CODE OF GOOD ORGANIC VITICULTURE AND WINE-MAKING

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Fig. 1: The ORWINE-Partnership – 2. Project- meeting FRICK, Dec. 2006

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FOREWORD

When this publication was first discussed with the project partners a sort of leaflet or a very short manual was planned. During the three project years it has been realised that quite a lot of knowledge has become available and that even more was already part of the organic wine-makers know-how and researchers background. Slowly the number of pages became 100, then 200 and more and still the result does not include all cases and possibilities a wine producer may encounter whilst performing their job.

Thus in these pages the reader will not find all the answers to organic viticulture or wine-making problems but, it is thought that something more useful and closer to the concept of sustainability is presented which requires from each of us the need to searchfor a personal and site/wine solution. This book is a tool that should support each wine-maker in finding their way to producing a high quality organic wine minimizing the use of external inputs in the vineyard and in the cellar as well as maintaining, in the final product, as much as possible of the character of the grape, of the farm and of the farmer.

It is not the only book that should be read before starting to make wine: it is a good compendia but needs to be correctly used and understood as well as a comprehensive knowledge of basic viticulture and oOenological concepts that may be found in several publications and courses.

Finally it is a good tool for interpreting the EU regulations on organic wine-making shortly coming into force and to put it into practice not only as a mandatorybureaucratic burden but, as it should be, as a tool for improvement of the industry and communication with the consumer.

If the total pages are now counted it is seen that there are more than 500, including fact-sheets and other documents reported in the CD. Don't be afraid of the volume, it is a long lasting book to be regularly consulted and not to be read just once. Keep it with you in your cellar, it will age with your best wine!

Cristina Micheloni
ORWINE overall coordinator

FOREWORD FROM DR DANIÈLE TISSOT BOIREAU (Project Officer)

The Policy oriented research activity – "scientific support to policies" of the Sixth Research Framework Programme had the overall objective to support the formulation and implementation of Community policies through a targeted scientific evidence based research agenda. With respect to this project the focus was upon the revision of the "Regulation on organic food and farming" and it's implementing rules where it was necessary to provide clear science-based practices for organic wines coherent with the principles of organic agriculture.
Accordingly, the ORWINE project has been exploring alternative methods to sulphite addition in the winemaking process coupled with improved management practices and application of selected optimised methods on pilot farms. The Commission is thus happy to report that the practical outcome of the project established and described here in this publication will strongly contribute towards the further development of best practice in organic viticulture and wine-making and meets fully the Treaty objective of strengthening the scientific and technological basis of the food and drink industry while encouraging competitiveness of the sector at an international level.

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This publication represents the ORWINE "Code of good organic viticulture and wine making" about task 5.2 in work package 5 "Regulatory proposal, stakeholder involvement, result dissemination" of the Integrated Project No 022769 "Organic viticulture and wine-making" (Sixth Framework Program for European Research & Technological Development (2002-2006) of the European Commission).
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They are also grateful to the numerous experts who have taken time to give information, complete the collected data and comment where necessary to elaborate the final report.

DISCLAIMER

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DEFINITION OF ORGANIC WINE

The International Federation of Organic Agriculture Movement (IFOAM) defines organic agri-culture including viticulture and wine-making as a “holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems.” (IFOAM 2005).
Organic wine is wine made from organically growing grapes without the help and need of synthetic fertilizers, synthetic plant treatments and herbicides.
Organic grapes come from vineyards grown under organic farming methods, as defined at European level, by the Council Regulation (EC) No 834/2007 and No 889/2008 on organic production and labelling of organic products the result of which is that the sole rules to be applied to wines processed from organic grapes are those contained in the EC Regulations 479/2008 (annexes 4 and 5) and 1622/2000, which define the oenological practices and treatments allowed for wines in Europe.
Moreover, organic vine-growers have developed specific approaches for processing their wines in a way they consider in compliance with organic farming principles. These private initiatives in the producing countries (i.e. Austria, Germany, Switzerland, Italy, Greece, France, and Spain) have taken the format of standards or charters belonging to producers groups, organic farming associations connected with certifiers, or national platforms.
A more detailed picture of the approach and intent of organic agriculture are provided by the following principles:

- To produce sufficient quantities of high quality grapes and wine.
- To work compatibly with natural cycles and living systems through the soil, plants and animals within the entire production system.
- To recognize the wider social and ecological impact of and within the organic produc-tion and processing system.
- To maintain and increase long-term fertility and biological activity of soil using locally adapted cultural, biological and mechanical methods as opposed to reliance on inputs.
- To use, as far as possible, renewable resources in production and processing systems and avoid pollution and waste.
- To support the establishment of an entire production, processing and distribution chain which is both socially just and ecologically responsible.
- Organic wines are obtained exclusively from certified organic grapes (respecting EU regulation 834/2007).
- All ingredients (sugar, alcohol, concentrated or rectified musts) used in wine-making must be from organic origins.
- The organic wine-making process excludes the use of genetically modified organisms (GMOs) as well as additives or processing aids produced by genetically modified organisms.
- The organic wine-making process must give preference, when possible to biological, mechanical and physical treatments and to avoid chemical processes.
- The organic wine-making processing must, as far as possible, preserve environment (energy and water resources) and avoid non sustainable practices.
- Organic wines must be safe for consumers’ health (if consumed with moderation): use additives only if necessary and to mention, allergenic products on labels.

Fig. 2: Doppel-Gold winner at BioFach Wine award. Organic wine as a high quality production.

SCOPE

The ORWINE Code of good organic viticulture and wine-making aims to fulfil the different vine-growing and wine-making conditions in Europe.
The background documents of this code are existing EU regulations for wine and for organic production:

- The Council Regulation (EC) 479/2008 on the common organisation of the market in wine (CMO-Wine) within the different wine-growing zones and the permitted and recommended oOenological practices and additives.
- The national AOC regulations in the different member states.
- The Council Regulation (EC) 834/2007 and 899/2008 for organic production with the definition of organic production and organic food processing.

The Code is the practical outcome of the activities done in the ORWINE-research project, which are summarised in the following reports:

- The research framework and literature survey,
- The Analysis of regulatory framework and standards (private and official standards for organic viticulture and wine-making),
- The results of the Producer investigation about current oenological practices,
- The results on market needs and perspectives as well as the results of the consumer expectations
- The results of the scientific research from WP3: Improved Management Practices in Wine-making and Experimental Testing as well as the results of WP4: On Farm Application and Testing of Innovative Methods.

The ORWINE Code of good organic viticulture and wine-making is not usable as a manual or an instruction book.
The concept of the ORWINE Code is to become more recognised and respected.
The ORWINE Code is a complementary tool to the new regulation for organic wine implementing rules under the (EC) Regulation 834/2007. It gives wine-producers a clear guidance on how to produce wine of high quality whilst reducing the use of additives.
The aim of this code is to contribute to the further development of practices for organic viticulture and wine-making in terms of increased safety, quality, transparency and success. The code summarises different traditional and innovative viticultural and oenological practices suitable, approved and acceptable for organic production.
Furthermore the code is not only for newcomers but should also help wine estates, co-operatives and wine-cellars that already produce organic wine to check and improve current production practices.
This code is useful in the development of HACCP-based food safety programs, ISO 9000 programs and total quality management programmes. This code alone is not a HACCP analysis, nor a quality assurance system and not a total quality management programme. It cannot, by itself, guarantee the safety of a product at the time of consumption.
Therefore, the ORWINE Code presented here should be regarded as a source of practical information for each wine company or cellar to adopt. The user has to select the viticultural and oenological practices appropriate under their specific climatic, traditional and recommended wine-making conditions. Therefore different strategic options are shown which can be taken into account depending on the local conditions as well as the personal wine-making concepts (philosophies).
The ORWINE Code has also the potential to be used as a reference tool in those certification systems, where more responsibility is given to the producer and wine-processor. As such it can help the control bodies to verify, which of the approved options have been followed by the wine-producer. For example if some of the options mentioned in the code are applied the wine-producer can explain their own production options in a similar way. Certainly if this code should become a reference tool for certifiers, e.g. as a "Code of Conduct" of an organization (e.g. a producer organization or a trading company), it would need to be further developed in a more certification-specific way (e.g. with check-lists). Nevertheless this code gives a good basis for such developments.
The Code is structured in such a way that the most relevant tasks or important features which exist in the relevant areas of activity are outlined in separate chapters:

- Organic viticulture
- Organic wine-making
- Technical notes
- Practical hints
- Research results
- Fact sheets (only on the CD)

■ CLIMATIC CONDITIONS FOR VITICULTURE IN EUROPE

Europe has a variety of climates, but most of the continent has mild weather.
The map (Fig. 3) shows what the climate is like throughout the continent.
According to this, European viticultural regions are classified into three different wine-growing zones. Each of these zones/regions have specific environmental wine-growing conditions. This includes different aspects of grape quality (like the sugar content) and of disease sensibility (e.g. Peronospora is mainly a problem in humid, Oidium in arid zones). The contemporary climatic zones are everchanging. They fluctuated in the past and they are shifting nowadays, mainly due to anthropogenic climate change effects. With this shift the regional conditions for wine-growing will change over all in border areas like Southern France or Northern Italy where significant increases of rainfall in early summer is correlated with an increase of Peronospora pressure.

Fig. 3: Climatic regions of Europe and viticultural zones.
Source: http://www.worldbook.com/wb/images/content_spotlight/climates/europe_climate.gif

1. Semiarid – subtropical dry summer climate Mediterranean Area

The Mediterranean zone (for example Spain, South of France, Italy or Greece) is one that resembles the climate of the lands in the Mediterranean Basin, which includes over half of the area with this climate type world-wide. In addition to the areas surrounding the Mediterranean Sea, this climate type prevails in much of California, in parts of Western and South Australia, in southwestern South Africa and in parts of central Chile, regions where mostly wine is a common agricultural product.
The climate is characterized by hot, dry summers and mild, wet winters. During summer, regions with a mediterranean climate are dominated by subtropical conditions with varying humidity, low rainfall with the exeption of an odd thunderstorm. As a result, areas with this climate receive almost all of their yearly rainfall during the winter season, and may have anywhere from 2-5 months during the summer without any significant precipitation.
These regions are ideally suited for the production of high quality red wine from regionaly adapted varieties as well as "international" varieties. In mediterranean areas, the "light" factor is not limiting except in cases of certain vine pruning and trellis systems where elevated vine vigour increases the number of shaded leaves. Nonetheless it is worth stating that it has been suggested, notably in the case of white grapes, that "air conditioning" of the bunches by appropriate management of their microclimate (shading of the bunches by leaves during ripening) could be useful in maintaining aromatic potential.
The wines are concentrated, full bodied with a high alcohol content and lower acidity.

2. Maritime – humid climate Atlantic/ Central Europe

The Continental and North Atlantic zone (for example: South western and eastern France vineyards, Germany, Swiss, Austria and parts of Hungary, Romania or Slovenia) is where the sugar concentration and the length of sun exposure seems to be the limiting factor during grape maturation. Precipitation is both adequate and reliable at all times of the year in oceanic climates. Overall temperature characteristics vary among oceanic climates; those at the lowest latitudes are subtropical , but more commonly a mesothermal regime prevails, with cool, but not cold, winters and warm, but not hot, summers. Summers are also generally much cooler than in areas with a humid subtropical climate. Average temperatures of the warmest month must be below 22 °C, and that of the coldest month above -3 °C.
In northern vineyards, climatic conditions favouring a forward growth cycle permit grape maturation during a warmer and sunnier period, thus benefiting grape quality. Temperatures during the fruit-ripening phase are moderate and consistent. These regions are ideally suited for the growing of fruity Chardonnay, Pinot blanc, Rhine-Riesling, Italian-Riesling, Grüner Veltliner and Pinot Noir or Blaufränkisch. Wines from cooler climates are characteristically higher in acids and highly aromatic. Micro-climatic differences influence the behaviour of cool climate varieties and the broad variation that can be found from vintage to vintage is an expression of that sensitivity.

3. Continental- humid to dry climate Central / East Europe

Continental climate is a climate that is characterized by winter temperatures cold enough to support a fixed period of snow cover each year, and relatively moderate precipitation occurring mostly in summer. Spring-like temperatures occur in this zone between early March in the southern parts of this zone to mid April in the far northern fringes. Annual precipitation in this zone is usually between 600 mm and1.200 mm, most of it in the form of snow during winter. Continental climates exist where cold air masses infiltrate during the winter and warm air masses form in summer under conditions of high sun and long days. The humid continental climate is marked by variable weather patterns and a large seasonal temperature variance. The seasonal temperature variance can be as great as 33° Celsius, but is typically about 15 - 22°C. The temperature difference between the warmest and coldest months increases as one moves further inland and away from the moderating influence of the ocean. The warm summer subtype is marked by mild summers, long cold winters and less precipitation than the hot summer subtype; however, short periods of extreme heat are not uncommon. These climatic conditions are favorable for fruity to full bodied white wines as well as concentrated high alcoholic red wines from different autochthones (indigenous) and international varieties.

Fig. 4: ORWINE-Farm-day at the Pilot farm "Rummel-Germany"

HACCP[1] – CONCEPT FOR ORGANIC VITICULTURE AND WINE MAKING (Barbier, J.M.)

In ORWINE-WP2, we proposed the application of a **HACCP** type methodology, normally used for grape processing in the cellar (oenology), to production of organic grape in the field (agronomy). The objective is to take advantage of the general principles of this methodology to offer **experts** from different grape producing areas an analytical framework, which which will give information on grape quality and of its potential control in the field through the choice of the more appropriate farming practices.

1. General principles of HACCP: logical sequence for its application

1. Assemble HACCP team

2. Describe product

3. Identify intended use

4. Construct flow diagram

5. List all potential hazards and consider control measures

6. Determine CCPs

7. Establish critical limits for each CCP

8. Establish a monitoring system for each CCP

9. Establish corrective actions

10. Establish verification procedures

11. Establish documentation and record keep-ing

2. Agronomic applications:

[1] **Hazard Analysis and Critical Control Points (HACCP)** is a systematic preventive approach to food safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCP's) can be taken to reduce or eliminate the risk of the hazards being realized. The system is used at all stages of food production and preparation processes including packaging, distribution, etc.
source: http://en.wikipedia.org/wiki/Hazard_Analysis_and_Critical_Control_Points

Evaluation of farming practices for grape production in the field

2.1. Feasibility of applying HACCP methodology

Several arguments support the HACCP approach in the evaluation of farming practices for grape production in the field.
Farming practices currently in use are not bad per se. It is their application in certain conditions (i.e., certain years, certain environments) and their inclusion in a certain sequence with other practices within a **sequence of technical operations** that can be a problem. It is thus the management, taken as a whole, which is questionable. As a result, a particular technique could be the source of potential danger for the production of a grape harvest of a given quality, yet the danger will occur only occasionally, in some conditions,
or rarely through the use of corrective practicesaccording to the the management programme. In the field, however, the management of the production process is imprecise because the effects of the management practices are not always fully known beforehand (i.e., due to various hazards, such as the climate for example) and the control systems are sometimes difficult to be put in place.
Although the "systemic" approach is usually highlighted in agronomy, it is, however, of interest to try to analyse, in the first instance, the grape production process (also called the crop management sequence), to identify **independent basic steps** and to assess for each of them possible consequences of certain technical choices on the consequent grape harvest . Do some technical choices represent a potential danger for the production step under study and for that specific type of decision or technical operation? Can they have **direct or indirect consequences** on the consequent harvest ?

2.2. Agronomic implementation

a. What to evaluate?
The objective is to assess **the grape production process in the field**. Throughout this process, it is necessary to locate the "critical" inputs (planting, soil maintenance, phyto-sanitary treatments, harvest….) which are the most commonly used management techniques (in organic farming) and are important in effecting the bio-physical-chemical status of the grapes. The evaluation concerns the final **harvest condition**.

b. Field applications to be evaluated
The steps, which are taken into consideration here, range from the land choice and land preparation for vine planting to the arrival of the grapes at the reception platform (including, the harvest and the transport).

c. Assessment of fruit yield and quality
It is crucial to define a view on the concept of **"quality"**, since this is the aspect which is to be evaluated. The harvest "quality" is defined by the whole range of its possible conditions: physical status (impurities, berry size, skin thickness….), health status (fungi….), degree of ripeness, chemical status (quantity of a given flavour precursor, nitrogen level…) and other conditions.
In the first phase of the evaluation, the experts are asked to express their opinions on the influence exerted by certain farming practices (including the fact that, due to the "organic" way of farming it is not possible to make use of certain practices) on the harvest quality and yield **independently of a given processing system and wine type.**

Secondly the grape harvest quality and yield may be judged "critical" if it leads to consequences in the way grapes are received and/or processed in the cellar (in order to assure the attainment of a final product, which complies with a given regulation and satisfies the organoleptic requirements).
The principle that is followed here is that the processing of an organic product (grapes) into another one (wine) must be done by involving the smallest possible number of interventions and in particular with the **fewest additions of exogenous components** (the reference being the 0 input or the most "natural" product possible). Therefore, it is considered that there is a "critical point", when a given farming practice is likely, under certain conditions, to cause the introduction of one or more supplementary components during the wine making process in order to correct a non satisfactory grape harvest condition. It is clear that the resort to "corrections" during the wine making process depends on the type of final product (wine type) that is to be produced and put on the market. This means that the same practice may prove critical for making a type of product and not for another. It is, thus, during this second phase, that the type of wine will be taken into consideration.

d. How to evaluate: definition of the items to be investigated

Production steps: A set of decisions to be taken and technical operations to be implemented in order to reach an objective that will accomplish a given result in the grape production process. It should be noted that in vineyard management, the same technique can contribute to different purposes (for example, tillage is useful for weeding, de-compacting, aerating…) and the same objective can be reached in different ways. It could also be expected that certain steps are not applied. Moreover, various technical operations are not always applied in the same chronological sequence through the season for.

Technical decisions: Within a production step, it is a matter of making a choice (for example, the choice of a clone), or of carrying out a given cultivation technique (this choice could be of not realising a certain act for that type of operation; for example: not to weed).

