that impact on its business model. Innovation is the one way to grow and gain market share. This innovation can occur in every part of the supply chain, from vineyards to consumers. The assessment of the needs for every step of the chain is the core of this paper. In vineyards, innovations include physical, biological and digital changes looking for a sustainable and productive viticulture. In wineries innovations are related to improving working conditions, reducing costs, and minimizing the environmental impact during the process of making and preserving wine. New technologies for fermentation are an essential issue in this step. For distribution and marketing, the greatest innovation deals with understanding the central role of consumers. New consumers and new requirements for existing one are defining the new scenario for the wine industry. In all, sustainability if the main driver of the industry and thus public and private actors need to work together for an adequate and comprehensive assessment.

Key words: innovation, wine, sustainability, supply chain

1.- What is innovation? Basic concepts

Productive societies that are preparing themselves for solving issues are considering innovation as the main strategy for the development of policies targeting both future and current issues. But, what is innovation and how can it help us finding solutions for our issues? Innovation is part of a major process in which creativity and the production of knowledge – both empirically and through the scientific system – are determining factors.

Joseph Schumpeter (1911), the controversial Austrian-American economist who popularized the term “creative destruction”, was the first economist to propose a conceptual system for explaining the creation, application and diffusion of knowledge (the innovation) in the capitalist productive systems. He differentiated three steps:

- invention, the discovery and understanding of a new chemical, physical or social process;
- innovation, the application of this new knowledge in productive, social and institutional systems; and
- diffusion, which is the innovation’s dissemination in the productive, social and institutional systems.

Inventions are clearly associated to knowledge and its production process. As such, inventions are especially linked to scientific, technological and academic systems that are part of the productive and social systems. On the other hand, while innovations are associated to the scientific and (especially) technological system, they depend on the social organization, the freedoms and collective attitudes towards change, success and failure, and financial and socio-economic incentives when taking risks. Thus, inventions are transversal to economic sectors, social organizations and policies. Israel is perhaps the best example of a country that presents the most favourable characteristics for innovation (Senor & Singer, 2012). This is evidenced by the great success achieved by the country's firms in diverse economic sectors such as water management, agricultural technology, biomedicine, and military technologies, among others.

Schumpeter (1911) considered different ways of innovation. For him, a new production process, the use of a new raw material, a new market, and the development of a new product, are all innovations. It is common to hear about innovation in the process, product or service; commercial innovation, which includes changes in the production, packaging and promotion of the product; and organizational innovation, which are changes in the way different players interact across the supply chain, such as vertical integration. In any case, innovations do not only depend on business, as there are also social and institutional innovations.

Diffusion – also referred as outreach – is strongly linked to the results obtained by the first adopters and the openness of societies towards change. The innovation cycle can be represented by a gaussian curve in which the first adopters are the real introducers of the innovation. Then, after looking at favourable results by those first adopters, most individuals or organisations adopt the innovation. Finally, so-called laggards are the last to adopt. This kind of cycle repeats. The final consequence of the innovation process is an improvement in the production system that is usually reflected in lower costs or more valuable products.

Innovation can be either a sudden or a continuous process. This is why it is common to hear about absolute and incremental innovations. Sometimes it is a combined process, in which an absolute innovation is followed by incremental innovations, and then by objectives and subjective differentiations. The Organization for Economic Co-operation and Development (OECD) Manual of Oslo (1997) represented an inflexion point in the systematization of the innovation knowledge and its promotion policies.

The adoption of an innovation depends on the cost-benefit and risk perception it may have. This is key to understand why some innovations are quickly adopted, while others just stay in its trial step. Some of the innovations that are currently under development – or that may be developed in the future – in the wine supply chain are explained in the following sections.

Innovations are usually motivated by key drivers. Some of the main drivers that motivate innovation in the wine supply chain are: a higher purchasing power for most worlds' consumers; a substantial increase in the population of Asia, Africa and Latin America; a decrease in the rural population favouring cities in coastal areas; premmiunization of consumption; and climate change and its consequences.

2.- Innovation in the vineyards

Like other sectors, viticulture is under constant strain and must deal with challenges in the medium and long term. One of them is environmental sustainability in a context of climate change that in a few years will affect the balance between the area of production and grape varieties and will change the impact of pests and diseases in vineyards. Growing conditions are predicted to change with higher average temperatures, water scarcity, and more pressure on land use from a growing population. Winegrowers will have to respect the environment, while competing with other new producing countries on a more globalized market. All these aspects represent a risk to the wine sector (Fraga et al., 2018). As a result, winegrowers will need to manage resources much more efficiently without comprising wine quality, knowing that wine consumers are increasingly aware of the environmental impact of viticulture (Costa et al., 2016; Martins et al., 2018).

As a consequence, it is important to identify and apply sustainable practices that help the sector mitigate and adapt to new scenarios and technologies. The introduction of new technologies for supporting vineyard management allows the efficiency and quality of production to be improved and, at the same time, reduce the environmental impact.

Viticulture should not be left out of the cutting-edge technology, such as Agriculture 4.0. The rapid evolution of information, communication technologies, and geographical science offers an enormous potential for the development of optimized solutions for distributed information for precision viticulture. Recent technological developments have allowed useful tools to be elaborated that help in the monitoring and control of many aspects of vine growth. Physical, biological, and digital innovations resulting from the exponential changing world allow reaching a sustainable and productive viticulture. Among physical innovation, precision viticulture seeks to exploit the widest range of available observations to describe the vineyard spatial variability with high resolution, and to provide recommendations for improving management efficiency in terms of quality, production, and sustainability.

Monitoring technologies are the basis of mapping spatial variability and consist in the acquisition of the maximum amount of georeferenced information within the vineyard. Wide ranges of sensors aiming to monitor different parameters that characterize the plant growth environment are employed in precision viticulture for remote and proximal monitoring of geolocated data.

Georeferencing is the process of establishing the relationship between spatial information and its geographical position. This technology allows to monitor phytosanitary, vegetative and productive situations, making it possible to all the players in the wine supply chain to have vineyard data available and the statistic in real time. Furthermore, it is useful in performing tasks requiring high precision, such as crop mapping, automatically driven farm vehicles, soil sampling, and distribution of fertilizers and pesticides at variable rates.

On the other hand, remote sensing techniques rapidly provide a description of grapevine shape, size, and vigor and allow assessment of the variability within the vineyard. This is image acquisition at a distance with different scales of resolution, able to describe the vineyard by detecting and recording sunlight reflected from the surface of objects on the ground (Hall et al., 2002). Remotely sensed data allows the plant physiology to be described by means of vegetation indices calculation, such as the well-known normalized difference vegetation index (NDVI). The three platforms mainly used in remote sensing are satellites, aircraft, and unmanned aerial vehicles (UAVs) with different application methods and types of sensors. The spatial resolution of satellite imaging systems has improved from 80 m with Landsat satellite to sub-meter resolution with GeoEye and WorldView, and the frequency has improved from 18 days to 1 day with new satellite platforms, with significant advances in sensor performances. The latest satellite, WorldView 3 is even capable of providing resolutions of 0.30 m in visible spectra, 1.30 m in multispectral, and 3.70 m in short-wave infrared, with a revisit frequency between 1 and 4 days.