Implementation of decisions: The technical choice and its practical application can contribute to the creation of a field situation at risk. For example, the re-greening of the vine inter-rows creates a potential danger, which becomes real if competing species are seeded in a superficial soil; a weak nitrogen content of the grape berries is the possible consequence, which generates a physiological danger.

Danger: This is the consequence on the grape harvest of the application of the chosen cultivation practice. How does it affect the grape harvest status? The danger has to be characterised and classified into different categories according to its nature: phyto-sanitary (i.e., Botrytis on the grapes), physiological (i.e., ripening heterogeneity, internal composition of the berries), chemical (i.e., treatment residues, for example: copper on the berries), physical (i.e., plant waste in the grape harvest)…
An effort has to be made in order to obtain all the information needed to evaluate the risk:

- ☐ Is the danger severe what is the size of the potential danger)?
- ☐ Is it recurrent? ☐ In which case?
- ☐ Is it detectable?

Hence the following parameters of **"dangerous situations"**:

Intensity, or severity: Scale of the consequences of a specific decision due to the danger when it can be seen in the vineyard (for example, the re-grassing can decrease the nitrogen content between 15 and 25% in comparison to areas where it is not practiced). In phyto-sanitary terms this scale can be reported in different ways: % of the grape harvest, % of damaged field surface, % of affected plants or grape bunches; severity of the attack on grape bunches. The assessment can also be only qualitative (i.e., weak, moderate, severe consequences).

Frequency: recurrence of the agronomic problem. This is the outcome of two factors.

1- It will depend on the stability of the cause-effect link between the cultivation practice and the consequences for the grape harvest. This link can be variable and can depend, for example, on the yearly climate conditions which cannot be controlled. In this case, it is interesting to specify the type of year, when this phenomenon becomes apparent. The link can also be unsystemic, as it depends on the farming conditions (i.e., environment….); in this case it is important to specify these conditions.

2- When the links between cultivation practice and the consequent grape harvest is relatively well known and consistent, then the frequency in occurence of the agronomic problem depends on the importance of the practice under question. The practice can be applied rarely, moderately widespread or frequently, It is also useful to specify whether the application of such practices is increasing or decreasing .

Detectability: is it possible to observe the actual appearance of the consequences of specific agronomic practices (possibly through some indicators) in order to set up corrective measures, during the grape production process (and, thus, before the arrival of the grape harvest at reception platform)?

1.ORGANIC VITICULTURE
(Hofmann,U., with contribiution of:v.d. Meer,M.; Levite, D.)

General principles of organic agriculture

Organic viticulture is defined as the application of organic agriculture practices to produce grapes and wine of best possible quality.
Organic viticulture focuses on the use of natural processes wherever possible for nutrient production and recycling as well as pest, disease and weed management. The organic vineyard is seen as an integrated system for converting solar energy, soil nutrients and water into grapes, with the end product reflecting the local terroir: the environmental conditions like hydrology, soil and micro-climate as well as traditional processing practices.
All aspects of organic viticulture such as canopy, soil, and pest and disease control are managed to maximise the quality and the healthiness of the organically produced wine grapes. They are the fundamental background of organic wine making.
Organic viticulture in the European Union is based on the Council Regulation (EC) 834/2007 laid down in the objectives and principles for organic production and the general production rules.

1.1. Soil Management

1.1.1 General principles of soil management in organic viticulture

The soil, like water, air and energy, is one of our most important resources: Our future living conditions will depend most fundamentally on how well we manage these nourishing resources. The soil, by its physical structure and chemical composition, directly affects root system development and therewith the supply of water and minerals. A serious endangerment of ecological soil vitality, caused by the effects of pollution and high external input management systems, can be confirmed at a global level. The development and application of ecologically appropriate soil and land management systems represents an urgent challenge and is imperative because it allows the long term-maintenance of ecological soil fertility.

Fertility

Organic viticulture aims to increase the on-site natural soil fertility. The soil fertility is supported by a positive and stable combination of soil organisms' activity, soil condition, organic/matter humus supply, soil structure, well-balanced nutrient content and water conservation. Soil fertility, defined as a capacity of the soil to sustain long term plant production, has to be maintained and as far as possible improved. Organic viticulture is based, on the "living soil" and on the preservation of this resource. The main issues of an appropriate soil fertility management are:

- to maintain or improve an adequate content of organic matter / humus in the soil;
- to encourage the soil micro-organisms activity with a rich and well-balanced soil fauna and flora;
- to preserve a stable aggregate soil structure to guarantee the necessary balance between water and air;
- to keep the soil covered (temporarily or permanent) to minimize the effects of soil erosion;
- to allow mechanical operations, avoiding soil compactions;
- to enrich the soil with nutritive elements (macro and micro nutrients);

The soil, by its physical structure and chemical composition, directly affects root system development and in these case the vine water and mineral supplies.

Fig. 4: Loose and well structured soil sample (spate-diagnosis), Soil profile of a « Braunerde – Terra Fusca », with a superposition of the horizons A/B-T/C.

Soil Structure and Organic Matter

A good soil structure permits roots to develop a wider and deeper area, accessing more water, nutrients, and oxygen for their metabolic processes. Furthermore, a good soil structure increases the number and the diversity of terrestrial organisms, reduces the development of harmful ones, and favours the process of nutrient release by organic matter. A living and well balanced soil guarantees the health of the plant and the terroir-expression of the wines.

Stable organic matter is a major factor in improving soil structure and fertility. It binds soil particles into structural units called "living aggregates" or "clay – humus complex", significantly limiting erosion, soil compaction and the formation of surface crusts and plough pans. Organic matter also improves the water holding capacity of the soil, making more water available to the plants and soil micro-fauna. Stable organic matter comprises the energy and nutrient source for soil micro-organisms, which through their normal metabolism break down and transforms organic materials. The diversity and abundance of soil micro-organisms depends on the type and quality of organic residues in the soil. If properly fed, beneficial soil micro-organisms may sucessfully compete with pathogenic ones through their antagonistic activity, thus preventing or reducing soil-borne diseases.

1.1.2. Soil cultivation / Tillage

Soil cultivation has a strong influence on soil fertility and preservation and, as consequence, to farming. Especially under arid or sub-arid (Mediterranean) climate and in areas where erosion and desertification are a real threat. The choice of the most suitable cultivation system with regards to machines, methods and timing is fundamental to preserve the soil. It is also important in order to achieve a good but sustainable production level (yield and quality) which means producing without adversely affecting future production. Soil cultivation should be minimized to allow the organic vineyard to gain most benefit from a structurally stable and biologically active soil.

Therefore, the emphasis of soil preparation is on the maintenance and improvement of the natural fertility of the soil structure, as well as on encouraging microbiological and earthworm activity. This is achieved by the use of cover crops (legumes and grass crops) mowing, mulching, green manure, organic fertilisers and soil preparation.

Different Climatic Conditions			
Soil management options			Related documents
Arid – sub-arid climate Mediterranean Area *Avoidance of water competition and soil erosion. Improvement of soil structure to avoid compaction. Increasing green manure and soil fertility to avoid Nitrogen deficiency in the juice or mush.* Shallow soil cultivation, tillage in early spring, discing from spring to autumn in every row, compost use ■ Deep ripping after harvest ■ Winter cover crop	**Maritime – humid climate Atlantic/ Central Europe** *Avoidance of water competition and soil erosion. Improvement of soil structure to avoid compaction. Increasing green manure and soil fertility to avoid Nitrogen deficiency in the juice or mush.* Shallow soil cultivation over summer every second row Cover cropping or Mulching with straw, bark chips compost use if possible: permanent cover crop in every row ■ Winter cover crop ■ Deep ripping in early spring	**Continental- humid /dry climate Central / East Europe** *Avoidance of water competition and soil erosion. Improvement of soil structure to avoid compaction. Increasing green manure and soil fertility to avoid Nitrogen deficiency in the juice or mush.* Shallow soil cultivation over summer every second row Cover cropping or Mulching with straw, bark chips, compost use ■ Winter cover crop ■ Deep ripping after harvest or in early spring	Reference: climate condition for viticulture Reference: Fertilization management Assessment of environmental impact Cover crop management

Regulatory framework:
Regulation (EC) No 834/2007: Article 5: " the maintenance and enhancement of soil life and natural soil fertility, soil stability and soil biodiversity preventing and combating soil compaction and soil erosion, and the nourishing of plants primarily through the soil ecosystem"
Article 12: (a) "organic plant production shall use tillage and cultivation practices that maintain or increase soil organic matter, enhance soil stability and soil biodiversity, and prevent soil compaction and soil erosion"

Additional comments: Deep ripping has to be carried out carefully; it is not adapted to all soils. It is not suited to shallow soils or to all clay types. The humidity of the soil determine the time of soil tillage.
Ripping can hurt the roots of the vines.

Environmental impact: prevention of soil compaction and soil erosion, increasing of soil organic matter and natural soil fertility, increasing of soil biodiversity

Different soil conditions and soil fertility			
Soil management options			Related documents
Pervious and shallow soils (sand , gravel – stones, skeleton, schist), loss in humus *Avoidance of water competition and soil erosion, improvement of soil fertility with green manure, compost, organic fertiliser to avoid Nitrogen deficiency in the juice or mush.* Shallow soil cultivation, tilling in early spring, disking from spring to autumn every second row, compost supply, mulching with straw, bark-chips, If possible: perennial cover crop in every second row Drip irrigation ■ Winter cover crop	**Deep soils (clay, loam, loess) rich in humus – crumbly and loose** *Avoidance of water competition and soil erosion, loosening of compaction, improvement of soil fertility to avoid Nitrogen deficiency in the juice or mush.* Deep ripping in early spring ■ Tilling and seeding of annual summer crops / perennial crops, shallow soil cultivation over summer every second row Compost- organic fertiliser supply if possible: perennial cover crop in every row ■ Winter cover crop	**Compacted soil** *Loosening of compaction, increasing of soil structure, water holding capacity and fertility Avoidance of stowing wet, drought condition,* Deep ripping after harvest or in early spring ■ Seeding of deep rooting cover crop plants every second row, Compost/ humus supply Shallow disking ■ Winter cover crop	References: Cover crop management

Regulatory framework:
Regulation (EC) No 834/2007: Article 11: (a) "organic plant production shall use tillage and cultivation practices that maintain or increase soil organic matter, enhance soil stability and soil biodiversity, and prevent soil compaction and soil erosion"

Additional comments: Deep ripping has to be carried out carefully; it is not adapted to all soils. It is not suited to shallow soils or to all clay types. The humidity of the soil determine the time of soil tillage.
Ripping can hurt the roots of the vines.

Environmental impact: prevention of soil compaction and soil erosion, increasing of soil organic matter and natural soil fertility. Under arid conditions, excessive tillage, including disking, can contribute to humus carbonization. Under humid conditions, deep ripping or excessive tillage can increase soil compaction.

1.1.3. Cover crop management

A temporary or permanent green cover crop should fulfil, in organic viticulture, benefits in addition to those cited in chapter 1.1.1 and 1.1.2:

- Improvement of soil structure and water conservation from the presence of a permanent root system
- Nutrient supply for soil organisms (earthworms, micro-organisms) as basis for improved biological activity and availability of soil nutrients
- Nutrient supply adapted to the growth of grapes by a specific cutting management
- seeding with herbs and nitrogen fixing plants
- Support and stabilization of the fauna in the vineyard ecosystem

In viticulture, the most commonly used herbaceous species for the production of green manure are):

- Leguminoseae: bean, pigeon bean, vetch, Egyptian clover, crimson clover, red and white clover, lupine etc.
- Gramineae; rye, oat, barley, fescue, Italian or annual ryegrass etc.
- Brassicaceae / Cruciferae: canola - rape, oil-radish, white mustard etc.

Fig. 5: Blooming crimson clover, Phacelia and mustard seed (summer-cover crop)

Fig. 6 : Winter cover-crop with rape, winter-vetch, winter-peas and oil reddish

A diversification of plants is essential. Organic vine growing basically uses a multi species plant mixture. The choice of seed mixture depends on the duration of green cover (annual, perennial), soil conditions, texture, pH, and humus supply, time of seeding as well as the management of mowing, cutting or rolling.
Regarding the composition of a locally required mixture, it should be kept in mind that:

- The mixture should consist of different nitrogen fixing plants (legumes), grasses and flowering plants
- The selection of green cover plants should include slow and fast germinating seeds as well as medium and high growing plants
- At least half of the plants should be deeply rooting
- The mixture should be adapted to the time of agricultural use and the location
- The amount of seeding should be at the lower level to allow local wild herbs to germinate and grow together with the green cover.

Fig. 7 : Different cover-crop systems for humid (permanent cover crop) and arid climate (winter-cover crop) with vetch or barley.

Fig. 8: Cover crops in the vineyard: Onobrychis viciifolia Scop. (GB: Sweetvetch, FR: Sainfoin, DE: Esparsette) was formerly grown as feeding for cattle and horses. Root system of Sweetvetch and the symbiosis with the Nitrogen-fixing bacteria's

Cover-crop management

Soil management options			Related documents
Winter cover crop	**Winter / summer cover crop**	**Perennial cover crop**	
Improvement of water infiltration and soil fertility	*Improvement of water infiltration and water holding capacity, soil fertility and organic matter*	*Improvement of bio-diversity, biological pest control increasing of humus content*	Reference: Fertilization management
Winter cover crop ■ mowing in early spring, tillage and soil cultivation green manure weed control ■ Winter cover crop	Winter cover crop seeding august or after harvest ■ Tillage in early spring seeding of summer cover crop ■ Tillage end of June Soil cultivation, green manure ■ Winter cover crop	Shallow soil cultivation and seeding of perennial crops after harvest or in early spring ■ mowing or cutting ■ self reproduction, blooming- reseeding ■ Deep ripping after harvest	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (b) "the fertility and biological activity of the soil shall be maintained and increased by multiannual crop rotation including legumes and other green manure crops"

Environmental impact: Contribution of slow-release organic nitrogen; Improvement of soil's permeability and structure; Enrichment of the superficial strata of the soil with humus; limitation of erosion, surface water runoff and nitrogen / nutrient leaching; Promotion of fauna reproduction; Elimination of compaction problems related to cultivation; Induction of a better temperature regulation and of the soil's water-strata; Improvement of better water infiltration and stabilisation of water-holding capacity (avoiding of water competition); Weed control; supporting and stabilizing the arthropod fauna in the ecosystem vineyard which can be useful in pest control.

Additional comment: In areas with higher frost potential in spring, cover crops can be risky as: cover crop induced humidity lowers the frost-point.

1.1.4. Under-vine weed control

In organic viticulture, weed problems are not resolved by the use of chemical herbicides, but rather by means of agricultural practices, such as:

- Mechanical cultivation between the rows and /or mechanical and manual inter-vine cultivation
- Seeding of low vigorous plants and subsequent mowing interventions to control the vegetation.

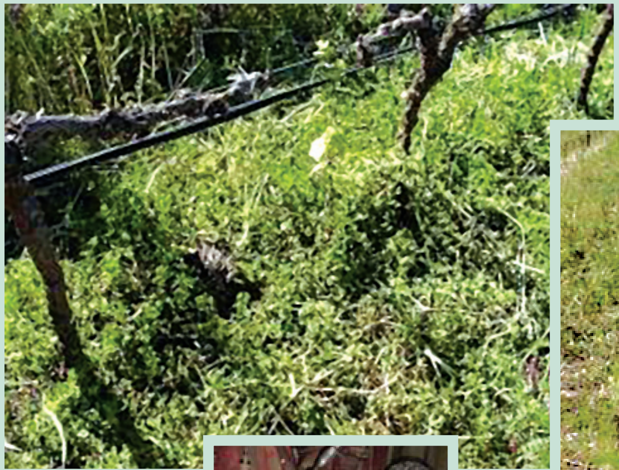
Apart from crop management, inter-vine cultivation plays an important role in the suppression of undesired competitors by an accompanying flora. The industry today offers a large range of different systems for the mechanical under-vine treatment from which the vineyard manager can choose according to the vineyard structure, soil, soil condition and slope of the vineyards. The machines used can be divided into:

1. Vine clearing ploughs with ridging and summer share, hilling and ploughing back share, hydraulic operated vineyard body with flat share and rotary tiller clearer.

2. Under-vine rotor mowers or under-vine clearer with rotating brushes.

Controlling the vegetation in the vineyard reduces the negative effects caused by competition with the vine for water and other nutrients.

A newer form of inter-vine weed control is the use of allelopathic plants. These plants release natural chemicals in the soil which impede or prevent the germination and/or the development of other plants. Currenty the most interesting allelopathic plants are *Hyeracium pilosella* and *Bromus tectorum*. They are especially interesting in arid climates because they have a (semi)dormancy period in summer, when the water stress is highest for the vines.



Under-vine cover-cropping with low vigorous clover

Straw mulch

Tournisol-under wine weeder

Under vine disk

Under vine flat-share

Fig. 9: Different biological and technical options for the under-vine weed control.

Under- vine weed control			
Soil management options			Related documents
Arid – sub-arid climate Mediterranean Area	**Maritime – humid climate Atlantic/ Central Europe**	**Continental- dry climate Central / East Europe**	
Avoidance of water or nutrients competition and soil erosion, *Improve suppression of undesired flora* Mechanical inter-vine cultivation, thermal weed control, hand hoeing Hilling up after harvest ploughing back in early spring or Seeding of *Trifolium subterraneum* or Medicago species as a winter crop, self reproduction, reseeding.	*Avoidance of water or nutrients competition and soil erosion, Improve suppression of undesired flora* Mechanical inter-vine cultivation, thermal weed control, hand hoeing Mulching with straw, bark chips, compost or organic matter Seeding of low vigour perennial clover (*Trifolium repens* var. Haifa; *Trifolium fragiferum*, *Medi-cago lupulina*; *Lotus tenuis, L. corniculatus*) Mowing with under vine mower or brusher, hand mowing	*Avoidance of water or nutrients competition and soil erosion, Improve suppression of undesired flora* Mechanical inter-vine cultivation, thermal weed control, hand hoeing Mulching with straw, bark chips, compost or organic matter If irrigation available: Seeding of low vigour perennial clover Mowing with under wine mower or brusher	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (b) “the fertility and biological activity of the soil shall be maintained and increased by multiannual crop rotation including legumes and other green manure crops”

Environmental impact: Contribution of slow-release organic nitrogen; Improvement of soil’s permeability and structure; Enrichment of the superficial strata of the soil with humus; Reduction of erosion; Promotion of fauna reproduction; Weed control; supporting and stabilizing the arthropod fauna in the ecosystem vineyard which can be useful in pest control.