The use of satellites in remote sensing therefore has great potential, but the spatial resolutions are not sufficient for precision viticulture due to the narrow vine spacing. Another limitation is the temporal resolution, and cloud cover that can occur at the time the satellite passes (Marçal et al 2007a, Marçal et al 2007b). Aircrafts allow ground monitoring with wide flight range and high payload in terms of weight and dimensions, thus providing the ability to manage a large number of sensors. The aircraft bypasses some limitations of the satellite application by programming the image time acquisition and providing higher ground resolution, depending on the flying altitude. However, the reduced flexibility of the time acquisition, due to the rigid schedule of flight planning and high operational costs, makes it economically viable only on areas of more than 10 ha. A new solution for remote monitoring, UAVs have fixed or rotary wing platforms capable of flying autonomously. These platforms can be equipped with a series of sensors, which allow a wide range of monitoring operations to be performed. The peculiarity of UAV application in remote sensing is the high spatial ground resolution (centimeters), and the possibility of highly flexible and timely monitoring, due to reduced planning time. These features make it ideal in vineyards of medium to small size (1–10 ha), especially in areas characterized by high fragmentation due to elevated heterogeneity. Despite these positive aspects, UAV platforms have an important limitation in terms of payload weight and operating times.

Within proximal sensing applications, there are many tools available for continuous measurements carried by moving vehicles, or instruments for precise ground observations made by an operator. The primary application of Wireless sensor network (WSNs) is the acquisition of micrometeorological parameters at vine canopy and soil level. In the last decade, the continuous innovation process has allowed the development of new kinds of sensors for plant physiology monitoring, such as dendrometers and sap-flow sensors, for the continuous measurement of plant water status for irrigation scheduling. The soil proprieties play an important role in vine growing, so knowing the spatial variability of soil characteristics within a vineyard allows improved understanding of vine physiological response variability.
Many systems have been developed for monitoring vineyards, which provide a high-resolution screening of the canopy side across the row coupled with a GPS system for data georeferencing, such as GrapeSense, GreenSeeker® , which supply information for vegetation indexes calculation. These sensors are mounted on machines and tractors allowing the acquisition of spatial data during the daily vineyard management. Thanks to these proximal monitoring systems, it becomes possible to analyze the spatial variability with higher resolution than provided remotely.

There are many systems to obtain georeferenced yield information, especially integrated on mechanical harvesters, such as HarvestMaster Sensor System HM570, Canlink Grape Yield Monitor 3000GRM, and Advanced Technology and Viticulture. These tools give the farmer the ability to map the vineyard productivity with a resolution never previously achieved. The yield maps realized with these sensors represent an excellent tool to verify the effectiveness of management practices applied in the vineyard.

The use of robotics in precision viticulture is still at a prototype stage. Nevertheless, many projects are already in the final stage of development, and some have already been put on the market.

To sum up, there are issues to overcome before widespread adoption of these technologies can take place, which are related not only to the need to further explore the potential of these tools, but above all to the ability of farms to train technicians capable to understand and properly use this type of technology.

Biological innovation implies the use of biotechnology which covers a very large number of applications to multiply or improve sanitary or genetic of grapevine varieties. Some of these technologies are now so integrated in the selection of rootstock and scion varieties that they are no longer debatable (Dalla Costa et al., 2019). Thus, without the use of apex culture or micrografting, that makes possible the elimination of pathogenic viruses or bacteria, it would not be possible to provide the industry with healthy clonal material. In terms of varietal innovation, the use of embryo rescue is widespread in the breeding of seedless table grapes, leading to a very innovative range of varieties (Dalla Costa et al., 2019). The latest biotechnologies, genetic transformation developed in the 1990s, and genome editing, also called NBTs (*new breeding technologies*), still under development, allow targeted modifications of the genome without deconstructs the phenotype of existing cultivars, such as elite cultivars sought-after by the wine market, NBTs technology has been successfully applied to generate edited grapevine plants. Two opposite attitudes towards green biotechnologies coexist: one is focused on the final product while the other pays more attention to the process through which a specific product has been generated. The first approach is followed by USA, Argentina, Australia and Brazil which have established that if no foreign genes or genetic material is present in a genome-edited variety, then it will not be subject to additional regulatory oversight and risk assessment as in the case of GMO. The second approach has been historically adopted by Europe, where, on 25th July 2018, the Court of Justice of the European Union ruled that organisms obtained by mutagenesis (including genome editing) are GMO within the scope of the GMO European Directive 2001/18/EC (Dalla Costa et al., 2019).

Finally, digital innovations consist in the use of Internet of Things (IoT), 5G technology, artificial intelligence, blockchain, and smart contract.

In conclusion, in recent years, one of the innovations in viticulture, the so-called precision viticulture has had a rapid development and greater applicability due to lower costs, ease of use, and versatility. In general, the application advantage of these innovative solutions is a cost reduction in crop management, through improving crop quality and yield production, process traceability and environmental sustainability with a rational use of chemical inputs. In terms of grapevine varieties breeding able to both limit inputs (e.g. pesticides, water, minerals) and cope with climate changes and abiotic stresses, NBTs will require significant technical progress and genetic knowledge to replace or complement classical breeding approaches.

3.- Innovation in the wineries

In recent decades, several research projects have focused not only on improving the quality of grapes and wine, but also on improving working conditions, reducing costs, and minimizing the environmental impact during the process of making and preserving wine. Nowadays, these are some of the main objectives of wine-related firms, which are forced to continuously improve their quality standards and to incorporate new processes in their manufacturing and commercial processes.

The main stage in winemaking takes place during wine fermentation. This is the most critical stage as it has crucial implications on the quality of the obtained wine. Innovations, such as “flash-detente” or “flash-release”, were introduced as pre-fermentation technologies more than two decades ago (Escudier et al., 1993), replacing the classic thermo-maceration process in most of the greatest wineries of the world. It is a variant of the thermo-vinification process, invented in France and patented in 1993 by the French National Institute for Agricultural Research (INRA). However, these technologies consume large amounts of energy and labour. It is estimated that approximately 64.3% of the total energy needed to produce a litre of wine is consumed during the maceration-fermentation stage (Genc et al., 2017). Based on this estimation, recent research seeks to establish different innovative technologies capable of enhancing the processes of extraction of phenolic compounds while reducing energy consumption at the same time.

The incorporation of non-thermal technologies such as Pulsed Electric Fields (PEF) (Maza et al., 2020; Puértolas et al., 2010), and ultrasound (US) (Bautista-Ortín et al., 2017; Ghafoor & Choi, 2009), have been recently studied with the aim of improving the process of extraction of polyphenolic compounds from grape skins in the pre-fermentation stage.

PEF technology has been tested at a semi-industrial scale (Maza et al., 2019). This technique consists in applying a potential difference intermittently and with a duration of one millionth of a second (μs), between two electrodes to the destemmed grape mass, causing an alteration in the permeability of the cell membrane (Cholet & Darné, 2004), favouring the extraction of intracellular polyphenolic compounds. The implementation of PEF in grapes improves the speed of extraction and shortens the maceration time (Maza et al., 2019). As such, wines with higher polyphenol contents are obtained even in shorter macerations. This reduces operating costs and energy use.

US technology, also known as “ultrasonic” acoustic energy, has been used in several industrial sectors for more than 50 years. However, its application in food processing is relatively new. Ultrasound is widely used in the food industry and is considered an innovative technology in the treatment of food products due to constant advances in research and development.

The technique consists in applying high intensity ultrasound through a liquid, which produces a phenomenon known as acoustic cavitation. Cavitation is responsible for increasing the transference of mass or the rupture of cells of microorganisms or of plant or animal tissues (Cravotto & Cintas, 2006). In this sense, the ultrasound applied to the grape after destemming causes a cell rupture due to the cavitation phenomenon and favours the extraction of phenolic compounds (González-Centeno et al., 2014).

Experiments have demonstrated the efficiency of this technique by obtaining wines with a higher concentration of polyphenols when grapes have been treated with ultrasound (Bautista-Ortín et al., 2017). This innovative technique has recently been approved by the OIV for use in the wine industry (OIV Res-616, 2019). Both innovative techniques are in evaluation stages in industrial implementation and have low energy consumption, which transforms them into an alternative to the pre-fermentative maceration techniques that are currently used (Maza et al., 2019).

4.- The role of consumers in marketing and distribution innovations

New consumers and new expectations from present consumers are defining a new scenario for all agricultural products. New markets are explained by demographic growth, growing urbanization, and increasing economic wealth. These three factors define the number and location of consumers, shaping distribution strategies. The growing population in Asia, with its increasing per capita income and higher concentration in urban areas, is one of the depicting characteristics of the current global scenario.

In this context, there is a need for improved (or even new) distribution strategies. The main trends refer to increasing concentration, growth of private label products, and new distribution schemes. With increasing concentration, the challenge for wineries is related to their ability to generate collaborative schemes for quality certification, shipping, customs, and promotional budget, among others. Regarding the growth of private label wines, the big challenge refers to the wineries ability to generate brand loyalty.

In new distribution schemes, innovation goes hand by hand with the combination of distribution channels. Omnichannel distribution refers to the multi-channel approach where a company intends to provide the customer an integrated experience. E-commerce combined with supermarkets, as Alibaba does, or Amazon integrating with Whole Foods, are clear examples of this type of strategy.

New expectations or requirements are mainly explained by four drivers: environmental awareness (highly motivated by a rising consumer interest on mitigating climate change), health, convenience, and new identity needs (Euromonitor, 2020). These four drivers are related and conditioned by a hyper connectivity trend, as a sharp increase in the interconnectedness of people and organizations.

Environmental awareness is reflected on the type of products consumers are willing to buy, and on the way these products are used or consumed. The weight of a wine bottle and its closing system are key factors defining consumers' willingness to pay. The water print and the carbon footprint can be useful for promoting the consumption of more environmentally friendly products. In many cases, however, these attributes are used as non-tariff barriers. Some wineries and countries should innovate on their communication strategies regarding the impact of their supply chain on the environment.

Regarding health, obesity is often considered the number one factor influencing consumption of most foods and beverages. For wine, same as for other alcoholic beverages, the impact and social consequences of excessive alcohol consumption is perhaps the main concern. The industry can help on this regard by lowering the percentage of alcohol on its products, or better, by promoting a moderate consumption. The European initiative "Wine in Moderation" is a clear example of an innovative effort to address excessive alcohol consumption.

Convenience was first defined by the wine price-quality relation, but the concept is now more broaden. It includes reduced purchasing time, the option of storing the products in smaller spaces, packaging containing lower quantities, and the possibility of buying other types of solutions.

New identity needs means consumers are changing the values they look for in companies and products. The increasing importance of geographical indications all over de world is a clear example of new attributes for which consumers are willing to pay (Menapace et al, 2011). The value of terroir and a region reputation in terms of history and quality often justify higher prices form a consumer point of view. Based on their perceptions and criteria, consumers are willing to pay more for products, brands, and the place in which products are made (Defrancesco et al, 2012)

The great impact of premmiunization of wine has led to a considerable differentiation in regions. Wine tourism has played a huge role on this process, shaping individual business strategies and even collective actions. Regions compete in order to sell more wine, at a higher price, while increasing their number of tourists. Hence, adding value to the wine supply chain. This scope is linked to the main components of sustainable development, which are environment (e.g. water, energy), economy, and society (Gennari et al, 2019).

Besides being segmented by more classical criteria such as their place of residence, gender, age, and other socio-economic characteristics, consumers can be segmented based on other types of characteristics and interests, including environmental demands, dietary requirements and preferences, and ways of living. These market segments have led to a supply of more varied and innovative products, including those for niche markets such as orange wine, natural wine, heroic wine, volcanic wine, and sole wine, among others (Steiner, 2002)
Further, wine has been paired with cultural activities. Examples of this pairings include wine and rock, wine and classic music, wine and yoga, wine and palaeontology, wine and arts, and wine and biodiversity conservation. Wine has also been paired with sports such as polo, golf, and football, and firms tend to promote their wines based on the sport that more closely relates to their targeted market segment.

Finally, wine and its differentiated regions have led to a fundamental association between wine and gastronomy (Schamel, 2006). In many regions, culture is very linked with wine, allowing the wine sector innovations to have an advantage when compared to beer, craft beer, and sider, among other beverages. Cocktails play an important role too, and not only still wine is used, but also sparkling wine and spirits made out of wine. These kinds of spirits are considered as a rediscovery in many countries, based on the increase in their production and commercialization.
Wine also plays an important role in cities that are not close to areas in which higher-quality wine is produced, such as London, New York, and Buenos Aires. The importance of wine in these cities has even forced a link between wine and tourism, such as the so-called Malbec wine path, that links wine bars in Buenos Aires. While historically the New World wine countries have been considered from a supply point of view, there is an increasing focus on New World wine countries from a demand point of view (Cardebat, 2015).

5.- Conclusions

Innovation is among the main instruments for public policies and business strategies oriented towards facing short- and long-run challenges in a wide variety of settings including the wine supply chain. The world is becoming more populated, and its population is becoming wealthier and is migrating to cities. In this context, the world's population is increasing its consumption, and this has a consequence in climate change. Satisfying population demands while mitigating the notorious effects of climate change is an imminent challenge that requires a great deal of innovation.

The agricultural sector is one of the most affected sectors by the negative consequences of climate change (Nordhaus, 2019). And, at the same time, is an activity that contaminates. As such, agricultural systems must change in order to reduce the negative consequences of climate change, simplified by greenhouse gases and global warming.

In order to quickly reduce its carbon emissions, the agricultural sector should focus (whenever possible) on carbon-positive processes (those that fix rather than emit carbon), and in a more conscious consumption of resources. Reducing agricultural emissions is hard in a global context in which there is no global authority able to agree goals and achieve them. Nevertheless, the wine industry is on its process of reducing its emissions.
Besides the willingness of some firms to be more environmentally friendly, or the demand of their costumers, the wine sector is motivated by the need of reducing the use of its resources, such as water and energy. Further, the winemaking process is favouring a more circular economy, as residues and water are often recycled.
Achieving sustainability is challenging based on what the concept implies (profitability, social welfare, and a preserved or improved environment for future generations). It is even more challenging if other two variables related to sustainability, cultural preservation and governability, are considered (Nilipour, 2020). However, with adequate incentives and public policies, integrating the wine supply chain actors, sustainability can be achieved.

7.- Bibliography

Bautista-Ortín, A. B., Jiménez-Martínez, M. D., Jurado, R., Iniesta, J. A., Terrades, S., Andrés, A., & Gómez-Plaza, E. (2017). Application of high-power ultrasounds during red wine vinification. International Journal of Food Science & Technology, 52(6), 1314–1323. https://doi.org/10.1111/ijfs.13411

Cardebat, J. M. (2017). Economie du vin. Editions La Decouverte, Paris.