1.1.5. Fertilization and plant nutrition

“Feed the soil and not the plant” is the main organic principle regarding plant nutrition. The intent of this approach is to imitate natural nutrient cycles which provide the soil with mineral nutrients upon the base of soil material and organic matter. Fertilization in organic viticulture is based on an as low as possible input of nitrogen. The mains types of manure used are: green manure and distribution of moderate quantities of ripe organic manure or compost, vine prunings and winery pressings during autumn-winter-spring.
The green manure practice consists of sowing seeds of a single species or of mixtures of herbaceous species, without aiming on the collection / harvesting of the products, but on the incorporation of the green biomass into the soil. The utility of green manure is traditionally recognized in viticulture as a post harvest or early autumn seeding, sowing winter cover crop (legumes like: vetch, beans, peas in combination with rape seed, gramineae, ray-grass or crimson clover), especially where fertilization is problematic due to environmental conditions. In regions with more spring-summer rain a summer cover crop with legumes, buckwheat, Phacelia, oil radish or mustard seed is also common. In case all these practices and products are not sufficient to support crop production or soil quality, a limited list of fertilisers and soil conditioners may be used. The sum of all nitrogen inputs should not exceed the limit of 170 kg N/ha per year (EEC Reg. 834/2007). Yet, this is a maximum value. When low levels of soil N are encountered it is important to assess the contribution by soil humus to the N levels. The recommended value of nitrogen is 50 – 70 kg/ha and year.


Fig. 10 :On farm compost production and the use of the Bio-dynamic Compost Preparations (502-507)

Allowed inputs:
- Animal manures and by-products such as fish meal, blood and bone meal
- Farmyard compost; composted or fermented household waste or mixture of vegetable matter
- Minerals from natural sources including gypsum, lime, clays, rock phosphate & pot-ash, crude potassium salts, potassium sulphate containing magnesium salt
- Biological preparations, organisms and their by-products
- Plant by-products such as wood chips, composted bark, wood ash and straw
- Seaweed and algal preparations
- Trace elements (only natural chelating agents allowed)

Different soil fertility			
Fertilisation options			Related documents
poor in humus (< 1,5%) low soil fertility - low vigour, stressed vines	**rich in humus (> 2,5%), high biological activity and soil fertility - high vigour**	**Specific mineral deficiencies**	
Improvement of soil fertility with green manure, compost or organic fertilizer to avoid Nitrogen deficiency in the juice or mush.	*Avoidance of nitrogen loss, reducing of vigour and disease susceptibility*	*Avoidance of nutrient un-balance Improve stabilisation of grape healthiness and ripeness*	References: Cover crop management Technical notes: Authorized organic fertilizer Annex I,IIA
Seeding of winter / summer cover crop as green manure, shallow soil cultivation Farmyard compost supply (high quantity), Addition of organic fertilizer	Seeding of perennial crops, Compost supply (low quantity) None addition of organic fertilizer Mulching with straw or bark chips	Soil or plant analysis Addition of specific authorized mineral fertilizer - gypsum, lime, clay, - rock phosphate & potash, - crude potassium salts, potassium sulphate - magnesium sulphate - trace elements	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (b) “the fertility and biological activity of the soil shall be maintained and increased by multiannual crop rotation including legumes and other green manure crops, and by the application of livestock manure or organic material, both preferably composted, from organic production;”
Authorized organic and mineral fertilisers included in Annex IIA
National regulations of manure and compost use

Environmental impact: increasing soil organic matter and natural soil fertility, avoidance of nutrient / nitrogen leaching

Additional comment:
A nitrogen deficit in grapes and in musts can affect not only all nitrogen components of the berry (NH4 and amino acids) but also, as an indirect consequence, certain aromas or flavour precursors such as the cysteine derivatives, which are for example present in the variety Sauvignon

1.2. Vineyard Management

1.2.1. Varieties

Viticulture in Europe has a long-term tradition with locally adapted Vitis vinifera varieties. They are adapted and appropriate to the local climatic and soil conditions from hot dry summer and winter rainfall to cool climate with warm and humid summer conditions. Some varieties grow quite well under hot and dry summer conditions but are sensitive to winter frost. Others, adapted to cool-moderate climate with high frost resistance, are sensitive to drought and water stress or sunburn.
One principle of organic viticulture is the use of varieties, species and rootstocks which are appropriate and suitable to the climate and general agricultural conditions. It is evidently best to choose local, autochthonous (indigenous) varieties, which usually have a greater inherited resistance to the main pathogens and pests of the regions. Pest and disease resistance or tolerance varies from one grape variety to another.
All Vitis vinifera varieties are exposed to a wide range of diseases and pests like Powdery Mildew (Erysiphe necator-Oidium), Downy Mildew (Plasmopara viticola), Grey-mould (Botrytis cinerea), Eutypa dieback, Esca and grape berry moths infection and need specific organic plant protection measures.
Currently, none of the existing varieties are able to resist an infection from the principal diseases. Yet, there are different degrees of susceptibility which range from "very susceptible "to" resistant" (Tab 1- 2). Amongst the traditional European varieties, one seldom finds varieties that would be classified at being no more than "tolerant". This means that there is resistance to a low disease pressure if plant protection measures are combined with canopy management.
In recent years, a new generation of disease-resistant vine varieties have been developed by cross-breeding different vitis species with Vitis vinifera varieties (Tab. 3). These so called "interspecific hybrids" or PIWI (from the German expression "pilzwiderstandsfähig" = apt to resist to fungi) are not accepted for Quality Wine production in some European countries, although the latest generation of these hybrids are accepted in some countries, e.g. Germany, Switzerland, Austria, Hungary and Czech-Republic.

Tab. 1: Resistance of common, widespread white grape varieties against the main diseases

The varieties assessed as "tolerant" or "robust" in the table beneath are not resistant against diseases; they are only less susceptible when grown in combination with optimal plant protection and canopy management. Varieties resisting or tolerating the most widespread diseases in the area should be chosen, if these varieties meet production and market requirements.

Variety	Downy Mildew Plasmopara viti cola	Powdery Mildew Erysiphe necator -Oidium	Grey- mould Botrytis cinerea	Black rot Guignardia bid-welii
White Grapes				
Pinot blanc,- bianco, Weißburgunder	Tolerant	Robust	Susceptible	Tolerant
Pinot gris, - grigio Grauburgunder, Rulandsky Bile	Tolerant	Tolerant	Susceptible	Tolerant
Chardonnay	Very Susceptible	Very Susceptible	Susceptible	Susceptible
Garganega	Tolerant - Susceptible	Tolerant	Tolerant	Tolerant
White Riesling, Ryzlink rynsky	Tolerant Susceptible	Tolerant Susceptible	Very Susceptible	Susceptible
Gray-Welschriesling Riesling Italico, Olasz Riesling	Tolerant	Robust	Susceptible	?
Viognier, Viogne	Tolerant	Tolerant	Susceptible	Susceptible
Grüner Veltliner	Very Susceptible	Susceptible	Tolerant	?
Trebbiano, Ugni blanc	Susceptible	Susceptible	Robust	Susceptible
Sauvignon blanc	Tolerant	Very Susceptible	Susceptible	Very Susceptible
Traminer, Clevner; Tramini piros	Tolerant	Robust	Tolerant	Tolerant
Semillon	Tolerant	Robust	Very Susceptible	Tolerant
Müller-Thurgau	Very Susceptible	Susceptible	Very Susceptible	Susceptible
Mauzac (F)	Robust	Robust	Susceptible	
Maccabeo (E)	Tolerant	Very Susceptible	Very Susceptible	
Furmint (HU)	Susceptible	Very Susceptible	Tolerant	Susceptible
Colombard	Tolerant	Very Susceptible	Tolerant	Susceptible
Chenin blanc	Tolerant	Susceptible	Very Susceptible	Robust

References:
Ambrosi, H. et al. 1998 Farbatlas Rebsorten, Ulmer Verlag
Lott, H. & Pfaff, F. 2003, Taschenbuch der Rebsorten, Fraund Verlag
Vitis International Variety Cataloque: http://www.vivc.bafz.de/index.php;
European Vitis Database: http://www.genres.de/eccdb/vitis/;
French Vitis database http://www1.montpellier.inra.fr/vassal/collections/liste.php;
Greek Vitis database: http://gvd.biology.uoc.gr/gvd/index.htm
US National grape register: http://www.ngr.ucdavis.edu/

Tab. 2: Resistance of common, widespread red grape varieties against the main diseases

The varieties assessed as "tolerant" or "robust" in the table beneath are not resistant against diseases; they are only less susceptible when grown in combination with optimal plant protection and canopy management. Varieties resisting or tolerating the most widespread diseases in the area should be chosen, if these varieties meet production and market requirements.

Variety	Downy Mildew *Plasmopara viticola*	Powdery Mildew *Erysiphe necator -Oidium*	Grey- mould *Botrytis cinerea Black rot*	Guignardia bidwelii
Red Grapes				
Pinot noir, - nero, Spätburgunder	Susceptible - Tolerant	Susceptible - Tolerant	Very Susceptible	Susceptible
Barbera	Susceptible - Tolerant	Susceptible - Tolerant	Robust	?
Cabernet Franc	Susceptible	Very Susceptible	Susceptible	Susceptible
Cabernet sauvignon	Tolerant	Very Susceptible	Robust	Very Susceptible
Canaiolo nero	Very Susceptible	Very Susceptible	Susceptible	?
Carignan noir, Carignano, Cainena	Susceptible	Very Susceptible	Very Susceptible	Susceptible
Cinsault, Hermitage	Susceptible	Susceptible	Susceptible	Susceptible
Malbec	Susceptible	Robust	Susceptible	Susceptible
Dornfelder Very	Susceptible	Very Susceptible	Tolerant	Susceptible
Gamay noir, Game	Susceptible	Susceptible	Susceptible	Susceptible
Grenache, Garnacha, Cannonau	Very Susceptible	Tolerant - Robust	Very Susceptible	Susceptible
Kadarka	Tolerant	Tolerant	Robust	Tolerant
Lagrein	Susceptible	Susceptible	Susceptible	Susceptible
Lambrusco	Tolerant	Tolerant	Susceptible	Tolerant
Lemberger, Blaufränkisch, Kekfrancos	Susceptible – Tolerant	Very Susceptible	Tolerant	Susceptible
Merlot	Very Susceptible	Tolerant	Tolerant - Susceptible	Very Susceptible
Monastrell, Mourvedre	Susceptible	Susceptible	Robust	Tolerant
Montepulciano, Uva Abruzzi	Susceptible - Tolerant	Susceptible - Tolerant	Tolerant - Robust	Susceptible

Variety	Downy Mildew *Plasmopara viticola*	Powdery Mildew *Erysiphe necator -Oidium*	Grey- mould *Botrytis cinerea Black rot*	Guignardia bidwelii
Red Grapes				
Nebbiolo	Tolerant	Very Susceptible	Susceptible	Tolerant
Nero d'Àvola	Susceptible - Tolerant	Susceptible	Susceptible	?
Pinotage	Susceptible	Susceptible	Susceptible	Susceptible
Portugieser, Portugalski modré, Oporto,	Very Susceptible	Very Susceptible	Susceptible	Susceptible
Saint Laurent	Susceptible	Very Susceptible	Very Susceptible	Susceptible
Sangiovese	Susceptible	Tolerant	Susceptible - Tolerant	Susceptible
Syrah; Shiraz	Tolerant	Robust	Susceptible	Tolerant
Tempranillo	Tolerant	Susceptible	Tolerant	Susceptible
Zweigelt	Susceptible	Susceptible	Susceptible	Susceptible

Fig. 11: White varieties: Cabernet blanc (PIWI- disease resistant), Pinot gris (Vitis vinifera)

Tab.3: Resistant – disease tolerant varieties

The list in the table beneath shows the evaluation of some resistant varieties by their breeders. The evaluation is based on field observation and assessed in five resistance levels (very low – low – medium – good – very good; "---" = "no declaration available"). This list is only a small extract of all available varieties; it shows varieties which are nowadays frequently grown, especially in Austria, Switzerland, Germany and East-Europe.

Colour	Variety	Resistance Peronospora Leaf	Resistance Peronospora Grape	Resistance Oidium Leaf	Resistance Oidium Grape	Resistance Coulure	Resistance Botrytis	Resistance Cold
Red	Baco noir	good	good	good	good	---	---	---
Red	Baron	good	good	good	good	medium	---	---
Red	Cabernet Carbon	v good	v good	medium	medium	v low	---	---
Red	Cabernet Carol	v good	v good	good	good	v low	---	---
Red	Cabernet Cortis	v good	v good	good	good	low	---	---
Red	Cabernet Jura (VB 5-02)	v good	v good	v good	v good	---	v good	v good
Red	Cabertin (VB 91-26-17)	good	good	good	good	---	good	v good
Red	Chambourcin	good	good	good	good	medium	---	---
Red	Chancellor	v good	v good	v good	v good	low	---	---
Red	De Chaunac	medium	medium	medium	medium	medium	---	---
Red	Landal	medium	good	good	good	low	---	---
Red	Léon Millot	medium	good	good	v good	low	---	---
Red	Marchéchal Foch	good	good	good	v good	low	---	---
Red	Monarch	good	good	medium	medium	low	---	---
Red	Pinotin	good	good	good	good	---	good	v good
Red	Plantet	good	good	good	good	---	---	---
Red	Prior	v good	v good	v good	v good	v low	---	---
Red	Regent	low	good	good	v good	medium	---	---
Red	Triumph vom Elsass	---	---	---	---	v high	---	---
Red	VB 91-26-4	good	good	good	good	---	good	v good
Red	VB 91-26-5	good	good	good	good	---	---	good
White	Bianca	good	good	good	good	strong	---	---
White	Bronner	good	good	good	medium	weak	---	---
White	Cabernet blanc (VB 91-26-1)	good	good	good	good	---	good	good
White	Helios	medium	good	v good	v good	low	---	---
White	Johanniter	medium	good	good	v good	low	---	---
White	Merzling	medium	medium	medium	medium	medium	---	---
White	Orion	medium	medium	medium	medium	medium	---	---
White	Phoenix	v good	v good	v good	v good	medium	---	---
White	Saphira	medium	medium	good	good	low	---	---
White	Seyval blanc	medium	good	good	v good	low	---	---
White	Sirius	good	good	good	good	---	---	---
White	Solaris	medium	good	v good	good	low	---	---
White	Soleil blanc	good	v good	v good	v good	low	---	---
White	Staufer	good	good	good	good	medium	---	---
White	Vidal blanc	medium	good	v good	v good	low	---	---

Source: PIWI-International (http://www.piwi-international.org/index.htm).

Fig. 12: Red Varieties: Pinotin (resistant), Blau-fränkisch – Kekfrancos, Merlot (Vitis Vinifera)

1.2.2. Trellis system and canopy management

In all European wine regions traditional training or trellis systems are adapted to the local climatic conditions. The production of quality wine grapes relies on two basic characteristics of the trellis systems.

- The first is adequate functional leaf area, which is the source of the soluble solids that are transported to the fruit. Therefore, a characteristic of a good vine training system is the ability to display a large amount of leaf area in a way that all leaves are well exposed to the sun.
- A second basic characteristic of a good vine training system is the exposure of fruit to the sun. This is most important in a cool to moderate climate because the temperature of the fruit during the period of its ripening directly influences the reduction of acid levels and increases the specific aroma profile in the fruit.

Open, airy canopies have higher levels of airflow and light interception, which aids foliage to dry and reduces diseases susceptibility.
Well structured canopies are easier to monitor than crowded “free-form” canopies.
It is easier to achieve thorough spray penetration and distribution throughout open, well structured canopies as opposed to dense, crowded canopies.
The management for an open well structured canopy includes, depending on soil fertility and climatic conditions:

- Careful winter pruning depending on trellis system, yield and quality.
- Disbudding of suckers and side shoots removal before blossoming.
- Shoot positioning, thinning, topping, trimming, leaf and bunch removal
- Increasing of loose-clusters (colouring) by spraying a sulphur / sodium silicate mixture in blooming time
- Cluster splitting and bunch thinning between the fruit setting and the beginning of the grapes’ closure.

Fig.12: Vineyard before and after shoot and bunch removal, cluster splitting and bunch thinning.

Fig. 13: Different appropriate vine training-systems (trellis system with horizontal cane, Lyra-system).

Fig. 14: Different appropriate vine training-systems (System-Guyot, System Goblet, bush-training).

Fig. 15: Different appropriate vine training-systems (umbrella system, minimal-pruning).

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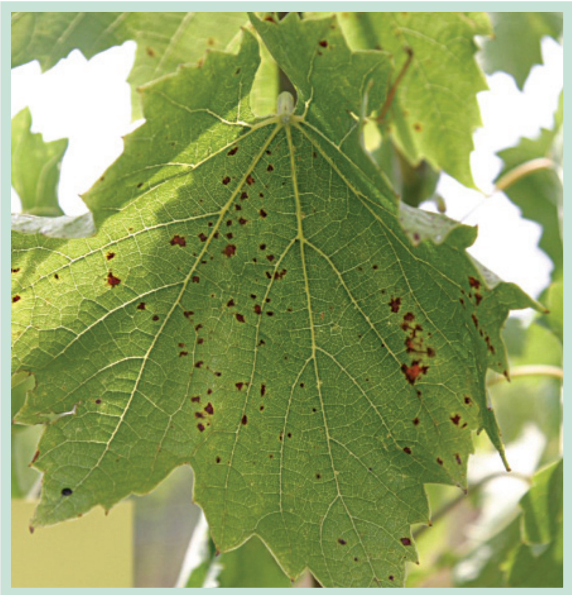

Fig. 16: Reaction of a PIWI –disease resistant variety to the fungal attack[2].

[2] The variety "Bronner" produces a suberization (hyper sensibility) reaction, what means that the plant defends itself by desiccating the fungi.