Cholet, C., & Darné, G. (2004). Evolution of the contents in soluble phenolic compounds, in proanthocyanic tanins and in anthocyanins of shot grape berries of *Vitis vinifera L.* during their development. Journal international des sciences de la vigne et du vin, 38(3), 171–180.

Costa, J. M., Vaz, M., Escalona, J., Egipto, R., Lopes, C., Medrano, H. & Chaves, M. M. 2016. Modern viticulture in southern Europe: vulnerabilities and strategies for adaptation to wáter scarcity. Agr. Water Manage. 164, 5–18.

Cravotto, G., & Cintas, P. (2006). Power ultrasound in organic synthesis: Moving cavitational chemistry from academia to innovative and large-scale applications. Chem. Soc. Rev., 35(2), 180–196. https://doi.org/10.1039/B503848K

Dalla Costa L., Malnoy M., Lecourieux D. , Deluc L, Ouaked-Lecouriez F., Thomas M., Torregrossa L. 2019. The state-of-the-art of grapevine biotechnology and new breeding technologies (NBTS). OENO One Journal. Vol 53 (2).

Defrancesco, E., A. Gennari & Estrella Orrego M. J. (2012). Would New World Wines Benefit from Protected Geographical Indications in International Markets? The case of Argentinean Malbec. Wine Economics and Policy, Vol. 1 N° 1, 2012, p.63 -72.

Escudier, J. L., Moutounet, M., & Cogat, P. O. (1993). Produit alimentaire, obtention et application à la fabrication de jus de fruits ou de vins. French Patent, 9313287.

Euromonitor “Top 10 Global Consumer Trends 2020, How to target consumers and build a competitive business strategy”.

Fraga, H., García de Cortázar Atauri, I. & Santos, J. A. 2018. Viticultural irrigation demands under climate change scenarios in Portugal. Agric. Water Man. 196, 66–74.

Genc, M., Genc, S., & Goksungur, Y. (2017). Exergy analysis of wine production: Red wine production process as a case study. Applied Thermal Engineering, 117, 511–521. https://doi.org/10.1016/j.applthermaleng.2017.02.009

Gennari, A., J. Estrella Orrego, L. Santoni y S. Riera (2019). El nexus agua, energía y bioeconomía. Modelos estratégicos de desarrollo. En II Congreso Agua, Energía, Ambiente. Montevideo, Asociación Universidades Grupo Montevideo, 27 al 29 setiembre 2019.

Ghafoor, K., & Choi, Y. H. (2009). Optimization of Ultrasound Assisted Extraction of Phenolic Compounds and Antioxidants from Grape Peel through Response Surface Methodology. Journal of the Korean Society for Applied Biological Chemistry, 52(3), 295–300. https://doi.org/10.3839/jksabc.2009.052

González-Centeno, M. R., Knoerzer, K., Sabarez, H., Simal, S., Rosselló, C., & Femenia, A. (2014). Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera L.*) – A response surface approach. Ultrasonics Sonochemistry, 21(6), 2176–2184. https://doi.org/10.1016/j.ultsonch.2014.01.021

Hall A, Lamb DW, Holzapfel B, Louis J. 2002. Optical remote sensing applications in viticulture – a review. *Australian Journal of Grape and Wine Research* 8, 36–47.

Marçal A.R.S., Cunha M. 2007a.Vineyard monitoring in Portugal using multi-sensor satellite images. In: Gomarasca MA, editor. *Proceedings of the 27th EARSeL Symposium*, *Geoinformation in Europe*. Rotterdam Millpress, 327–335.

Marçal ARS, Gonçalves JA, Gonçalves H, Cunha M. 2007b. Analysis of the temporal signature of vineyards in Portugal using vegetation. In: Bochenek Z, editor. *Proceedings of the 26th EARSeL Symposium*, *New Developments and Challenges in Remote Sensing*. Rotterdam: Millpress, 377–384.

Martins, A. A., Araújo, A. R., Graça, A., Caetano, N. S. & Mata, T. M. 2018. Towards sustainable wine: comparison of two Portuguese wines. J. Clean Prod. 183, 662–676.

Maza, M. A., Martínez, J. M., Delso, C., Camargo, A., Raso, J., & Álvarez, I. (2020). PEF-dependency on polyphenol extraction during maceration/fermentation of Grenache grapes. Innovative Food Science & Emerging Technologies, 60, 102303. https://doi.org/10.1016/j.ifset.2020.102303

Maza, M. A., Martínez, J. M., Hernández-Orte, P., Cebrián, G., Sánchez-Gimeno, A. C., Álvarez, I., & Raso, J. (2019). Influence of pulsed electric fields on aroma and polyphenolic compounds of Garnacha wine. Food and Bioproducts Processing, 116, 249–257. https://doi.org/10.1016/j.fbp.2019.06.005

Maza, M., Álvarez, I., & Raso, J. (2019). Thermal and Non-Thermal Physical Methods for Improving Polyphenol Extraction in Red Winemaking. Beverages, 5(3), 47. https://doi.org/10.3390/beverages5030047

Menapace, L. & G. Moschini (2011). Quality certification by geographival indications, trademarks and firm reputation. European Review of Agricultural Economics, 17, 1- 28.

Nilipour, Azadeh (2020). Introduction to Social Sustainability, in Social Sustainability in the Global Wine Industry. Concepts and Cases. Switzerland, Palgrave Macmillan, p. 1 – 14.

Nishitani C., Osakabe K., Wada M., Komori S., Malnoy M., Velasco R., Poli M., Jung M.H., Koo O.J., Viola R. and Kanchiswamy C.N. 2018. CRISPR–Cas9-mediated genome editing in apple and grapevine. Nat. Protoc., 13, 2844-2863.

Nordhaus, William (2019). Le Casino Climatique. Risques, incertitudes et solutions économiques face á un monde en réchauffement. De Boeck Supérieur SA. 352 p. 2019.

OCDE (Organización para el Desarrollo y la Cooperación Económica), 1997. Medición de las Actividades Científicas y Tecnológicas. Directrices propuestas para recabar e interpretar datos de la innovación tecnológica: Manual de Oslo.

O.I.V. (2019). Organización internacional de la vid y el vino. Res: 616-2019 http://www.oiv.int/public/medias/6826/oiv-oeno-616-2019-en.pdf

Puértolas, E., López, N., Condón, S., Álvarez, I., & Raso, J. (2010). Potential applications of PEF to improve red wine quality. Trends in Food Science & Technology, 21(5), 247–255. https://doi.org/10.1016/j.tifs.2010.02.002

Schamel, G. (2006). Geography versus brands in a global wine market. Agribusiness 22 (2) 363 – 374.

Schumpeter, Joseph Alois (1911). Teoría del desenvolvimiento económico: una investigación sobre ganancias, capital, crédito, interés y ciclo económico. México, Fondo de Cultura Económica (FCE), 2° ed en español, 1997. ISBN 9789681602093.

Senor, Dan & Saul Singer (2011). Start up Nation. La Historia del Milagro Económico de Israel. Buenos Aires, Publiexpress SA, 2012.

Steiner, B. (2002). The valuation of labelling attributes in a wine market. In AAEA – WAEA (agricultural & applied economic association & western economics association), 2002, Long Beach, California, 29 july.

Advances in flow cytometry, which benefits for enology

Gilles Bourdin [1] **and Federico Sizzano** [2]

[1] Groupe Œnologie, Agroscope Changins, Route de Duillier 50, 1260 Nyon – Switzerland

[2] Flow cytometry, Biobanking and Data Management department, Nestlé Research, Route du Jorat 57, 1000 Lausanne 26 – Switzerland

Abstract: Nowadays, the needs for understanding the biotransformation happening during the wine making process can benefit from the development of tools like flow cytometry. This powerful technique allows a quick and reliable detection and enumeration of microbial populations. Thus, by using the appropriate fluorescent dyes, specific probes and antibodies, flow cytometry can deliver information on metabolism and physiology of the cells or target proteins. Applications like the follow-up of alcoholic and malolactic fermentations as well as the detection of spoilage microorganisms are some examples of the potential of this technique. A large variety of kits can help the assessment of the viability and the vitality of microorganisms in wine. What is the future of such technology and what can we learn from the use of the flow cytometry in other application fields?

Keywords: flow cytometry, metabolism, physiology, cells, microorganisms, wine

The future of the South African grape and wine industries in the context of the Fourth Industrial Revolution

Albert E Strever [1]*

[1] *Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland, South Africa*

Abstract: The fourth industrial revolution is a hot topic of discussion on all levels of society, from both positive and negative perspectives. This article outlines its relevance to agriculture, but more specifically the grape and wine industries, emphasizing technology uptake across its value chain. The context of technologies in an ecosystem of labour, policy, resources, economy and environmental framework are important to consider when technologies are exchanged not only between countries, but also between industries. Each industry's specific needs/challenges as well as its consultation networks and platforms need understanding before technologies are implemented/developed. It is therefore important to get key players (i.e. companies, researchers, government) in the same discussion in order to optimize technology solutions towards sustainable grape and wine production within this new "Agri Renaissance".

Keywords: Technologies, 4IR, grapes, wine

1. Introduction

Industrial revolutions occur when new technologies and world views introduce significant shifts in economic systems and social structures. The current reality is that technological advancement is increasingly transforming the way we work, live, communicate, travel and socialize, which, at the rate it is going, could fundamentally alter life as we know it (Schwab, 2016a). From a scale, scope, and complexity perspective, the transformation will be unlike anything humankind has experienced before, and humankind now finds itself at the genesis of a revolution considered to be the Fourth Industrial Revolution (4IR) (Schwab, 2016b). Even though this era presents almost unfathomable opportunities, it also presents a set of realities for developing countries such as South Africa. Opportunities include wider access to renewable energy and digital connectivity offering financial inclusion for many people still stuck in the second industrial revolution (Harvey, 2017). Anticipated risks for developing countries include rising unemployment and inequality with its related social effects, including possible amplification of biases by artificial intelligence algorithms (Harvey, 2017; Hamann, 2018).

The grape and wine industries are not unique to any other agricultural industries, from the perspective that it is at a relatively low level of digital maturity, with apparent slow uptake of new technologies related to the 4IR, compared to other industries such as the financial services sector (WCG, 2017). This article will briefly introduce a perspective on disruptive technologies in agriculture that may be of relevance to the

*Corresponding author. E-mail: aestr@sun.ac.za

grape and wine industries, and give a view on its development in the South-African context.

2. Matrix of technologies, systems and applications, and its context

2.1. Constructing a technology matrix

In the work by the WCDoA (2018), base technologies were identified from literature, i.e. from De Wilde (2016), and then classified into technologies that may apply to the agriculture and food production industries. Apart from including related industries (indicated in grey shading on the circle) to agriculture (indicated by green shading), the contextual framework of technology was also emphasized (Figure 1).

For instance, labour policy may affect how robotics will develop, while unmanned aerial vehicle applications may be executed different, or be more difficult to use, depending on legislative policies. Climate and weather are also seen as contextual, as it is an overarching natural system in which technologies function, and it may play a role in assessing, acting on and predicting future scenarios. It was already found that i.e. hyperspectral sensors designed for European conditions, could not be used as-is in South Africa due to much higher temperatures combined with higher radiation intensities.

Although there are numerous technologies that may offer significant advantages to industries, including agriculture, there is also a “hype cycle” linked to these technologies

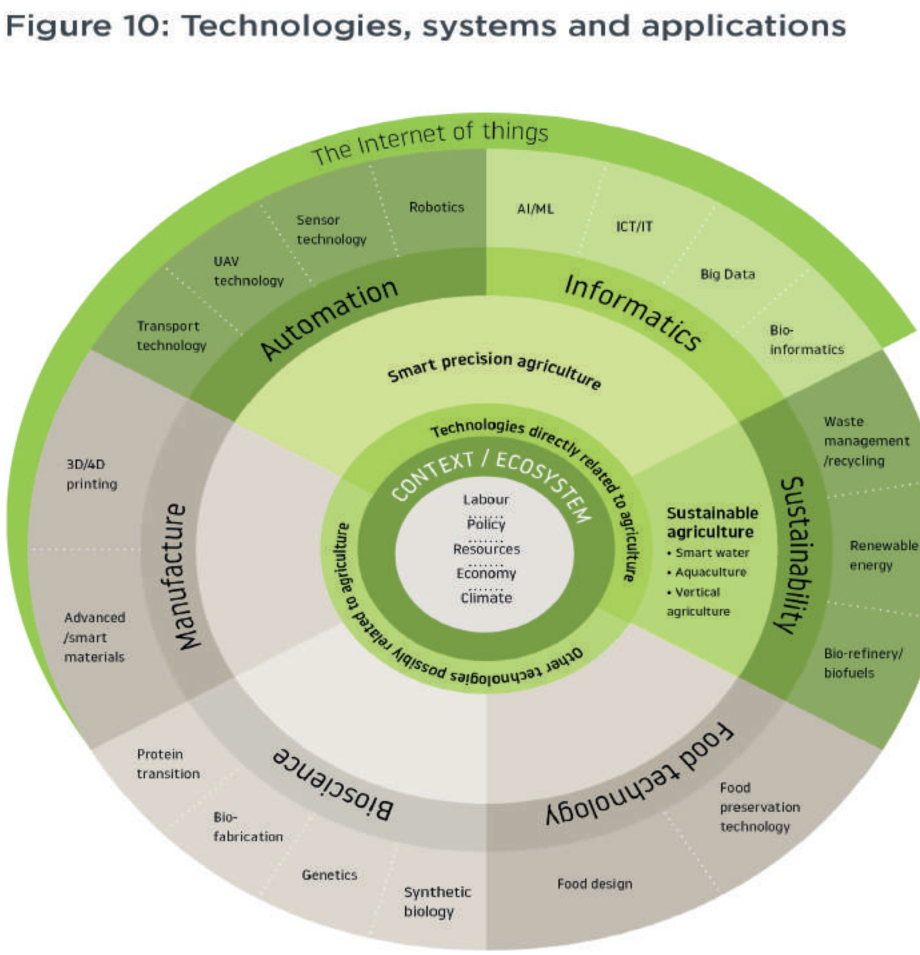


Figure 1. Technologies, systems and applications in Agriculture (WCDOA, 2018).

(also known as the “Gartner hype cycle”). However, as Brian Burke, Research Vice President of Gartner put it: “Technology innovation is the key to competitive differentiation and is transforming many industries” (Panetta, 2019).