1.3. Plant protection

In organic viticulture, there are five main principles of plant protection:

- fertility and health of the soil
- viticulture practices, appropriate varieties and training systems
- timing of the protection measures and application methods
- Encouragement of plant vigour to enhance natural defence mechanisms
- biological pest control and habitat management.

The knowledge of the fields and of the soil's characteristics, the weather conditions and seasons which affect the vineyard, also influence plant protection measures.

One of the primary interests in organic viticulture is to grow healthy and disease resistant plants. Most of the widely used cultivars are not resistant to fungal infection (see chapter 1.2.1). With the help of plant health enhancing products like plant strengtheners and natural fungicides, which are accepted by organic standards and with the correct soil and plant management the control of fungal diseases through the induction and enhancement of the plant's own defence mechanisms should be implemented. This does not involve the application of toxic compounds to plants.
For example vine management techniques such as inter-row and under-vine planting, herbal leys, green manure, mulching, mowing, soil amelioration, compost applications, choice of suitable varieties and rootstocks, training and pruning techniques as well as an appropriate canopy management should be implemented to enhance the health and quality of the vine and its fruit.
Organic fungicides like copper, sulphur or acid clays should be used to manage fungal problems as a last resort as it is these products that are the only organic weapons which reliably protect the plants against fungi attacks.
The use of copper is problematic because of its poisonous effect on the soil's flora and fauna. However it should be remembered that it is an oligo-element which is necessary for essential life-processes not only in mammals but also in plants. Plants lacking copper results in an inability to build up certain proteins. This incapacity necessitiates a "copper-input" of up to 5 kg Cu/ha every 5-8 years.

The producer investigation (WP 2.2.) has indicated different disease levels in European Organic Vineyards and Regions.
Many scientists agree that an attack of over 10% by a mould disease can adversely affect wine quality.
According to producers declarations this disease level is rarely reached in countries like Spain, Italy and France, but is reached more frequently in others like Germany, where 70% producers must face this situation at least every 3 years. This depends of course on the climate, but has also a big influence on the wine-making technology and specifically on SO_2 additions.

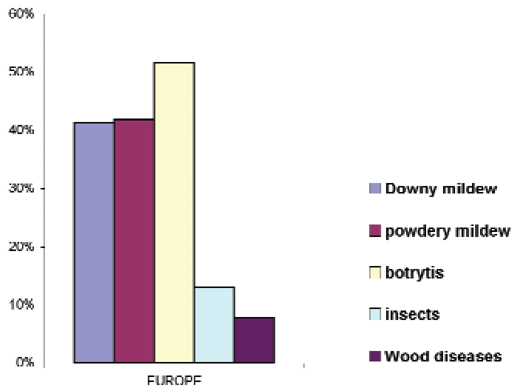

Fig.17: Different disease levels in European winegrowing areas.

Source: Micheloni, C.; Trioli, G. (2006): Producer investigation about current oOenological practise. www. Orwine.org

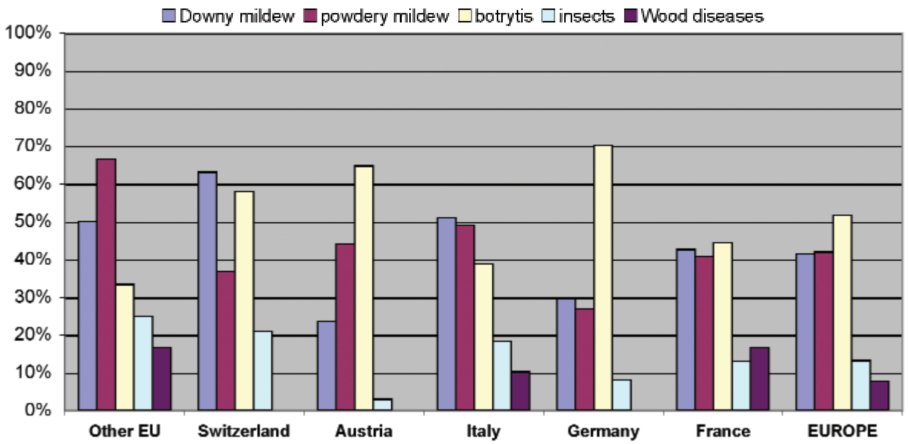

Fig.18: The most frequently induced plant-protection problems in organic vineyards in Europe.

Source: Micheloni, C.; Trioli, G. (2006): Producer investigation about current oOenological practise. www. Orwine.org

Where grape diseases are more frequent - Botrytis is the major problem as in, Germany, Austria and Switzerland. This means that in these countries a reduction of total SO_2 in organic wines will be more difficult to obtain.
Powdery mildew, the second disease in terms of potential negative effect on wine quality, is also a major concern in Spain, Italy, and France, as well as in other EU-countries.
Interestingly, insect problems and wood diseases are very secondary concerns for Germany and Austrian producers.

1.3.1. Major diseases

1.3.1.1. Downy mildew or Peronospora (*Plasmopara viticola*)

Downy mildew is one of the most harmful grapevine diseases in all European wine growing zones. The pathogen can infect all of the vegetative organs of the vine such as leaf, tip, flower, cluster, stalk and young fruit. Various symptoms can be observed, corresponding to the different stages of the disease cycle: the “oil spots”, the whitish mould and the necrotic tissues. There can be numerous infections during the season. The most critical phases for downy mildew infection and yield loss are from beginning of blooming to fruit set.
The greatest damage done by the fungus is the infection of the cluster of young berries and the stalks with an extremely high fruit loss. The infected and damaged berries dry out and drop. There is a minimal influence on wine quality. A late downy mildew attack can cause total leaf loss in certain very susceptible varieties. Almost all of the *Vitis vinifera* varieties are susceptible to downy mildew. At the moment, organic viticulture cannot exclude direct plant treatments, but weather and infection forecasting models[3] can help to establish the plant treatment and can reduce the number of treatments required.

Fig. 20: A “LUFFT” weather station which can be used to model the attack-pressure of fungi-diseases.[4]

[3] Diseaseforecasting models: Switzerland: http://www.agrometeo.ch ; Germany: Viti Meteo Plasmopara; http://www.dlr-rheinpfalz.rlp.de

[4] The “LUFFT” weather station can be installed and removed easily. The measure software is easy to handle. The station is driven electrically, the energy being produced by solar panels.

Fig. 21: Leaf infections by downy mildew (Oil spot and new Sporulation).

Fig. 22: Flower and berry infections (with new Sporulation) by downy mildew

Control

Indirect measures: As grape cultivars vary in their susceptibility to downy mildew, selecting the least susceptible cultivars may reduce the overall risk of this disease (see chapter 1.2.1). Management interventions such as sucker removal, defoliation and thinning around bunches, are not directly effective against the pathogen, but they are effective in reducing crop treatments.

Direct measures: The principal antifungal agent used in organic viticulture is copper, in its different chemical formulations (oxychloride, hydroxide, tribasic sulphate, oxide, and oxalate). Recently, the use of copper in organic viticulture has been limited to 6 kg / ha and year of metal copper (30 kg in the average of five years) (EEC Reg. 834/2007; some national plant protection laws are more restrictive). In some member states the use of Potassium-Phosphonate in combination with amino-acids and oligosaccharids (algae-extracts) is allowed as a plant strengtheners or a foliar feed. Potassium-Phosphonate works as a trigger / stimulator that encourages the plant self defence mechanism (production of phytoalexine). The use is recommended in the extreme growing period between the start of flowering and fruit set.
The use of plant strengtheners such as sulphuric acid clay or lime stone products is possible and successful. They can help to reduce the copper content per ha and year.

Plant protection strategies			
Downy mildew			Related documents
- no risk	low infection pressure	high infection pressure	
Planting of high resistant grape varieties (PIWI) reduces the use of cop-per treatments	*Dry weather conditions, low rainfall, no dew, low humidi-ty < 40%; late primary infection (after blooming) day temperature > 30° night temperature < 10°*	*Wet and warm weather conditions, high or perma-nent rainfall, dew, high hu-midity > 95% early primary infection day temperature < 30° night temperature > 20°*	Reference: canopy management
Two plant treatments with low copper content or plant strengtheners (like sulphuric acid clay) before and after blooming Well structured canopy	Weather forecasting system Well structured canopy Application methods, timing of the treatments Spraying every second row Treatments with low copper content (100 – 500 g Cu/ha per spray) or plant strength-ener	Weather forecasting system Well structured canopy Application methods, timing of the treatments Spraying every row, weekly treatments with high cop-per content (500 – 1000 g Cu/ha per spray), 3 applications of Potassium-phosphonate between pre-flowering and fruit set maximum use of Copper: 6kg Cu/ha *(30 kg in the aver-age of 5 years)*	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, culti-va-tion techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Additional comments:
Selective harvesting, sorting and destemming are necessary, infected berries can influence the quality of the wine (mush-fermentation by red wine)
Copper has a negative role on the expression of sulphuric aromas such as the « thiols ».
Copper treatments increase the skin thickness; this thickening favours a better resistance to the diseases occur-ring at the end of the year: grey rotting and acid rotting.

Environmental impact: Copper is a heavy metal which remains in the soil and which is toxic for some micro-organisms. Long term strategies to reduce the amount of copper are necessary.

1.3.1.2. Powdery mildew, Oidium, *Erysiphe necator*; *Oidium tuckeri*

Grapevine powdery mildew or Oidium is a widespread fungal disease which attacks the leaves, flo-wers, grape-berries and shoots of the vine. The infection can cause crop loss and reduce vine growth, fruit quality and wine quality. It is the economically most important grapevine disease wor-ldwide.
As the fungus grows, and especially when it produces spores, it gives infected tissue an ash grey powdery appearance. The fungus grows during the whole vegetation period and can penetrate the cuticle of the grape-berries or of the leaves.

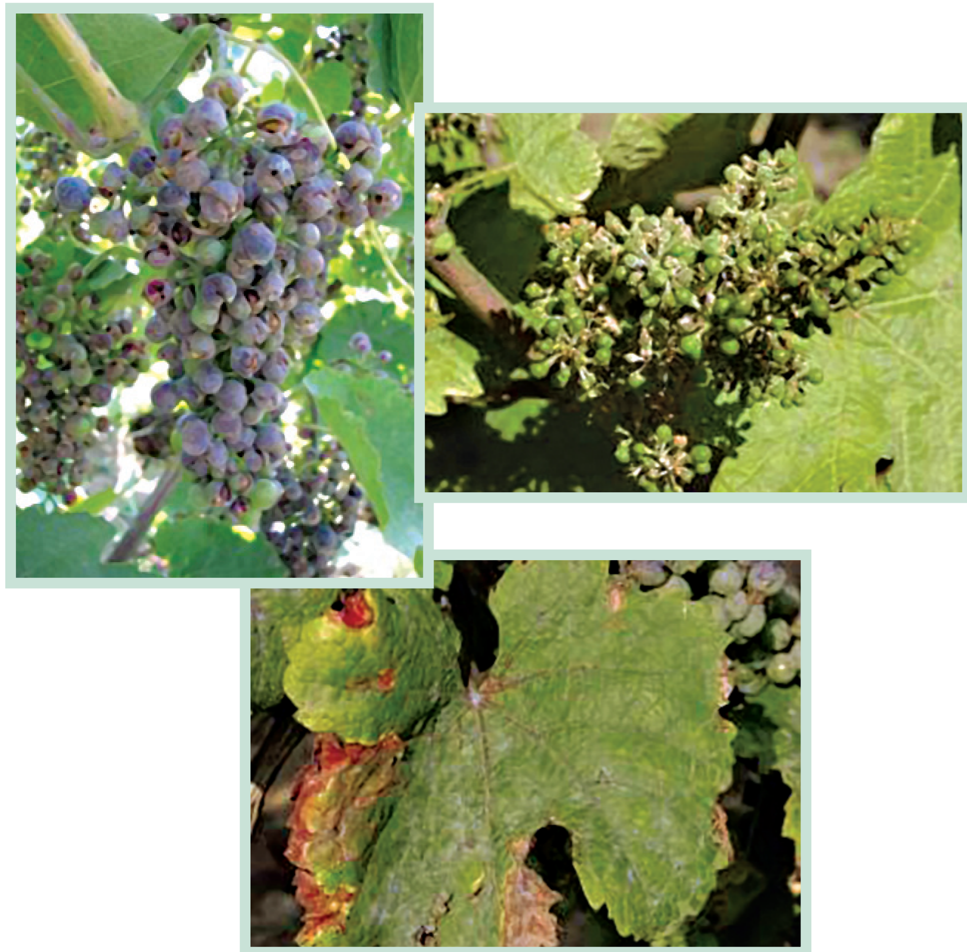

Fig.23: Leaf, flowers and berry infection by powdery mildew

Control

Indirect control measures: As grape cultivars vary in their susceptibility to powdery mildew, selecting the least susceptible cultivars may reduce the overall risk of this disease (see chapter 1.2.1). Management interventions such as sucker removal, defoliation and thinning around bunches, are not directly effective against the pathogen, but they are an effective help in the distribution and accumulation of the plant treatments.

Direct control measures must be initiated early, immediately after sprouting, to lower the number of spores present in the vegetation and prevent attacks. This is true especially in vineyards in which this pathogen has caused serious damage in the previous year.

In organic vine growing Oidium control is essentially based on the use of sulphur in forms as powder (raw, ventilated, activated and copper) and wettable (micronized, colloidal, liquid). Other effective methods to control powdery mildew are the use of an antagonist fungus (Ampelomyces quisqualis AQ10), of Potassium-bicarbonate (baking powder), of plant extracts (fennel oil, equisetum-extract, soya-lecithin) or of sodium / potassium silicate.

Fig. 24: Ladybird beetle (Thea vigintiduopunctata) it´s a powdery mildew hyphen eating beneficial, established in Mediterranean and Central European vineyards.

Plant protection strategies

Powdery mildew			Related documents
- no risk	**low infection pressure**	**high infection pressure**	
Planting of highly resistant grape varieties (PIWI) reduces the use of sulphur treatments.	*Dry weather with low humidity < 30%, Rainfall with high humidity > 90% Temperature <7° or > 35° windy*	*Wet and warm weather conditions, dew, humidity 70 – 90% day temperature < 27° night temperature > 15° High infection in the year before, flag shots in early spring*	Reference: canopy management
Two plant treatments with sulphur (wettable or dust) or plant strengtheners before and after blooming Well structured canopy	Weather forecasting system Well structured canopy Optimal air flows Application methods, timing of the treatments Spraying every second row before blooming Treatments with sulphur or plant strengthener (Potassium-bicarbonate, plant extracts, soya lecithin, sodium silicate), Ampelomyces quisqualis AQ10 Bacillus subtilis	Weather forecasting system Well structured canopy Application methods, timing of the treatments Spraying every row, weekly treatments with sulphur (wettable 4 – 10 kg) 3 – 4 application with sul-phur dust (30 kg/appl.), Two "bunch-washing" –application (1000l water with K-soap only in the berry zone after blooming and before bunch closure) Potassium-bicarbonate in combination with plant extracts (fennel oil) and sul-phur Ampelomyces quisqualis AQ10 Bacillus subtilis	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cultivation techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Additional comments: Infected berries and bunches: influence the quality of the wine, are the starting point for secondary rot infection, destroy the typical grape flavor, increase a "mushroom" flavor, increase the necessity of specific wine making practices. Infected berries have to be avoided in wine making by selective harvesting and sorting the grapes, destemming and whole bunch pressing for white/rose wine.
Sulphur residues on the berries might induce "off-flavours" in the wines; late treatments with sulphur against Oidium are not practiced (these, except in case of accident, do not go beyond the closed grape stage).

Environmental impact: Excessive use of Sulphur can induce environmental imbalances in the vineyards by destroying useful predators like phytoseiidae or parasitic wasps, which are essential for biological pest control. Sulphur can help to control spider mite infestation.
Plant extracts/oils can increase the population of predators; K-bicarbonate has a side effect against leafhoppers. Soya lecithin can lead to phytotoxicity in the vines.

1.3.1.3. Botrytis cinerea - mould (Botrytis blight, Botrytis bunch rot, Sour rot)

One of the principal causes of crop quality degradation is grape rot. The most important fungal pathogen responsible for bunch rot of grape berries is *Botrytis cinerea*. This fungus can grow on any plant material that is succulent, stressed or dead on an extremely wide host range. It is especially problematic as a result of relative high humidity and frequent rainfall that has created a microclimate suitable for fungal development. Times when disease pressure is evident can occur from bunch closure right through to harvesting time.

Bunch rot infection by B. cinerea alone or associated with other micro-organisms like acetic acid bacteria, natural wild yeasts eg. *Kloeckera apiculatus*, *Metschnikowia pulcherima* or *Candida ssp*. as well as *Penicillium sp*. *Aspergillus niger*, *Cladiosporum sp*. fungi is one of the major problems in organic viticulture encountered in the last few years, due to climate change. In some regions and some years this complex of micro-organisms induced disease has dramatically decreased wine quality and influenced the implementation of specific wine making practices. In contrast to noble rot, grey rot often causes aromatic defects.

The so called "Noble rot" requires specific environmental and weather conditions. In a few areas of the world, particular conditions permit *Botrytis cinerea* to develop on mature grapes. This process results in an over ripening which increases the sugar concentration and so improves the wine quality, conferring sweet white and rose wines their specific qualitative aromas.

Fig. 25:Bunch and sour rot induced by Botrytis cinerea.

Control

Currently there is no really efficient control measures against Botrytis in organic viticulture. Most of the products and methods mentioned below are in a more or less experimental state, sometimes working, sometimes not.

Indirect measures: Since Botrytis spores have specific environmental requirements for germination and growth, control can be obtained by creating a canopy microclimate that restricts disease development. The objective is to increase the exposure of the grape clusters to air and light so that they dry out more quickly after a wetting. Indirect measures can include the selection of trellis systems, pruning methods, shoot positioning, leaf plucking, shoot thinning in the fruit zone, increase of loose-clusters (coulouring) or cluster splitting and bunch thinning as well as irrigation, fertilizer strategy, avoiding nitrogen excesses, rootstock selection, clonally selection or planting density.

Fig.26: Red grape variety with open cluster, canopy management with cluster splitting.