2.2. *The grape and wine industry context*

In recent years the grape and wine industries in South Africa, supported by industry bodies such as Winetech, are increasingly focusing on development of innovation along with its strong research focus. As an example Winetech pioneered “pitching den” events in 2018 and 2019 to find ideas and solutions from entrepreneurs and businesses that could gear the industry towards optimal sustainability in the context of the 4IR. These events, also forging international collaborations with i.e. France and the Netherlands, have the advantage of engaging entrepreneurs while also linking back to research being done i.e. by Stellenbosch University in grape and wine sciences. It is important that each technology related to the 4IR is contextualized for the specific industry in question and it is envisaged that these government/business/academic partnerships could add practical value to technology applications.

Although this is not an exhaustive list, Table 1 show some technologies that may be applicable to the industries within the 4IR context.

In the next sections, some brief examples are provided of advancements in the grape and wine industry related to these technologies.

2.2.1. *Viticulture/grape production innovations*

There are several promising innovations in both long-term decision making (i.e. land suitability analysis) as well as short-term management in viticulture. Artificial intelligence solutions such as Wine Australia’s GAIA (projectgaia.ai) use deep neural networks to monitor crop conditions, fruit quality and classify vineyards in Australia. In South Africa, mapping solutions such as CapeFarmmapper and the Terraclim tool are the first steps in enabling i.e. multicriteria decision models in future that can be used for matching cultivar, site and wine goal. This is especially possible with the recent significant improvement in resolution of satellite products down to even 0.5 m at relatively low cost, as well as availability of soil electromagnetic scanning solutions also now offered by local companies. South African fruit and grape producers are well-familiar with satellite field management platforms, with the Fruitlook product already being operational from 2011 locally. These solutions play a crucial role to supplement irrigation decision making in a severely drought-stricken industry as was experienced in the recent past. There are also solutions for real-time monitoring of canopy/grape condition (biotic or abiotic stress conditions) in the context of weather prediction i.e. Grape Compass for biotic conditions and a new in-situ vineyard thermal camera system currently being developed in a Stellenbosch University/ Winetech innovation collaboration. This also includes local testing of a mobile hyperspectral stress detection solution by a Dutch company (Polariks).

Wearable devices and RFID for labour management, traceability and observation registration during manual grape harvesting are also locally available, mostly now being used in the table grape industry (Adagin Technologies), including tracking solutions such as Farmtrack (Etse electronics) for tractor and spraying optimisation.

Although there are some cases of use of self-driving tractors for spraying internationally in orchards, as well as vineyard mechanization robots tested abroad, this is still not seen in South Africa.

2.2.2. *Technology for improved natural resource sustainability*

Several environmental solutions incorporating clean energy, biofuel or wind energy may add to lowering the environmental footprint of grape/wine producing businesses, with increased prevalence expected in future – especially considering South Africa's current energy crisis. Waste management technologies (water conservation, eco-friendly packaging, nutrient recycling etc.) are not only important from a wine cellar/grape pack house perspective, but also increasingly on farm level, with expected larger focus on these aspects in certification schemes.

The wine and grape consumer increasingly asks for traceability, which can be augmented by i.e. blockchain technology, RFID tagging or even virtual reality technologies. Consumers also want to see responsible use of pesticides/fungicides or other chemicals in processing, which makes technologies for weed control (i.e. Vitirover) or efficient spraying (i.e Diimotion) as well as plant defense augmentation (i.e. UVboosting) attractive for the future.

2.2.3. *Oenological innovations*

There are several innovations related to wine processing technologies ranging from online sensing of anything from oxygen to sulphur to phenols, to connected tanks and barrels that monitor fermentation and ageing through various sensor types. On the sensory side, it is speculated in Marr (2019) that artificial intelligence (AI) today enables vision and natural language capabilities – and that it may soon also develop smell and taste. After all, the aroma and taste of a wine comes from a range of chemical compounds, for which the real-time analysis may become much faster and cheaper than current methods on offer. For a review on many other applications of AI, also refer to Johnson (2018).

2.2.4. *Market access or advancement innovations*

Various food and beverage marketing systems relying today on digital platforms, sometimes incorporating "big data" analytics as well as social media platforms in marketing initiatives. There are also an increase in the use of "virtual sommeliers" by consumers and according to Marr (2019), more than 25% of wine drinkers use apps to help them decide on which wine to purchase. Several wine recommendation applications have therefore seen the light (i.e. AskJean, WineRing, WineStein and others). Even the popular Wine Spectator is written by software, which is something many readers do not even realise (Marr, 2019). In future, the use of wine sommelier robots may become more commonplace, especially at the tasting room door, or in other hospitality areas, perhaps combined with wine dispensing units.

There are also wine labels (i.e. LaVi in Italy) that are "smart", with the QR code showing the wine's journey, in this case a full account of the wine's DNA, when and where the grapes were harvested, how the wine was treated (i.e. sulphites), bottling date, lot number and more. All of this are recorded into a blockchain. This of course needs proper data capture at each stage of production, which could be prohibitively expensive for the smaller producer.

Although some of this is already in place and slightly controversial, the future may see more "designed" wines, where components may be removed or added selectively using different techniques to target a specific product profile for a specific consumer taste. From the commonplace use of chemicals like Sulphur in winemaking to the advanced techniques for removing specific off-flavors or just making low or zero-alcohol wines, it remains to be seen where the future is heading with respect to consumer pressure and preference, where maybe organic or biodynamic wines could become even more prevalent, in a world where everything seems to become more synthetic (see i.e. Buranyi, 2018).

3. Conclusions

It is important to consider that the grape and wine industries in South Africa are sub-sections of the agricultural sector, and like their peer industries, they are affected by government policies and actions in response to the challenges of the 4IR. The risks related to this revolution (also affecting agriculture) will require that academics, businesses, and civil society actors attend to the role of new technologies in the context of a developing country. Governments ought to carefully assess the above risks in their national context and then establish corresponding policies and programs. This includes national skills development and work placement platforms, intellectual property and competition policies, and local technology adaptation and development (Hamann, 2018). Although local manufacture of technologies cannot be feasible in all contexts related to the 4IR, the adaptation of technology to fit its ecosystem is crucial, and in some cases there are local agricultural engineering solutions that are much cheaper than the imported alternatives. Apart from local manufacture and support, some key aspects that need improvement that were synthesized in the report by WCDoA, 2018, include:

- Improving data accessibility in rural areas
- Digitally and spatially enabling viticultural and wine processing equipment
- Proper training of producers in the technology use
- Data protection policies need to be in place for the consumer and business
- Solutions should preferably be open-source and compatible across software and hardware platforms
- Proper data visualization, storage and processing should enable user-friendly interpretation

Acknowledgements/disclaimer

Names of companies and/or products are used here exclusively for providing examples of some relevant technologies and products and no endorsement or support for these products/companies are intended.

The support of Stellenbosch University, Winetech and VINPRO are acknowledged to the grape and wine innovation platform at the University.

Support of the Western Cape Department of Agriculture (WCDoA) and the University of Stellenbosch business school are acknowledged while drafting “The future of the Western Cape agricultural sector in the context of the Fourth Industrial Revolution” together with the project team.

References

Buranyi, S. 2018. Has wine gone bad? The Guardian (theguardian.com), 15 May 2018.

De Wilde, S. 2016. The Future of Technology in Agriculture. STT Netherlands Study Centre for Technology Trends (https://stt.nl/).

Hamann, R. 2018. Developing countries need to wake up to the risks of new technologies. The Conversation Africa (theconversation.com), 4 January 2018.

Harvey, R. 2017. The ‘fourth industrial revolution’: potential and risks for Africa. The Conversation Africa (theconversation.com), 30 March 2017.