Direct measures: Applications of silica in the form of sodium silicate, equisetum extract or Potassium-bicarbonate can harden the cuticle and protect the berries from bunch rot infection. Copper application has the same thickening effect. Some biological fungicides based on antagonist fungi, *Trichoderma herzianum* or *T. viride*, *Ulocladium oudemansii* or bacteria, *Bacillus subtilis sp.*, which develops to the detriment of the pathogen, is also used in organic viticulture.

Plant protection strategies			
Grey-mould, Sour rot			Related documents
no risk	low infection pressure	high infection pressure	
dry, warm weather with low humidity < 50%, windy, favourable ripening conditions balanced fertilisation, avoiding of nitrogen excess	*dry to humid weather conditions, low rainfall, low night temperature < 10° balanced fertilisation, avoiding of nitrogen excess*	*Wet and warm weather conditions, dew, foggy, permanent humidity 70 – 100% day temperature < 25° night temperature > 15° in harvesting time*	Reference: canopy management. Oidium protection, Grape berry moth protection
varieties of low susceptibiity, open loose clusters well structured canopy, canopy management (shoot, leave, bunch removal, plugging thinning), coulouring – cluster splitting low vigour, optimal berry moth protection	varieties of low susceptibiity, open loose cluster by coulouring – cluster splitting, well structured canopy, optimal air radiation low berry moth infection	varieties and clones of very susceptibiity, compacted bunches, crowded canopy – no canopy management nitrogen excess in case of late soil tillage, high vigour, high attack of grape berry moths, wasps, birds, vertebrates, hail or heavy rainfall after véraison, late infection of powdery mildew	
	Treatments with plant strengthener (Potassium-bicarbonate, plant extracts, sodium silicate) or Copper for skin thickness	Treatments with plant strengthener (Potassium-bicarbonate, plant extracts, sodium silicate) or Copper for skin thickness, Bacillus subtilis, Trichoderma viride – T. herzianum	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cultiva-tion techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Additional comments:
Grapes infected with bunch or sour rot, acetic acid bacteria or Penicillium sp. cannot be used to make wine. Their presence in the vineyard must be detected as soon as possible and the grape clusters should be eliminated. If there are visible infections of sour rot or other fungi infection, the grapes should be harvested by sorting healthy from rotten grapes by hand picking. Multiple selective manual harvesting optimises wine quality.
The oenological consequences are serious: oxidations by specific enzymes, degradations of colour and aro-mas, losses of Thiamine and fermentation and clarification difficulties, higher need of SO_2.
The grapes and wines obtained are frequently marked by characteristic mould or undergrowth odours. The contaminated grapes are often extremely bitter and contain higher acetic acid content.

1.3.2. Major pests

1.3.2.1. Grapevine moths (*Lobesia botrana* – grape berry moth; *Eupoecillia ambiquella* – European grape berry moth)

In all European vine growing areas, one or both moths are present. *Lobesia botrana* is more easily found in warmer and sunnier vine zones, whereas *Eupoecillia ambiquella* is characteristic of cooler areas. In the last few years, due to climate change and global warming, *Lobesia botrana* has also established itself in northern vine growing zones.
There are two to three generations of these insects which can cause damage to flower organs (first generation) and to grape bunches during the larval stages (second and third generation). Damages to the berries can subsequently promote the development of Botrytis bunch rot and decrease the quality of the vine.

Fig. 27: Moths of Lobesia botrana and Eupoecilia ambiquella, second/third generation (sour worm).

Control
Recently, on-line sites modelling the development of the pests' lifecycle have been created[5]. These sites allow a more target-oriented application of pesticides.
The refining of monitoring techniques for these pests, with the help of pheromone traps, yellow traps and Tortrix moth warning systems, has allowed the establishment of precise and efficient direct methods using permitted organic insecticides authorized by Reg. CEE 834/2007.
Bacillus thuringiensis preparations and Spinosad (microbial based insecticide) are generally recommended and allowed in all European vine growing areas. They must preferably be applied in the evening or under cloudy conditions in combination with molasses/sugar or vegetable oil preparations.
Natural Pyrethrums are only allowed in Mediterranean areas.

[5] Pest forecasting models: Switzerland: http://www.agrometeo.ch; Germany: Viti Meteo Insercts; http://www.dlr-rheinpfalz.rlp.de, Austria: www.wickler-watch.at

Other control techniques such as mating disruption, sexual confusion with pheromones are very common and successful. Mating disruption is a pest management technique that "floods" the crop with a synthetic version of a pest's sex pheromone. Mating disruption has no effect on non target organisms such as beneficial species because pheromone activity is specific to each species.

Fig. 28: Pheromone trap and different systems for mating disruption (pheromone dispensers)

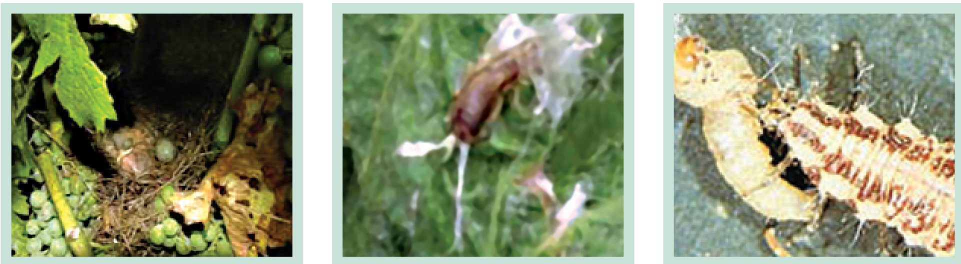

Fig. 29: Breeding birds, common earwig (Forficula auricularia) and lacewing larva (Chrysopa carnea) are very effective predators against berry moths.

Plant protection strategies			
Grapevine moths: (Lobesia botrana – Eupoecillia ambiquella)			Related documents
Arid – sub-arid climate Mediterranean Area	**Maritime – humid climate Atlantic/ Central Europe**	**Continental- dry climate Central / East Europe**	
three to four generations of Lobesia botrana	*two to three generations of Lobesia botrana and / or Eupoecillia ambiquella*	*two to three generations of Lobesia botrana and / or Eupoecillia ambiquella.*	
Habitat management, landscaping, increasing of biological corridors, biological pest control by established antagonists Monitoring by pheromone traps	Cover crop management, landscaping, increasing of biological corridors, biological pest control by established antagonists, use of parasitic wasps. Monitoring by pheromone traps, tortrix moth warning systems Mating disruption with pheromones	Habitat management, increasing of biological corridors, biological pest control by established antagonists, use of parasitic wasps Monitoring by pheromone traps Mating disruption with pheromones	
2-3 applications of Bacillus thuringiensis per generation 2 applications of natural pyrethrum (second, third generation)	2 applications of Bacillus thuringiensis or Spinosad - second/third generation	2 applications of Bacillus thuringiensis or Spinosad pro generation	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cultiva-tion techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Additional comments: Grapes attacked by berry moths' larvae increase bunch or sour rot, acetic acid bacteria or *Penicillium* sp. and therefore cannot be used to make wine.
The presence of grapevine moths can encourage the infection of skin injured berries with *Aspergillus carbonarius*. This fungus has been recognized to be one of the major reasons of OTA-development in wines.

Environmental impact: Spinosad is very dangerous for bees, not usable in the time where blooming cover-crops are in the vineyard or in the surroundings.

1.3.2.2. Spider - Mites (*Panonychus ulmi* red spider mite; *Tetranychus urticae* two spotted spider mite; *Calepitrimerus vitis* – vine mite; *Colomerus vitis* – grape blister mite)

Mite infestation is a result of environmentally unbalanced vineyard systems which is often associated with cultural intensification and excessive use of pesticides in vineyards, including natural insecticides such as rotenone or pyrethrum. Infestation of *Calepitrimerus vitis* – vine mite, is often observed in young vines where no natural enemies are established. The principal symptoms affect the leaves which become deformed, necrotic and turn red, grey or yellow-brown depending on the mite. In longterm organic managed vineyards, natural control of mite infestation is guaranteed by different species of natural enemies such as predatory mites (phytoseiidae), pirate bugs, lacewings and ladybird beetles.

Fig. 30: Tetranychus urticae, Calepitrimerus vitis and infected leafs

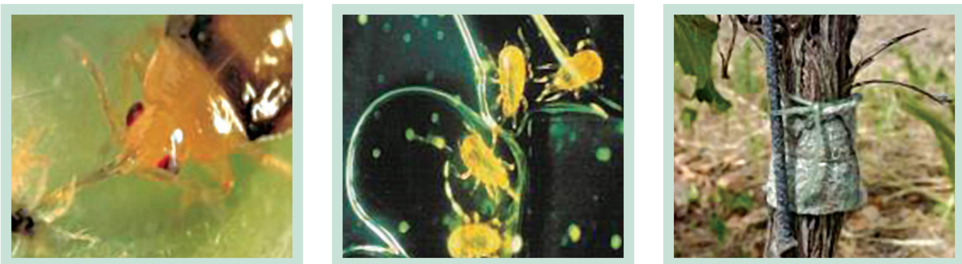

Fig.31: Pirate bugs and predatory mites are the most effective protection against mites. Establishment of predatory mites with over-wintering females.

Control

Indirect measures: Increase of biodiversity on the vineyard by cover-crops and/or creation of habitats for the predator colonies.

Direct measures: In case of serious infestation, it can be useful to intervene before sprouting with a mixture of rotenone or pyrethrum (only in Mediterranean areas) or potassium soap combined with pure alcohol. The use of sulphur against powdery mildew and some preparations of sodium /potassium silicate can reduce the infestation in early spring. Mineral or paraffin oil are useful before bud break.

Plant protection strategies			
Spider mites			Related documents
Panonychus ulmi Tetranychus urticae	**Calepitrimerus vitis**	**Colomerus vitis**	
Excessive use of pesticides, insecticides, - no natural enemies are present, unbalanced vineyard mangement (too high vigour in case of excessive nitrogen supply), no cover crop	*Excessive use of pesticides, insecticides,new plantation - no natural enemies are present,unbalanced vineyard management*	*Excessive use of pesticides, insecticides, new plantation - no natural enemies are present, unbalanced vineyard management*	
Colonisation and protection of predatory mites Safeguard the biodiversity around the vineyard, landscaping, increasing of biological corridors, biological pest control by establishing of antagonists Reduction of dust in the vineyard	Colonisation and protection of predatory mites Habitat and cover crop management, landscaping, increasing of biological corridors	Colonisation and protection of predatory mites Habitat and cover crop management, landscaping, increasing of biological corridors, biological pest control by establishing of antagonists	
Use of Mineral-Oils, Rote-none, Pyrethrum Potas-sium soap Sulphur	2-3 applications of sulphur from bud-break to leave unfolded	2-3 applications of sulphur from bud-break to leave unfolded	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cultiva-tion techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Additional comments: An interesting solution in case of infection danger is to introduce a Phytoseiidae (Typhlodromus) population from another vineyard by cutting vine shoots with leaves or winter pruning wood which can then be placed in the canopy of the endangered vineyard.

Environmental impact: The use of rotenone or pyrethrum as natural insecticides can decrease the population of predators, the natural enemies of the pests. Excessive use of sulphur can decrease the population of predatory mites and parasitic wasps.

1.3.2.3. Leafhopper – cicadellidae
(*Empoasca vitis* – green grape leafhopper; *Scaphoideus titanus; Hyalesthes obsoletus*)

Leafhoppers are vine pests of the Mediterranean areas which, in the last five to ten years, have spre-ad to the northern regions of European vineyards.
Both adults and nymphs of ***Empoasca vitis*** – grape leafhopper - feed on leaves by puncturing leaf cells and sucking the contents. As injury increases, photosynthetic activity declines, heavily dama-ged leaves lose their green colour, dry up, and fall off the vine. The damage is normally minimal: Most of the vines can tolerate up to 20 % leaf loss, provided leaves are not removed until about a month after fruit set.

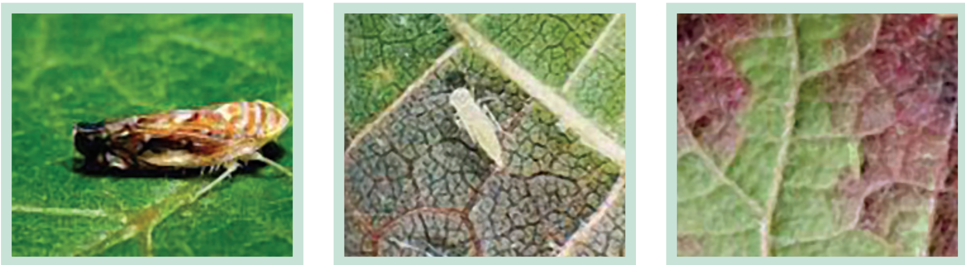

Fig. 32: Scaphoideus titanus and Emphoasca vitis (adult and nymph) and infected leafs.

Fig. 33: Predators of cicadelle like Anystis agilis and spiders and a lot of cicadelle-adults are captured in the cobweb.

Control
Empoasca vitis can be controlled by natural enemies such as the parasitic wasp *Anagrus* spp. These parasitic wasps are particularly valuable because of their ability to locate and attack grape leafhoppers eggs. Their short life cycle also permits them to multiply far more rapidly than the lea-fhopper population. Other parasitic wasps attack the nymphs of the third to fifth instar. Several general insect predators prey on leafhopper adults and nymphs of all stages during all seasons. Among the most abundant are *Chrysopdae* spp. – green lace-wing, *Orius* spp. – minute pirate bugs, different ladybird beetles and spiders. The predaceous mites *Anystis agilis* also attack first-instar nymphs. The use of Potassium–bicarbonate (bak-ing powder) against powdery mildew has a very good side effect against the cicadelle-infection.

***Scaphoideus titanus* (American grape leafhopper)** feeds on leaves and damages the vine by transmitting the pathogenic agent responsible for **flavescense dorée** (FD) a phytoplasma – xylem-clogging micro-organism. The FD phytoplasma is acquired by the vector insect during nutritional processes on infected vines and subsequently, after about one month, it can be transmitted to other vine plants. The symptoms on an infected vine are manifested from the following year onwards. Serious infections of this disease have been observed in different Mediterranean vine growing areas.
The symptoms of this disease are highly variable and complex and concern the entire plant. A relia-ble diagnosis can only be obtained only through laboratory analysis.
Infected vines have to be cut of and burned to reduce the infection potential of FD-Phytoplasma. All European countries affected by epidemics of FD defined strict rules, primary con-cerning the control measures against leafhopper *S. titanus* and eradication of infested plants. Monitoring and control of the vector, as a monophageous species leading to epidemics in vineyards with severe losses, represents the most important measure of control and prevention.

Control
In organic viticulture the vector control can be done by using organic insecticides such as roteno-ne or pyrethrum if they are allowed by national legislations. However the use of these insecticides has undesirable effects on the endemic insect population so they must be used carefully. The use of vegetable oils or coniferous resins can increase a synergistic effect with pyrethrum products. The use of Potassium–bicarbonate (baking powder) against powdery mildew has a very good side effect against the cicadelle-infection.
All natural enemies which attack the grape leafhopper (see above) also attack *Scaphoideus titanus*.

Plant protection strategies

Cicadellidae Leafhopper			Related documents
Emphoasca vitis	**Scaphoideus titanus -Flavescence dorée**	**Hyalesthes obsoletus -Stolbure / Black wood disease, boir noir**	
Warm and dry summer, no strong winter frost,evergreen plants as winter host plants,grass cover or permanent tillaged soil, unbalanced vineyard mangement,no natural enemies present	*Hot and dry summer, no winter frost,vector infected with FD-phytoplasma,grass cover or permanent tillaged soil, unbalanced vineyard mangement,no natural enemies are present,"wild vineyards" or mother-plants with infected vines in narrow or wider surroundings*	*Warm and dry summer, no strong winter frost,vector infected with stolbur-phyto-plasma, natural host and over-wintering plants esta-blished in or around vine-yards (convolvulus arven-sis- bindweed, urtica ssp. – stinging nettle, Cardaria draba – hoary cress)*	
Safeguard the biodiversity around and in the vineyard, landscaping, implementation of biological corridors, multi-species cover crop system, biological pest control by establishing of antagonists, (parasitic wasps, lacewings, spiders, pirate bugs...) Potassium-bicarbonate (used against Oidium)	Safeguard the biodiversity around the vineyard, land-scaping, implementation of biological corridors, biologi-cal pest control by establi-shing of antagonists (parasitic wasps, lacewings, spiders, pirate bugs...) Monitoring by yellow sticky traps Winter application with Mineral-oils 2-3 applications of Rote-none, Pyrethrum (May – August), Potassium-bicarbonate (used against Oidium)	Safeguard the biodiversity around the vineyard, land-scaping, implementation of biological corridors, biological pest control by establishing of antagonists in the soil to reduce the larvae population, Monitoring by yellow sticky traps Destroying of host plants by mechanical weed control in spring and autumn, cover cropping to suppress the host weeds Potassium-bicarbonate (used against Oidium)	

Regulatory framework:
Regulation (EC) No 834/2007: Article 12: (g) the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cul-tiva-tion techniques and thermal processes;
(h) in the case of an established threat to a crop, plant protection products may only be used if they have been authorized for use in organic production
Authorized organic plant treatments included in Annex IIB,
National regulations of plant protection

Environmental impact: the use of rotenone or pyrethrum as natural insecticides can decrease the population of predators, the natural enemies of the pest.
It is nowadays known, that rotenone can affect human health. Pyrethrum is therefore the more suitable pesti-cide, showing best results to fight against FD.
Excessive use of sulphur can decrease the population of parasitic wasps which are very sensitive.

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2. ORGANIC WINE-MAKING

2.1. WHITE WINES PRODUCTION (Trioli, G. with contribiution of: Cottereau, P.; Hofmann, U.; Werner, M.; v.d. Meer, M.; Levite, D.)

2.1.1. Introduction

It is almost impossible to produce a high quality white wine without inputs. Although, it is possible to significantly reduce the use of additives and adjuvants (processing aids) through a precise planning of the wine-making strategy.
The modern consumer's minimal requirements for white wines are: intense and clean aroma, yellow-green colour, absence of cloudiness. White wines especially have a great potential to express the "terroir" effect by reflecting the particularities of the local climate and soils (also called "minerality"). Excellence is defined on the basis of varietal aroma expression and taste balance.