Johnson, T. 2018. Artificial vintelligence: AI gets taste of wine industry. Vonvino (vonvino.com), 11 June 2018.

Marr, B. 2019. The incredible ways the fourth industrial revolution and artificial intelligence are changing winemaking. Forbes

(Forbes.com). 3 July 2019.

Panetta, K. 2019. The Gartner Hype Cycle highlights the 29 emerging technologies CIOs should experiment with over the next year. Smarter With Gartner, 29 August 2019, www.gartner.com/smarterwithgartner.

Schwab, K.M. 2016. Welcome to The Fourth Industrial Revolution. Rotman Management Magazine, The Disruptive Issue (Fall 2016):18-24.

Schwab, K.M. 2016. The Fourth Industrial Revolution. World Economic Forum, Geneva, Switzerland.

The Western Cape Department of Agriculture (WCDoA), 2018. The future of the Western Cape agricultural sector in the context of the Fourth Industrial Revolution. Synthesis report by the University of Stellenbosch Business School.

The Western Cape Government (WCG). 2017. Western Cape Sector Digital Disruption Impact Assessment, (https://www.westerncape.gov.za).

World Economic Forum (WEF) (in collaboration with McKinsey and Company), 2018. Innovation with a Purpose: The role of technology innovation in accelerating food systems transformation, (https://www.weforum.org).

Table 1 Mapping of technologies related to the 4IR for the grape and wine industries (adapted from WCDoA, 2018).

Categories (WEF, 2018)	Technologies (WEF, 2018)	Technologies (WCDoA, 2018)	Most applicable to the grape/wine industries?
Advances in science	• Next-generation biotechnologies and genomics • Gene sequencing • Efficient energy technologies	• Protein transition • Biofabrication • Genetics • Synthetic biology • Bioinformatics • Food design • Food preservation technology • Renewable energy • Biorefinery and biofuels • Recycling and waste management	Genetics and bioinformatics are applicable from a cultivar development and resistance trait perspective, as well as investigating cultivar and environment interactions for improved management. Synthetic biology is applicable in micro-organisms such as yeasts and bacteria or viruses, both from a beneficial or detrimental point of view in the vineyard and cellar. Food preservation technology, recycling and waste management technologies become important in a winemaking environment.
Digital building blocks	• Big data and advanced computing systems • Internet of Things • Artificial intelligence • Machine learning • Blockchain	• Artificial intelligence/ machine learning • Information and communications technology/ information technology • Internet of Things • Big data • Blockchain • Cryptocurrency	All of these technologies are relevant on many levels from planning phases, to grape production to marketing and sales – therefore across the whole value chain of grape and wine production.
New physical systems (hardware)	• Autonomous vehicles • Unmanned aerial vehicles • Robotics • Manufacturing advancements • (3D and 4D printing) • Advanced materials • Nanotechnology	• Unmanned aerial vehicle technology • Transport technology • Sensor technology • Robotics • 3D and 4D printing • Advanced/smart materials	Although 3D printing and advanced/smart materials are still further in the future, all the other technologies may be relevant in the value chain of grape and wine production. Bio-sensors become increasingly interesting for plant stress sensing as well as wine and grape process monitoring.

Digital wine tasting as a direct marketing instrument

Veaceslav Kunev

Gustos.Life LTD, 2 Negruzzi blv, Chisinau, Moldova, MD2001

Abstract: Historically speaking, wine tastings are one of the oldest marketing instruments used by winemakers to boost their brand awareness and, thus, sales. Nowadays, with all recent digital trends the marketing effect of such activities is constantly declining, while the producers are shifting towards modern instruments and techniques in their business activities. Yet, is there a way to help public wine tastings and professional international competitions become an integral part of modern commercial and marketing processes again? Yes, there is. It is not an idea or a concept, but a fully functional and commercially tested solution.

Keywords: Wine tastings, Competitions, Informational technologies, Marketing, Sales

1. Introduction

In this article, I will present my vision of how wine tastings, both public ones and professional "blind" ones, can embrace new trends, as well as describe how the synergy between traditional ways and informational technologies can transform wine tastings into a major digital marketing instrument.

2. What's in a medal?

Any winemaker submitting samples to a professional international competition, even the ones who do get medals and awards, will always have questions left to ask: why did a sample receive a medal and the other one didn't? Are these results totally trustworthy or was the evaluation affected by subjective circumstances? What exactly did the jury members like or dislike about the tasted sample? Was the jury opinion unanimous? And one of the main questions - if we did not get a medal have we wasted our time and money?

Most of the hundreds of major and small competitions taking place yearly all around the world leave those questions unanswered. Thousands of wine samples are awarded medals, thousands of others are not, leaving many participants empty handed and frustrated by the complete lack of feasible results or any other feedback, which would give them a hint about their product flaws.

3. Modern Solutions

Over the last three years Gustos. Life developed, tested and launched a professional wine tasting IT solution, successfully used during several major international competitions, such as Eurasia Wine and Spirits Competition 2018, 2019 and 2020; Wine and Spirits Ukraine 2019; Odessa Bay (Одесский Залив) organised by LSA ONAFT in 2019 and the upcoming edition in 2020.

*Corresponding author. E-mail: aestr@sun.ac.za

This software offers an easy, transparent and user-friendly way of organising, conducting and reporting “blind” wine tasting activities during professional wine competitions. Besides solving all the issues mentioned above, this solution provides a whole set of other functional and informational advantages benefiting both the participants and the organisers.

3.1. Complete transparency

One of the major advantages of Gustos. Life solution is real-time tracking of the competition process using blockchain technology. This way the scores the samples receive can’t be deleted, edited or altered in any other way. The authenticity of each database entry can be cryptographically audited anytime.

For even higher transparency, trustability and security of the competition results, we always offer the organisers to live-stream the wine tasting process, together with real time database entry log files, like we did during Odessa Bay 2019 and Eurasia Wine and Spirits Competition 2020. These videos are always available on our youtube channel, while the live stream for Odessa Bay 2020 will be also available right after the start of the competition.

3.2. Detailed report and professional feedback about flaws

Gustos.Life software brings extra value to participation in competitions, regardless of whether the sample was awarded a medal or not, by giving the winemakers or third-party representatives access to complete and detailed information about the results of the wine tasting, as well as specific comments from jury members about the eventual product flaws.

The evaluation of each sample will result in a detailed report, stating each score of each jury member, for each descriptor analysed according to the current evaluation methodology.

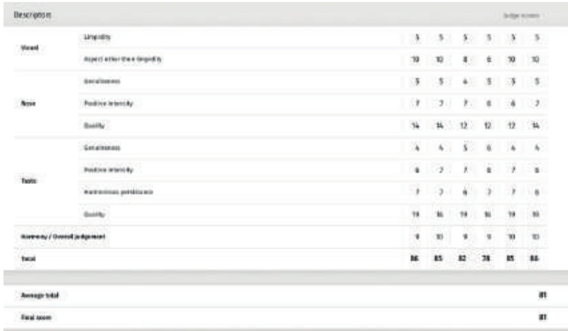


Figure 1. Example of scoring report of a still wine sample with descriptors according to O.I.V. methodology.