To meet these goals, two main enemies must be taken under control in every phase:

- **oxidation** of aromatic compounds (leading to loss of varietal aromatic intensity, appearance of oxidized notes) and phenols (causing colour browning); the main strategies in wine-making are to limit oxygen contact with sensitive compounds, to add antioxidants to stop oxidative reactions, to maintain low temperature and to selectively eliminate oxidative enzymes and some of the more oxidisable phenols.
- **microbial spoilage** with off-flavor appearance, mainly due to development of bacteria and non-*Saccharomyces* yeasts on juice. The most common practices against microbial contamination are careful hygiene, temperature control, physical treatments to reduce microbial populations and the use of antimicrobial additives.

Prevention is the keyword of low input white wine production: once microbial contamination or oxidation has started it is impossible to recover the original potential quality of the wine. Some of the oxidative reactions are extremely quick (in the order of seconds) and require very low amounts of oxygen to get started. Even a very limited microbial population can develop within days or even hours in uncontrolled conditions, and produce evident off-flavor.

The **original grape** defines most of the wine-making strategies. Some varieties are rich in phenols sensitive to oxidation and require safer strategies. Mouldiness, especially by Botrytis, introduces into the system oxidative enzymes, unstable proteins, microbial contamination and unbalanced starting composition.

Consistency is another golden rule. Once a strategy has been started, it is very risky to switch to another one. For instance, if "reductive wine-making" with total oxygen protection is applied at the beginning of the wine-making process, the wine will be very sensitive to oxidation, and later lack of protection (i.e. during storage or bottling) can completely jeopardize wine quality.
Similarly, if there are no additions of preservatives there is a need for a constant control of the microbial population as well as the capability of a rapid intervention with physical means to control contaminants.

In the following chapters different options are described for each step of the wine-making. No-input options are included (green colour); as well low input ones (yellow colour) together with practices making use of all additives and additives allowed by wine regulation (red colour).
Organic wine-making aspires to limiting the use of external inputs: although, the choice of the lowest input option in each step of wine-making can expose the producer to a **level of risk** which is unacceptable.

A good knowledge of the health of the grape and its composition as well as a constant sensory and analytical control of the wine can help the wine-maker follow the best track to suc-ceed in producing a quality wine which is safe for consumer and environment friendly.

2.1.2. Harvesting

The most important prerequisite for high quality organic wine is the harvesting of healthy and physiological and technologically matured grapes. First and foremost, the grapes should be protected from fungi or insect attacks and contaminations such as Botrytis sour rot, Oidium etc., right up to the harvest. If there are visible infections of sour rot, Oidium or other fungi infection, the rotted grapes should be sorted out by hand picking at harvest: Only healthy grapes achieving the desired maturity level are picked. Infected grapes are eliminated in the vineyard. This is the most effective sorting method.

Rotten grapes infected by bunch and sour rot, Oidium or other fungies, except "Noble rot", are not usable for wine production.

Fig. 34 : White grapes infected by "bunch or sour rot " and "Noble rot" induced by Botrytis cinerea.

White grapes should be harvested at a temperature below 20°C. In warm climates, harvesting has to occur by night or in the early morning. This is the best way to stabilise and conserve the typical white grape aromas which are very volatile and decreased by high temperature.
Another important prerequisite for optimal wine quality is the optimal physiological and technological maturation of the grapes which depends on grape variety, environmental and climatic conditions and the type of wine which the wine-maker wants to produce.
Thus a perfect knowledge of véraison conditions - optimal relationship between sugar, acid content and pH of the juice as well as the colour of the berries, the smell and taste of the grapes and juice, will permit the vine-grower to organize the harvest according to the various maturity periods. Maturity monitoring complements this information.

Fig, 35: Hand-picking and in-vineyard selection of healthy grapes;

The grape crop should be harvested by hand or mechanically under favourable climatic conditions, with sorting in the vineyards or on sorting tables in the winery. Thanks to its speed and ease of use, the mechanical harvester permits a rapid harvest of grapes at their optimal quality level and at the most favourable moments, but manual grape-picking can be even more selective and qualitative. Unfavourable climatic conditions at harvest can lead to quality and yield loss in a very short time. Under these conditions mechanical harvesting can be recommended without a selection of the grapes by hand picking.
Such unfavourable conditions consequently requires the implementation of specific wine-making practices (see chapter 2.1.3., 2.1.2.)
In certain regions or appellation zones and vineyards, quality concerns prohibit mechanical harvesting. Harvest transport is linked to the organisation of harvest work (harvesting by hand or mechanical) and the winery's technological installations. From the quality and wine-making viewpoint the grapes should arrive at the winery without delay and in good condition. If necessary the grapes and the must should be protected from oxygen and microbial infection by using SO_2, carbon dioxide or dry ice.
The exaggerated brushing and crushing of grapes has to be avoided by:

- using shallow transport container, vats or bins;
- using easily cleaned material to ensure proper hygiene;
- dumping the grapes into the destemmer, crusher or press directly.

Fig. 36: Typical cellar-road in Austria, Czech-Republik or Hungary.

2.1.3. Grape Processing

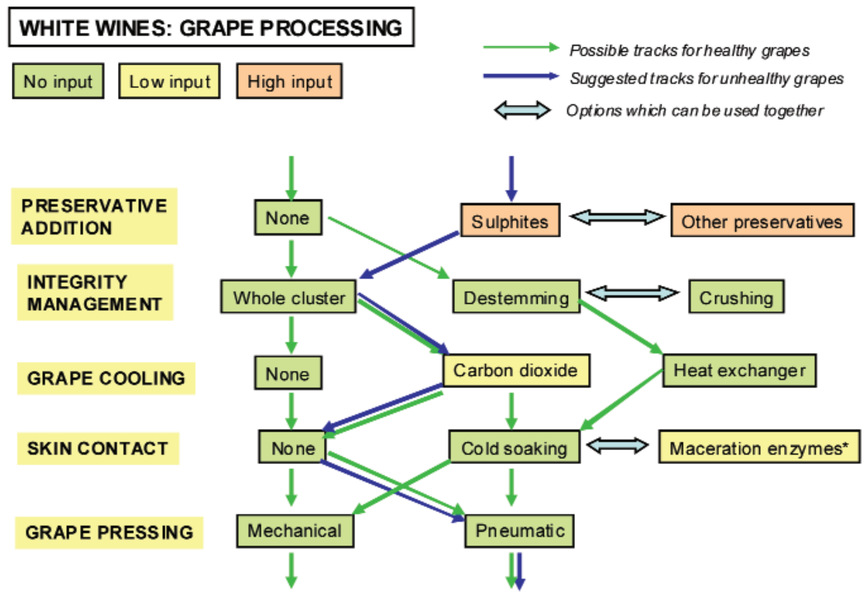

Fig. 37: White wine making – grape pressing options

General principles

The healthy state of the grapes, coupled with the knowledge of its varietal traits, defines the strategy to be used in grape processing.
In case of limited mouldiness and hand picking, the preliminary sorting of the grapes is an expensive but very useful practice. In case of mechanical harvest or reception of grapes from third parties, a careful selection of grape lots can be very valuable. Suitable chemical and spectrophotometric tools to determine grape quality are available or under development.
As a general rule, the wine-maker must promote a selective extraction of positive elements from grapes (varietal aroma, macromolecules etc.) avoiding the solubilisation of potentially dangerous compounds (i.e. oxidative enzymes, excess of polyphenols, micro-organisms, negative aromas, etc.). Consequently, a grape at perfect ripeness will allow routine extraction whereas an unhealthy or unripe grape will require careful and rapid processing.

2.1.3.1. Addition of Preservative

Principles

The addition of preservatives depends on the health of the grapes and on the overall technological level of the winery.
Healthy grapes with low oxidation potential and in good condition can be quickly processed without preservative addition.

Wine-making options			Related docu-
"No Input" Oenology	**Low-Input Oenology**	**High-Input Oenology**	
Healthy grapes are pro-tected from oxidation and microbial spoilage. Not possible on grapes with mouldiness, which have lost their integrity during harvest and transport, with varieties rich in polyphenols.	***Ascorbic acid** (vitamin C) is an antioxidant which can support SO_2 action.* ■ Add together with SO_2 ***Tartaric acid** reduces pH of released juice and reduces microbial development.* ■ Add to released juice on the bottom of trucks or to re-ceiver of mechanical har-vesting machines	*Avoid oxidation of grape aroma and phenols; reduce develop-ment of bacteria and yeasts.* Distribute sulphites on grapes as soon as berry integrity is lost. Dosages ranging from 10 to 50 ppm depending on the state of grapes. ■ Preferred sulphite form depends on when the addi-tion is done (powder on trucks or receivers, solution of gas on-	Practical hint: reductive wine-making Technical note: Oxidation of must and wine Microbial contamination
Inputs			
Necessary: none	Necessary: ascorbic acid, tarta-ric acid,	Necessary: P- metabisul-phite, Gaseous SO_2	
Regulatory framework: Tartaric acid addition is allowed only in some UE regions (zone C), Not allowed in zone A and B			**Fact sheets** #: SO_2 #: P-metabisulphite #: ascorbic acid #: tartaric acid
Additional comments: SO_2: Several small additions in different steps of the process allows better efficacy at the same final doses.			

The presence of mouldiness (especially from Botrytis), the loss of berry integrity with consequent juice liberation, the distance of transportation and the lack of temperature control will necessitate the need to protect the juice against oxidation and contamination through preservative addition. The dosage will be proportional to the level of damage of the grapes.

The following planned steps will also define the need of preservatives. Flash-pasteurisation, hyperoxygenation and treatment of the obtained juice can result in a lower need of preservatives. Alternatively, when is not possible to protect the wine through the whole wine-making process by other means, it is advisable to slightly increase the dosages of SO_2 and ascorbic acid.

1.1.3.2. Integrity Management

Principles

In whole berries enzymes and substrates are kept separate, oxygen is practically not pre-sent; micro-organism presence is limited to berry surface and no significant development occurs.
As soon as the berry integrity is lost (mould attack, mechanical damage, grape processing etc.) chemical and enzymatic reactions start, substrates are exposed to oxygen and micro-organisms start feeding on juice sugar and nutrients.
Whole cluster pressing ideally give juices with no oxidation and no contamination.

Wine-making practices		
Whole clusters	**Destemming**	**Crushing**
Avoid oxidative reactions and micro-organism development. Hand picking and transport in small cases ■ Charge of the press by hand or through movement belts	*The elimination of stems causes some berry integrity loss but allows the use of pumps for grape movements, the treatment of the pomace, and higher loading of presses* Hand or mechanical harvest ■ Quick transportation to winery ■ Destemming machine	*Complete berry integrity loss allows easier movement and treatment of pomace in the winery.* Hand or mechanical harvest ■ Quick transportation to winery ■ Crushing machine
Inputs		
Necessary: none	Necessary: none	Necessary: none

The management of whole clusters is very labour-intensive, requires special equipment (i.e. transportation belts) and space in the winery (i.e. reduces press loads), which limits the practice .
The wine-maker can decide to lose grape integrity in order to be able to process increased volumes and to apply other useful technologies (eg. cooling). Between the two extremes outlined here it is possible to manage the integrity of the grapes to the desired level ie. only destemming without crushing, use of pumps respecting berry integrity etc. In some cases grapes are crushed but not destemmed to increase juice drainage at pressing.

Fig. 38: Modern destemmer and crusher for white and red grapes (large and small wineries).

2.1.3.3. Grape Cooling

Principles

Oxidative reactions and microbial development are temperature dependent phenomena. It is preferable to harvest grapes when environmental temperatures are low (early morning, during the night for mechanical harvest). The temperature of grapes at the winery reception can be too high to allow their processing without quality loss. In destemmed grape processing, the easier way to cool the pomace is to refrigerate it through a heat exchanger, assuming that the diameter of the tube-in-tube holes are big enough. Special systems using CO_2 under pressure to be injected on line or to be sprinkled on grapes using dry ice have been recently developed. Due to the fact that carbon dioxide is heavier than air, these systems give the additional advantage of creating a protective coat above the grapes thus reducing their contact with oxygen.

Wine-making options			Related docu-
"No Input" Oenology	**Low-Input Oenology** **Carbon dioxide**	**Low-Input Oenology** **Heat exchanger**	
Grapes are picked at low environmental temperatures and protected against oxidation and contamination.	*Dry ice or carbonic snow is mixed with grapes when received at the winery or in the press* *Whole clusters* ▪ *Addition of dry ice (10 g of dry ice reduce by 1 degrees the temperature of 1 kg of grapes)* *Carbon dioxide under pressure is injected on line* Destemmed grapes ▪ Passage in a special CO_2 cooling equipment	*Grapes are sent through an heat exchanger to lower temperature* Destemmed and crushed grapes ▪ Refrigeration	Technical note: Oxidation of must and wine Microbial contamination
Inputs			
Necessary: none	Necessary: none	Necessary: none	

Additional comments: Contact with dry ice or carbon dioxide under pressure can damage skin and reduce berry integrity In the no-input and low-input options, the use of cooling and heating should be limited in a way which can be considered as low-level energetic.

2.1.3.4. Grape Skin Extraction

Principles

Immediate pressing of whole clusters results in a very limited extraction of grape skin constituents. If desirable for the production of bases for sparkling wine production or in the processing of damaged or unripe grapes, the low extraction can become a loss of potential quality in the production of still white wines.

Wine-making options		
No-Input Oenology	**Low-Input Oenology** **Cold soaking**	**Low-Input Oenology** **Maceration enzymes**
Grape composition and state make skin contact not advisable. Grapes are directly sent to pressing phase Whole clusters, destemmed and/or crushed grapes ▪ To press as quickly as pos-sible	*Destemmed / crushed grapes are left soaking in released juices at low temperature for a certain time to increase extraction of positive components* Pomace at low temperature (6-12°C) ▪ Soaking for a defined time (4-24 hours) ▪ To pressing	*Special enzymes are added to crushed grapes to accelerate extraction and increase free run juice yield* Destemmed and crushed grapes ▪ Addition of enzymes (0,5 – 3 g/hl) ▪ Temperature control ▪ To pressing
Inputs		
Necessary: none	Necessary: none	Necessary: none

A controlled soaking of the skins in the juice can allow solubilisation of varietal aroma, poly-saccharides, minerals and phytosterols into the juice, which contribute to the sensory profile of the wine and its nutrient enrichment which enhances alcoholic fermentation .
Nevertheless, grape skin extraction is a very delicate practice. If time, temperature and general conditions are not well managed it can result in an excess of polyphenols, negative aromas and microorganisms in the juice. Also, it requires special containers and equipment not always available in the winery.
Useful tools in this phase are the so called "maceration enzymes", pectolytic enzymes with some extent of hemicellulase, cellulase and protease properties which accelerate vegetal structure degradation and the release of some components. It's use can be considered as an alternative to cold soaking to increase juice yield and obtain a certain increase in the extraction of grape components during pressing.

2.1.3.5. Grape Pressing

Principles

As for conventional wine-making the pressing of grapes aims to separate an economically reasonable amount of juice from the pomace. This should be carried out in a way that promotes the extraction of desirable compounds and to leave in the pomace the grape components which lead to poor quailty wines High pressure increases the juice yield, but also extracts undesired components from grapes (herbaceous compounds, acidity, potassium, phenols etc.).

Wine-making practices			Related documents
Mechanical pressing	**Pneumatic pressing**		
Pressing is done by applying mechanical pressure on grapes (vertical, dish or continuous presses)	*The pressure is given by a membrane progressively filled with air or water. Absence of friction between grape and equipment.*		Practical hint: reductive wine-making Technical note: Oxidation of must and wine
Avoid complete filling of the press tank	Avoid complete filling of the press tank		
Reduce frictions between equipment and grapes	Preferably apply more cycles and steps at lower pressure		
Preferably apply more cy-cles and steps at lower pressure			
Inputs			
Necessary: none Useful: CO_2	Necessary: none Useful: CO_2	Necessary: none Useful: CO_2	
Additional comments: Some press models are equipped for CO2 protection against oxygen during pressing SO_2 added on grapes is almost completely washed out with first free run juice. Add another portion of sulphite if a longer protection is needed (SO_2 fractioning)			

As organic wine-making aspires to a low input technology it is important to manage juice extraction in such a way that will reduce the need of later additives or treatments.

Juice fractioning (separate wine-making of free run and pressed juices, by following different strategies) can be of great help.

Fig. 39: Historical – tree – busket press

Fig. 40: Vertical internat screw press

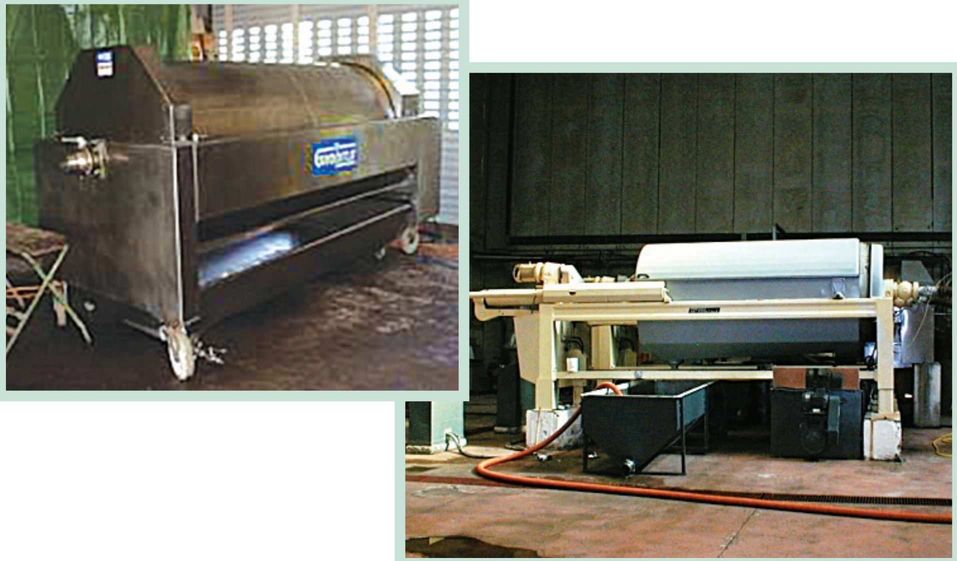

Fig. 41: Modern pneumatic tank-presses

2.1.4. Juice Processing

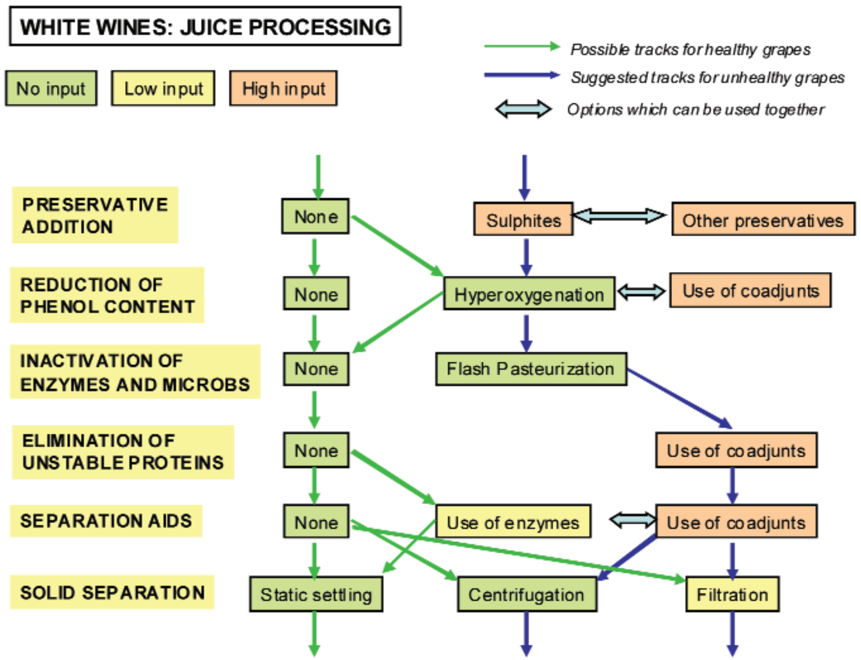

Fig. 42: White wine making – Juice processing options

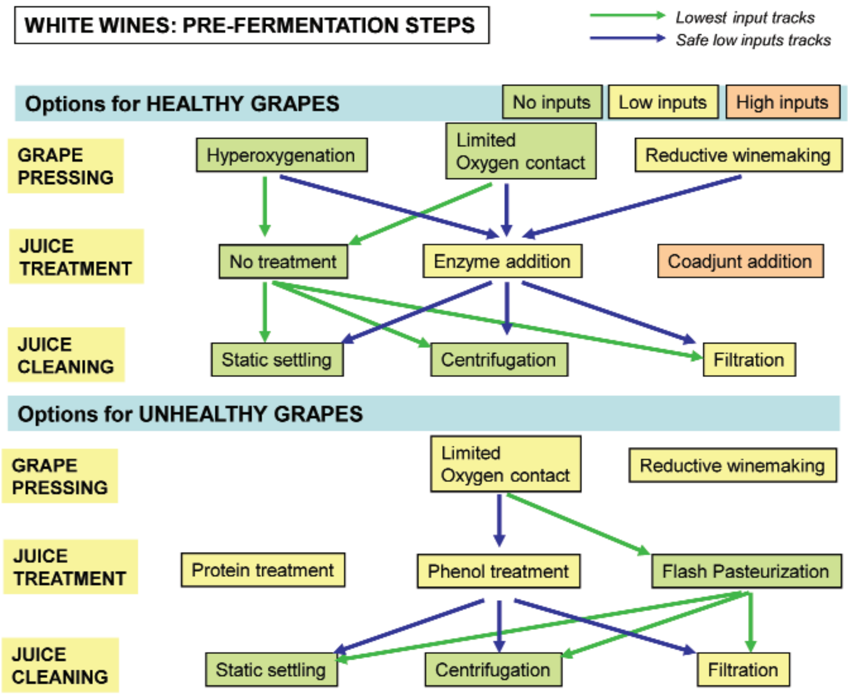

Fig. 43: White wine making – Juice processing options (differentiated for healthy and damaged / unhealthy grapes)

General principles

The treatment of juices from white grapes aims to eliminate polyphenols and proteins which are responsible for instability of the wine at later stages.
When juices are contaminated by a high population of wild yeasts and bacteria, treatment is useful to reduce contamination and allow suitable alcoholic fermentation.

In organic wine-making this step is implemented in order to reduce as much as possible the future use of processing aids, especially those which are potentially allergenic or of synthetic origin (casein, PVPP).
Botrytis and Powdery mildew infections on delivered grapes requires the elimination of chemicals dangerous for wine quality (laccase, specific off-flavours, excess of unstable proteins).
Whatever practice the wine-maker decides to apply the golden rule is to it carry out the initial processing as fast as possible. Even at low temperatures, microflora continues to grow and oxidation reactions proceed. In risky situations (highly oxidable or contaminated juices) the fast initial processing is critical.

2.1.4.1. Addition of Preservatives

Principles

The decision to add preservatives depends on the condition of the grapes, whether there has been previous additions of preservatives on the technology applied in the whole process and on the desired style of wine. It might or might not be necessary to add preservatives at this stage.

Acidification with tartaric acid is an option at this stage (when needed and allowed). The goal of the practice is not strictly to preserve the wine but the reduction of pH in the first stages of processing results in a reduction of the development of spoilage micro-organisms.

In addition to SO_2 and ascorbic acid, some wine-makers add at this stage Oenological tannins to reduce polyphenol-oxidase activity and bacterial development. The combination of ascorbic acid and tannin has been proposed as an alternative to sulphite addition in this phase of wine-making. The use of sulphite excludes the positive wine-making practise of hyperoxigenation.

Wine-making options			Related documents
No-Input Oenology	**Low-Input Oenology Other preservatives**	**High-Input Oenology Sulphites**	
Juices are protected from oxidation and microbial spoilage by other means. Not possible on juices extracted from unhealthy grapes or with high content of spoilage micro-organisms or poly-phenols.	***Ascorbic acid** (vitamin C) is an antioxidant which can support SO2 action.* ■ *Add together with SO2* ***Tartaric acid** reduces pH of released juice and reduces microbial development.* ■ *Add according to acidification needs* ***Oenological tannins** reduce polyphenol oxidase activity and bacterial growth* ■ Add suitable amount and type of tannin, by considering sensory side effects	*Avoid oxidation of juice aroma and phenols; reduce develop-ment of bacteria and yeasts. Dosages ranging from 10 to 50 ppm depending on the quality of juice.* *Add sulphite solution and mix the liquid mass, or inject on line during juice move-ment* ■ Preferred sulphite form depends on dimension and equipment of the winery	Practical hint: reductive wine-making Technical note: Oxidation of must and wine Microbial contamination Research note: ascorbic + tannins
Inputs			
Necessary: none	Necessary: ascorbic acid, tarta-ric acid, tannins	Necessary: P-metabi-sulphite, Gaseous SO_2	
Regulatory framework: Tartaric acid addition for acidification is allowed only in some UE regions (479/2008), the tartaric acid has to be agricultural origin (mostly coming from grapes) EU reg. 1622/2000			**Fact sheets** #: SO_2 #: P- metabisulphite #: ascorbic acid #: tartaric acid #: tannins
Additional comments: SO_2: Several small additions in different steps of the process allow better efficacy at the same final doses. The use of tartaric acid is not fitting to organic wine production philosophy as it is a massive intervention on the wine's taste, affecting especially the millésime typicity.			

2.1.4.2. Reduction of Phenolic Content

Principles

Some varieties have a naturally high content of phenols, which can be easily oxidised during wine-making, giving a brown colour and catalysing chain reactions on aromatic compounds. Grape mouldiness, bad management of the grape processing, excessive pressing can enhance the problem.

Wine-making options			Related documents
No-Input Oenology	**Low-Input Oenology Hyperoxygenation**	**Low-Input Oenology Use of adjuvant**	
Phenol content in juice and their sensitivity is judged acceptable.	*Promote complete oxidation of juice polyphenols in order to eliminate them with clarification Do not use sulphur before hyperoxygenation.* *Speed up pressing operations and implement hygiene procedures to avoid microbial contamination* ■ All of the juice quantity should be treated with O_2 ■ Saturate juice with oxygen ■ Quickly proceed to juice clarification phase	*Excess phenols are eliminated by absorption on adjuvant and subsequent elimination during clarification stage.* Select the most suitable adjuvant among the following: casein, gelatin, ov-albumin, plant proteins, Identify preferable dosage ■ Properly prepare the product and add to juice, making sure the adjuvant is well homogenized in the whole mass ■ Quickly proceed to juice clarification phase	Practical hint: hyperoxygenation Research note: hyperoxygenation
Inputs			
Necessary: none	Necessary: none Useful:O_2, adjuvant	Necessary: one or more among casein, gelatin, ov-albumin, plant proteins	**Fact sheets** #: casein #: ov-albumin #: plant proteins #: gelatin
Additional comments: The use of caseine, P-caseine and ov-albumin has to be labeled as an allergenic compound. The raw-material of the plant proteins has to be free of genetically modified plants, if there are some aller-genic compounds in it – it has to be labeled.			

In these cases, when is not possible to completely protect juices and wine from contact with oxy-gen, it is preferable to eliminate part of the phenols from the system.
The conventional way is to add adjuvants able to absorb phenols which will then be eliminated later by racking or filtration. Adjuvants with this function are casein, albumin, gelatin and some plant proteins. Some of these adjuvants vary in their ability to absorb specific phenols.
Another option for organic wine-making as an alternative to the use of adjuvants is the practice of hyperoxygenation. This is the injection into the juice of a measured amount of air or pure oxygen. A complete oxidation and precipitation of sensitive phenols is achieved. These phenols can then be eliminated from the system in the subsequent extraction phases. It is possible to combine hyperoxygenation with a limited use of adjuvant, but the use of sulphur dioxide should be avoided before any oxygen treatment. Oxidation does affect varietal aromas which are lost to some extent. For this reason hyperoxygenation should not be applied to every variety and type of wine.

2.1.4.3. Inactivation of Enzymes

Principles

Botrytis infection of grapes causes the synthesis of laccase, a polyphenol oxidase with very high activity. Its presence in the juice can be limited by correct grape management and pressing but these treatments can still be insufficient to reduce the oxidation risk below an acceptable level. Similarly, the microbial population in the juice can be too high to allow the necessary degree of management of the fermentation processes.

In these cases, the best alternative to a massive addition of SO_2 is a thermal treatment of the juice by normal pasteurisation. Modern equipment heats the juice at relatively high temperatures for a very short period of time (flash-pasteurisation, short-time high heating), a combination strong enough to denature laccase and kill most of the microbes but not the elimination of varietal aromatic compounds and other beneficial components of the juice.

Wine-making options			Related documents
No-Input Oenology *Enzyme and microflora levels are considered acceptable.*	**Low-Input Oenology** **Flash-pasteurisation** **Normal-pasteurisation** *Juice heating (75°C) for short time (20-30 seconds) allows laccase denaturation and micro-organism inactivation* Avoid presence of vegetal material ■ Continuous process ■ Quickly proceed to juice clarification phase		Research note: Flash-pasteurisation
Inputs			
Necessary: none	Necessary: none		
Regulatory framework:			
Additional comments: Flash pasteurisation only requires low energy input and is very effective in reducing the SO_2 input, but it requires the addition of selected yeast and bacteria in combination with yeast nutrients to start the fermentation.			

2.1.4.4. Elimination of unstable Proteins

Principles

Some grape varieties (i.e. Sauvignon blanc, Grüner Veltliner, Riesling) as well as unhealthy grapes (i.e. attack of powdery mildew) have a typically high content of proteins which can precipitate once the wine is bottled. Unstable proteins are eliminated through the use of Bentonit,a clay which absorbs positively charged molecules from wine. Bentonite fining also reduces aroma intensity. Oenological tannins also reported to have some effect in the elimination of unstable proteins.

Some experts prefer to apply Bentonite to unfermented juice in cases when it is definitely known to be required instead of waiting to apply to the resulting wine. It is reported that a lower dose of Bentonite is needed to get the same result and fermentation aromas are not affected. In some cases, Bentonite is added to juice during alcoholic fermentation.

Wine-making options			Related documents
No-Input Oenology *Historical records on the wine don't make certain the need of protein treatment*	**Low-Input Oenology** **Use of adjuvant** *Bentonite (or tannins) are used to eliminate part of the unstable proteins* Properly prepare the adjuvant ■ Add to the juice and mix carefully ■ Quickly proceed to juice clarification phase		
Inputs			
Necessary: none	Necessary: Bentonite Useful: tannins		Fact sheets: #: Bentonite #: tannins
Regulatory framework:			
Additional comments: It is recommended to use Bentonite with low iron content. Bentonite has to be completely removed before fermentation.			

2.1.4.5. Elimination of Off-Flavours

Principles
Botrytis infection on grapes or specific environmental conditions (e.g. attack of powdery mildew) causes mouldiness off-flavours and off-tastes.
The precursors of these off-flavours are eliminated to use of charcoal, a pulverized activated carbon of vegetable origin with correspondingly varied inner surface and a selective adsorption capacity. It is also useful for the reduction of tannins and polyphenols.

Wine-making options			Related documents
Natural Oenology	**Low-Input Oenology** **Use of adjuvant**		
The health of the grapes did not need the use of clarification adjuvant	*Charcoal is used to eliminate pre-cursor of mould, rotten off- flavour* Properly prepare the adjuvant ■ Add (10-30g/hl) to the juice and mix carefully ■ Quickly proceed to juice clarification phase		
Inputs			
Necessary: none	Necessary: charcoal Useful: Bentonite, gelatine		Fact sheets: #: Bentonite #: charcoal
Regulatory framework: Admitted according the presently national laws and regulations for wine making.			
Additional comments: Adsorption is definitely terminated within one day. The sediment of activated carbon should be separated / filtered as soon as possible. Charcoal is completely removed by filtration before fermentation.			

2.1.4.6. Separation Aids

Principles
Regardless of whether activated charcoal is used as described above the grape juice very often needs to be clarified to obtain an acceptable level of clarity before going to alcoholic fermentation. Solids separate from liquids according to Stock law: the smaller the dimension of the solid and the higer the viscosity of the liquid phase, the greater the time required for separation.

Wine-making options			Related documents
No-Input Oenology	**Low-Input Oenology** **Use of enzymes**	**Low-Input Oenology** **Use of adjuvant**	
No addition is needed to allow complete and rapid separation of solids	*Pectolytic enzymes degrade pectin's and reduce viscosity. Beta-glucanase can degrade glucans produced by botrytis* Properly prepare the enzymes ■ Add to the juice and mix carefully ■ Control temperature and time parameters (the lower the temperature, the slower the enzyme action)	*Bentonite, kaolin, tannins, silica gel, react with wine or added proteins forming heavy flocks* Properly prepare the adjuvant ■ Add to the juice and mix carefully. The order of additions can be important ■ Wait for flock formation and quickly proceed to juice clarification phase	
Inputs			
Necessary: none	Necessary: pectolytic enzymes Useful: beta-glucanase	Necessary: one or more among Bentonite, kaolin, tannins, silica gel	Fact sheets: #: Bentonite #: tannins #: kaolin #: silica gel #: pectolytic -enzymes #: beta-glucanase

Speed is a key factor in this stage of wine-makingand it can be helpful to reduce juice viscosity or to add adjuvants which allow the formation of bigger and heavier flocks. Pectolitic enzymes reduce the pectins in solution in the juice and reduce its viscosity allowing a faster separation of solids. Glucans can also contribute to viscosity of juices derived from botrytis infection, and beta-glucanase preparations are commercially available.
Another strategy is to add substances to the juice which interact with juice components creating

larger flocks. Negatively charged Bentonite, silica gel, kaolin or tannins can interact with positively charged natural or added proteins. The use of large doses of adjuvants for phenol stabilization imposes the need for the addition of negatively charged substances to eliminate them completely from the system. The formation of flocks, which are further eliminated by separation techniques, are the main causes of phytosterol deficiencies in clarified white grape musts.

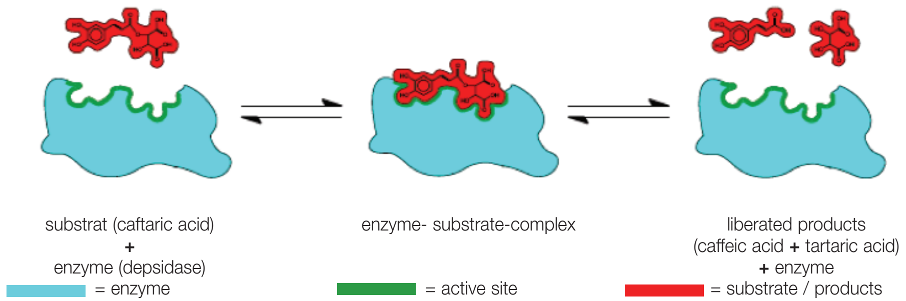

Fig. 44: Example for the function of enzymes. (Source: Haßelbeck, G.; Stocké, R. (2002) Enzyme –Werkzeuge des Oenologen. das deutsche weinmagazin 18; with the permission of Erbslöh, Geisenheim)

2.1.4.7. Solid Separation

Principles

The elimination of solids from the juice can be obtained through different technologies.
Settlement of the juice is initiated by leaving it undisturbed for 12 - 24 hours until most of the solids have sedimented and can be eliminated by racking. Enzyme treatment accelerates the process. The advantages are the low cost and the possibility for separating gross lees whilst keeping part of the fine lees in the system.