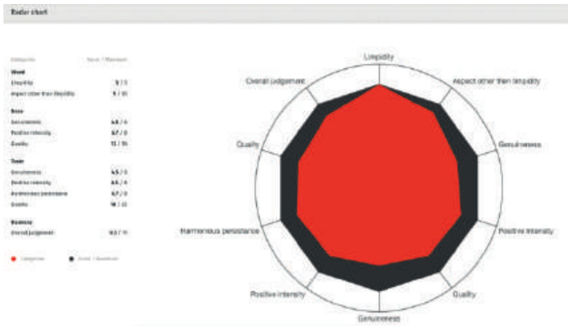


Figure 2. Radar chart report of a still wine sample with descriptors according to O.I.V. methodology.

Moreover, each jury member is advised to leave an explicit comment for each descriptor, he gives a below-average score to. All these comments are structured and appended to the scoring report where the jury members are anonymised.

3.3. Methodological Compliance and flexibility

The first version of the solution was designed for conducting competitions in full compliance with official O.I.V (International Organisation of Vine and Wine) recommendations. Yet, the technical architecture allows integrating any number of other competition methodologies - not only in terms of the scoring scale and descriptors, but also in terms of specific organisational aspects. Different methodologies and extensions have already been used during bulk wine evaluations and en primeur wines aging potential assessments.

3.4. Detailed statistics

All the information about all the events using our solution are stored in the database, while all the stakeholders of the competition, depending on their role, have access to a full or limited share of this data. The organisers have access to detailed results of all conducted events. Jury members have access to all their scores in the events they took part in. The participants can review and download the reports for each of their samples from all the events they took part in.

The amount of gathered information is already enough for big data analysis, giving the possibility to offer a large variety of instruments that grant the participants analytical information about their results, depending on different criteria - competition methodology, jury qualification, vintage of the wine and so on.

Another example of such an instrument would be "personal metrics and rating of the wine tasters", giving both the experts and the competition organisers information about average deltas between each jury member score and the total score of the sample per any category of alcoholic beverage.   .

3.5. Integration with other marketing solutions

The information about the scores received during professional competitions is always available for winemakers in their Gustos. Life accounts. This information can be easily made public by making it available in an integrated database, connected via API with other gustos.life modules and third party apps and services. For example, a retail online wine marketplace, where the clients will be able to compare these professional ratings with "public opinion ratings", giving them extra information during their decision process.

4. Synergy between public wine tastings and sales

Professional competitions aren't the only way the wine tastings are used for marketing purposes. Wine producers, wine merchants and other wine industry actors often organise public wine tastings. Such events rarely have the purpose of direct sales of the tasted wines and, generally, just raise brand awareness or present new products on the market.

Gustos.life developed a designated "casual wine tastings" module for such events, giving them a simplified scoring system just for sample comparison purpose and making the sample anonymization optional.

But the main difference of this module is its direct integration with retail sales features, giving the users not only the possibility to rate and comment on the tasted samples, but also to add them to the "wishlist" or directly buy the favorite wines.

5. Conclusion

The digitalisation of wine tastings is an inevitable step in the era of mobile technologies and e-commerce. New technological solutions such as Gustos.Life will not only grant such practices a second life, but also bring them to a whole new level of marketing importance and commercial feasibility, thus benefiting all stakeholders - the organisers, the participants and the jury, as well as giving the mass wine loving audience free and open access to valuable information about wine quality assessments.

Contacts

For additional info please contact: vk@gustos.life
Veaceslav Kunev – Tél. +373 794 51 868.

10th International Symposium

Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation.

SCIENTIFIC COMMITTEE

Volodymyr Pechko - Albert Mas, Monika Christmann - Andrew Waterhouse - Davide Slaghenaufi, Jacques-Olivier Pesme - Darko Jaksic - Alla Plachkova - Shigeaki Oda - Sofia Catarino - Tristan Richard - Markus Herderich - Zhanwu Dai - José-Miguel Martinez Zapater - Alejandro Gennari - Gilles Bourdin - Albert Strever - Veaceslav Cunev

ORGANISATION AND CONTACT

Pierre-Louis Teissedre - pierre-louis.teissedre@u-bordeaux.fr
Tel. +33 (0)5 57 57 58 53
http://www.oenoviti.com
Université de Bordeaux - Institut des Sciences de la Vigne et du Vin
210 chemin de Leysotte - 33882 Villenave d'Ornon cedex - France

SECRETARIAT

Agathe Lairy - agathe.lairy@u-bordeaux.fr
Marion Charvet - marion.charvet@u-bordeaux.fr

ISBN **978-2-9509403-2-2**
EAN **9782950940322** **à changer ?**

CONCEPTION GRAPHIQUE/*GRAPHIC DESIGN*

Agence MEDIACROSSING (Bègles)
www.mediacrossing.fr

IMPRIMÉ PAR/*PRINTED BY*

Imprimerie Escourbiac (Graulhet)
www.escourbiac.com

DÉPÔT LÉGAL/*LEGAL DEPOSIT*

2e trimestre/2th trimestre 2021

COVER PHOTO CREDIT

Adobe stock ©JOZSITOEROE **à changer ?**

The **OENOVITI INTERNATIONAL network** is 11 years old and the first and only international network for training and research in oenology and viticulture. It aims to promote exchanges of expertise and know-how between stakeholders in the academic and industrial winemaking worlds. The network offers its members a high level of visibility on the international scene, enabling them to maximize opportunities via joint research and training projects, as well as discussions. The OENOVITI INTERNATIONAL network has nearly 61 partners across the globe, forming an international consortium of institutions known for their excellence in the field. The joint OENODOC doctoral program was created within this framework with the aim of developing an international doctorate specific to the oenology and viticulture sector. This network, coordinated by the University of Bordeaux, enables the academic and industrial worlds to come together to take up the many challenges of oenology and viticulture research.

This 10th network symposium is dedicated to ***« Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation. »*** : This topic is of great importance for the wine sector to organize, adapt and preserve wine quality production. Several questions are approached during this symposium:

- **Overview of winemaking and implications for appellations and authenticity,**
- **Wine Appellations and Wine Tourism with resources preservation, typicality and system,**
- **Authenticity with analytical approaches,**
- **Winemaking and winegrowing innovations and novelties including vine and modelling, genetical approaches, vine-wine chain and consumers, vine protection and new processes, digital and marketing.**

Wine market, wine tourism economy, consumers expectations, innovations for the wine industry sector in the context of climate change, with new consumers demands, as well as product identity certification are the major points and questions of this 10th symposium with recognized scientists and researchers in this field.

All these topics are developed in this 10th symposium of the OENOVITI INTERNATIONAL network and adaptation, innovations, alternatives and research to develop wine market and tourism, wine authenticity, quality and identity need to be encouraged.

This international symposium enjoys support from the OIV, Château Pichon-Baron (AXA Millésimes), the Foundation Bordeaux University (original interface between the university and socioeconomic worlds), the University of Bordeaux, ISVV, and all of the OENOVITI INTERNATIONAL network's academic and industrial partners. We would like to thank all our partners and backers for their help in supporting the development of research in oenology and viticulture in order to collectively overcome the new challenges arising in this field. We wish all the best for the network for the next 10 years!

The 10th OENOVITI INTERNATIONAL Symposium on "Challenges in viticulture and oenology: Wine Appellations, Authenticity and Innovation." has been supported by the following institutions and firms:

ŒNOVITI INTERNATIONAL

CHÂTEAU PICHON BARON
GRAND CRU CLASSÉ EN 1855
PAUILLAC

FONDATION BORDEAUX UNIVERSITÉ
S'engager pour l'avenir

EXCELLENCE INITIATIVE Université de Bordeaux

OIV