Wine-making practices			Related documents
Static settling and racking	**Centrifugation/ Flotation**	**Filtration**	
The lees are left to separate out at the bottom of the tank and the juice is separated by racking Temperature control ▪ Overnight sedimentation ▪ Racking of the clean juice (target turbidity and protection from oxygen according to the overall strategy) ▪ To alcoholic fermentation	*Continuous process, to quickly eliminate suspended solids from microbiologically contaminated juice and /or for processing large volumes* Centrifugation ▪ To alcoholic fermentation Flotation Use of gelatine in combination with N or O_2 ▪ To alcoholic fermentation	*Use of vacuum filters or cross-flow filtration to separate solids. Because of reduced flow rates and very clean exiting juices, this practice is usually restricted to the less problematic juices* Filtration ▪ To alcoholic fermentation	Technical note: Microbial contamination Temperature control
Inputs			
Necessary: none	Necessary: gelatine, use of pure nitrogen or oxygen	Necessary: perlite, diato-maceous earth, cellulose	**Fact sheets:** #: perlite #: diatomaceous earth #: cellulose #: gelatine
Additional comments: For the cross-flow filtration used for must, it is absolutely necessary to increase the turbidity before adding yeast.			

Centrifugation can continuously separate solids and is suitable for large volumes. Oxygen solubilisation must be avoided during this step. Vacuum filtration or cross-flow filtration are also extensively used in modern wine-making. In the first case some filtration coadjuvants are needed and the juice can result in being too clean for a good fermentation. Vacuum filtration is then often limited to the last press juices. Cross-flow filtration is a continuous process which does not require adjuvants, and represents an interesting alternative to the use of centrifuges.

Wine-making practices / type of filter for grape must			Related documents
vacuum rotating filter *Use earth, available in different particle sizes* Can work with very turbid products like heated musts, lees… Significant flow	**press filter** *Use earth, available in different particle sizes* Can work with very turbid products (lees, mires) Small flow	**Cross flow filter** *Very thin filtration, sterility reached after the filter* Very small flow	Related documents Technical note: Hygienic standards
Inputs			
Necessary: cellulose, dia-tomaceous earth, perlite	Necessary: cellulose, diato-maceous earth, per-lite		**Fact sheets:** #: perlite #: diatomaceous earth #: cellulose
Regulatory framework: No recommendation / alimentary contact materials / membranes (classic or cross-flow) are obtained by organic synthesis.			
Additional comments: Centrifugation can be used / Flotation can also used in the case of heated musts. This technique requires some flocculants like gelatine for example. / Some cross-flow membranes are minerals.			

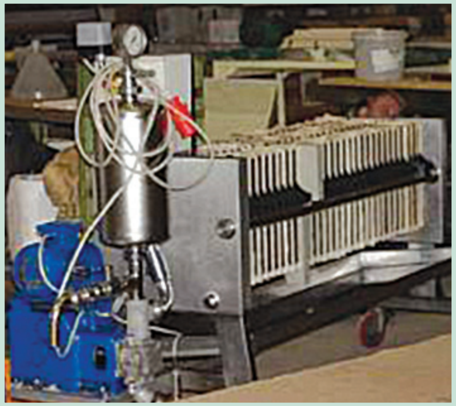

Fig. 45: Grape must Press-Filter and Vacuum Rotation Filter with Cellulose, Diatomaceous earth or perlite

2.1.4.8. Deacidification

Principles

When grapes do not reach complete maturity grape acidity can be considerable. In these conditions, malic acid concentrations are almost always higher than those of tartaric acid. When the biological degradation of malic acid is not desired due to the traditional wine-making practices and the sensorial changes of the wine, the juice must be chemically deacidified. The chemical deacidification can also help to trigger malolactic fermentation in the case of higher pH.

The acids in juice or wine decompose the carbonate into carbonic acid, which is released in the form of CO_2. The potassium and calcium combines with the tartaric acid to form an insoluble salt which then precipitates.

Wine-making options			Related documents
No-Input Oenology **None** *A balanced composition of the grapes is obtained through a better management of the vineyard and the specific ripening conditions in autumn*	**Low-Input Oenology** **Use of adjuvant** *Calcium carbonate alone or in combination with potassium-tartrate is used to correct very high acidity* Properly prepare the adjuvant ▪ Add to the juice, mix carefully and make sure the adjuvant is well homogenized in the whole mass ▪ Quickly proceed to juice clarification phase	**Low-Input Oenology** **Use of adjuvant** *Potassium hydrogen- carbonate is used for slight acidity correction* Properly prepare the adjuvant ▪ Add to the juice, mix carefully and make sure the adjuvant is well homogenized in the whole mass ▪ Quickly proceed to juice clarification phase	
Inputs			
Necessary: none	Necessary: Calcium Carbonate, Potassium tartrate	Necessary: Potassium-hydrogencarbonate	**Fact sheets:** #: calcium carbonate #: potassium hydrogencarbonate
Regulatory framework: Reg. UE 479/2008 - annexe V - defines precise rules for the deacidification practice. The UE regulation does not impose must deacidification limits, but there is a limit of 1 g/l of the total acidity as tartaric acid for wine.			
Additional comments: In white wine processing, the chemical deacidification should be carried out after must clarification but before fermentation.			

2.1.5. Fermentation

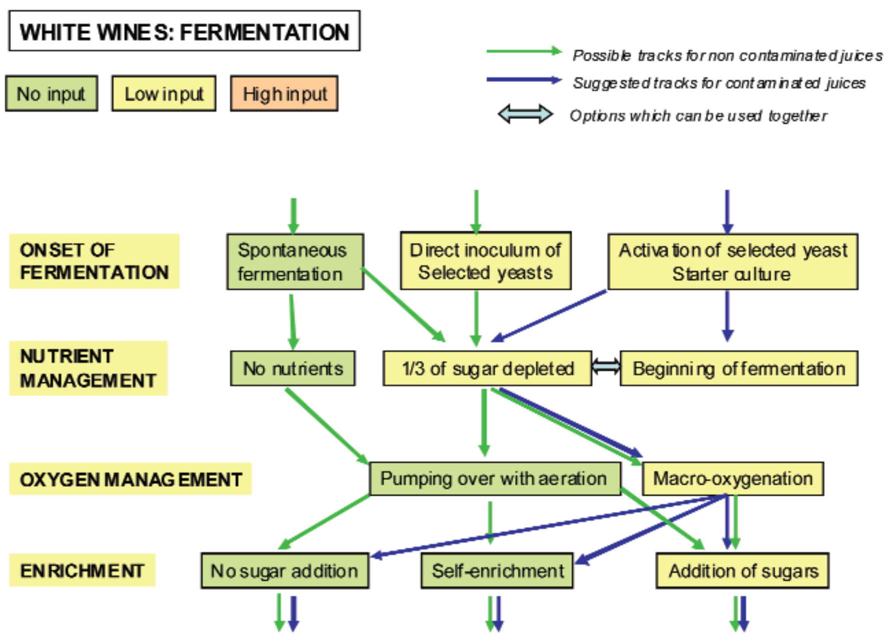

Fig. 46: White wines – fermentation options

General principles

Good management of alcoholic fermentation can limit the need for inputs and treatments. During the fermentation the wine is protected against oxidation and spoilage. Wine yeasts quickly utilise all oxygen present and can effectively compete against contaminating micro-organisms. The protection from oxygen continues even after the complete depletion of sugars untill the yeast lees are present in the system. In organic wine-making it is important to quickly promote the start of fermentation and to make sure the process is dominated from the start by strains of suitable quality yeasts (by avoiding high SO_2 or H_2S producing strains). It is also necessary to assure good nutrition and development of the yeasts in order to be able to use yeast lees without the appearance of off-flavours and to avoid the risk connected with stuck or sluggish fermentations.

The use of selected yeasts and nutrients for fermentation management can be easily counterbalanced by a much lower need of additives and adjuvants in the later phases of wine-making.

2.1.5.1. Start of Fermentation

Principles:

Alcoholic fermentation is an important step of the wine-making process. Complete sugar depletion, without intervention of undesired micro-organisms and without metabolic aberrations, is the basis for the production of a quality wine.

In organic wine-making yeast fermentation can assume a key role. By promoting a healthy and quick development of good wine yeasts it is possible to drastically reduce the risks of oxidation and microbial contamination without addition of inputs and limiting the need for intervention. The early dominance of yeast strain(s) with desired characteristics controls the competition for nutrients with the development of other contaminants. Carbon dioxide produced by yeasts prevents oxygen entering into the system and slows down chemical and enzymatic oxidative reactions.

Importand notes

The organic wine-making process excludes the uses of genetically modified organisms (GMOs) like genetically modified yeast strains (e.g. *Saccharomyces cerevisiae* strain

Moreover, a healthy and suitable yeast population at the end of alcoholic fermentation offers different options of "on lees" practices, with direct favorable effects on wine quality and indirect advantages in terms of protection from oxygen.

The main factor which defines the strategy of fermentation management is the microbial contamination level of the juice to be fermented. This can range from very low to extremely high. High microbial contamination (total population > 10E5 UFC/ml) results from mouldy grapes or grapes which have lost integrity during harvest and transportation, grapes and/or juices which have been kept too long without antimicrobial additives, lack of temperature control in some phases and spoilage by winery equipment with poor hygiene practices.

Low microbial contamination of juices (total population < 10E5 UFC/ml) can be obtained by: processing healthy and sound grapes, speeding up all phases of juice winning and by treatment and controlling juice temperature at every step.

Contaminated juices treated by physical methods (flash-pasteurization, centrifugation, vacuum or cross-flow filtration etc.) will have a low microbial population at the onset of fermentation. However these juices will have lost a major proportion of natural constituents (e.g. assimilable nitrogen and micronutrients) which will result in the need for special attention in management of the fermentation process.

The choice of a known yeast strain to dominate fermentation can be of major importance: some strains can produce up to 100 mg/l SO_2 or more, negating all efforts at reducing preservative addition during wine-making. Some strains can produce high amounts of volatile acidity and/or hydrogen sulphide, which can compromise the final quality of the wine.

6 Yeast description is available on the web side of IFV (Institut Français de la Vigne et du Vin (ENTAV-ITV France) www.vignevin.com in "OUTILS EN LIGNE"

Hundreds of selected wine yeast strains are now commercially available in their dry form[6]. After proper rehydration and seeding, these products allow a quick onset of fermentation and assure the dominance of a strain with good characteristics. Activation of the culture – inoculation of the whole dry yeast dose in a portion of the juice 24 hours before - allows an even more rapid start of fermentation and dominance of the right strain on the indigenous unknown micro-flora.

Those who don't want to use commercial cultures of yeasts can rely on spontaneous fermentation. Given that the dominant strain is of unknown characteristics, this practice can give uncertain qualitative results. If the initial contamination of the must by indigenous population of yeasts is low – e.g. positive conditions – the fermentation take some time before a real start. To partially avoid these problems, some wine-makers promote spontaneous fermentation in several small volumes of juices coming from different vineyards, and to choose the one to be used as starting culture on the basis of sensorial and analytical results. Modern technologies permit in-house yeast strain selection to be inexpensive. Pure cultures can then be used for inoculation of the wines instead of the commercial preparation.

Wine-making options			Related documents
Spontaneous fermentation, Pied du cuvee *Leave natural yeast population present in juice to develop and to dominate fermentation, seed the juice with self selected yeast* Temperature control ▪ Check for volatile acidity and off-flavor development	**Direct inoculation of selected yeasts** *Seed the juice with a significant population of selected wine yeasts* Properly rehydrate dry yeasts in suitable dosage (15-25 g/hl) ▪ (temperature acclimatisation step if juice to be seeded is below 15°C) ▪ Integrated rehydrated yeast suspension added to the juice to be fermented ▪ Temperature control	**Activation of selected yeasts starter culture** *Activate yeast development 24 hours in advance in a portion of the juice, to accelerate fermentation start in the juice and to guarantee dominance of desired micro-organisms* Prepare 12-24 hours in advance a portion of juice equivalent to 5-10% of the final volume ▪ After proper dry yeast rehydration, seed this portion with 200-400 g/hl of dry yeast ▪ After 12-24 hours, use the fermenting portion to seed the whole volume of juice ▪ Temperature control	Practical hint: yeast seeding with activation
Inputs			
Necessary: none	Necessary: selected yeasts	Necessary: selected yeasts	
Regulatory framework: Use of selected yeasts is allowed by most private standards			**Fact sheets:** selected dry yeasts
Additional comments: It is recommended to use yeast strains with a low SO_2 production and if possible with a low nitrogen consumption.			

2.1.5.2. Nitrogen Management

Principles

Organic grape juices can have a lower content of YAN (Yeast Assimilable Nitrogen) compared to those produced by conventional viticulture. (See chapter soil management / fertilization and HACCP). In addition, a reduced use of preservatives like SO_2 in the pre-fermentation phases can induce a higher microbial contamination of the juice which reduces nitrogen availability for Saccharomyces cerevisiae.
As a general rule, the yeasts need over 170 mg /l YAN to complete fermentation: nitrogen requirements increase with sugar content in the juice. Aside from the quantity, the timing is also important. Yeasts need a minimum of YAN at the beginning of fermentation to develop a suitable cell population, then they still need YAN at the end of exponential growing phase to reinforce the cells which will be active at the end of fermentation.

Wine-making options			Related documents
No-Input Oenology	Low-Input Oenology	Low-Input Oenology	
No nutrient addition *Yeasts are left developing on the natural reserve of YAN in the juices – if sufficient.* Check the YAN availability of the juice ▪ Check fermentation activity, volatile acidity and sulphur compound production	**Addition at beginning of fermentation** *In very low YAN juices, nitrogen is supplemented to allow a sufficient growth of yeast population* Check the YAN availability of the juice ▪ Add thiamine and nitrogen nutrients ▪ Check fermentation activity, volatile acidity and sulphur compound production	**Addition at 1/3 – 1/2 of sugar depleted** *Nitrogen available at this stage is used by yeasts to produce enzymes which maintain their activity untill the end of fermentation* Check the YAN availability of the juice ▪ Follow sugar depletion ▪ Add thiamine and nitrogen nutrients ▪ Check fermentation activity, volatile acidity and sulphur	Technical note: Yeast nutrients and their different functions
Inputs			
Necessary: none	Necessary: ammonia salts Useful: thiamine, yeast hulls	Necessary: ammonia salts Useful: thiamine, yeast hulls	
Regulatory framework: Ammonium phosphates are allowed in most EU private standards			**Fact sheets:** #: Di-ammonium phosphate #: thiamine #: yeast hulls

Addition of nitrogen nutrient at beginning of fermentation is then recommended only for very low YAN juices (< 150 mg /l). An addition of nitrogen nutrient at between one third and a halfway through the fermentation progress is helpful in the large majority of cases. Later additions are useless or dangerous. The addition of 30 g/hl of ammonium salts increases YAN by 60 mg/l.
The addition of ammonium salt and thiamine is an important part of the SO_2- reduction strategy in organic wine-making and is also necessary to avoid stuck fermentations.

2.1.5.3. Reductive Sulphur- Off-Flavour corrections

Principles

The occurrence of reductive sulphur off-flavours is linked to many different factors during alcoholic fermentation. Deficiency in nitrogen, pantothenate and pyridoxine (vitamins), residues from the application of wettable sulphur and other stress-inducing factors can lead to the formation of volatile sulphur compounds such as hydrogen-sulphide (H_2S), mercaptans, disulphides and their esters. Among many points, nitrogen deficiency seems to be the most important factor. This is why the content of Yeast Assimilable Nitrogen (YAN) in the must should be controlled before every fermentation. Nitrogen is needed as well as sulphur which is naturally present in every must in the form of sulphate for the formation of amino acids like cysteine and methionine.
Addition of nitrogen nutrient at the beginning of fermentation is then recommended only for very low YAN juices (< 150 mg /l). An addition of nitrogen nutrient at between one third and halfway through the fermentation progress is helpful in the large majority of cases. Later additions are useless or dangerous. The addition of 30 g/hl of ammonium salts increases YAN by 60 mg/l.
The addition of ammonium salt and thiamine is an important part of the SO_2- reduction strategy in organic wine-making and is also necessary to avoid stuck fermentations.
Wettable sulphur is a very important fungicide in organic viticulture. If it is present in the must it will be reduced to hydrogen sulphide by yeasts during fermentation. Maintaining an adequate interval between the last application and harvest will diminish the residues of elemental sulphur on the grapes. Additionally, a good sedimentation and racking of the solids and residues in organic grape juice will minimize the risk of sulphide formation by this source.
The addition of ammonium salt is not only a helpful strategy to avoid sluggish fermentations in organic must, but it will also help to avoid the formation of H_2S. The formation of H_2S should be detected by a regular tasting of the fermenting wine. Usually the formation of H_2S stops as soon as nitrogen is added as ammonium salt in the first half of the fermentation. Later additions of nitrogen will not normally be taken up by the yeasts. If volatile sulphur compounds cannot be avoided during fermentation and a sulphur off-flavour decreases the quality of the final wine, a copper treatment ($CuSO_4$*$5H_2O$) can eliminate hydrogen sulphide (H_2S) and the mercaptans (ethyl mercaptan and methyl mercaptan). Unfortunately other volatile sulphur compounds which may contribute to the off-flavour cannot be removed.
In organic wine processing, the technique of "blue fining" (potassium-hexacyanoferrate) is generally not allowed for the elimination of excessive copper in the final product. The maximum legal dose of copper sulphate as an additive is 1 g/hl. The maximum legal concentration of residual copper in wine is 1 mg/L (Cu^{++}).

Wine-making options			Related documents
No- Input Oenology No nutrient addition *Yeast assimilable nitrogen (YAN) is sufficient. Only healthy grapes were picked. No heat treatment of the juice.*	**Low-Input Oenology Addition of nutrients** *YAN of the juice is very low (< 150 mg N/l). High sugar content. Heat treatment of the juice. Low fermentation temperature.*	**High-Input Oenology Addition of nutrients plus copper treatment** *YAN of the juice is very low (< 150 mg N/l). Sluggish fermentation. Persistent occurrence of S-off-flavours.*	Technical note: Yeast nutrients and their different functions
Check the YAN availability of the juice ■ Check fermentation activity. Check sensory quality during fermentation. If no formation of volatile sulphur compounds, no input of nutrients and treatments is needed.	Check the YAN availability of the juice ■ Add nitrogen containing nutrients ■ If fermentation activity is acceptable and the beginning of production of volatile sulphur compounds can be avoided by nitrogen, no further input is needed.	Check the YAN availability of the juice ■ Add nitrogen containing nutrients ■ Observe course of fermentation ■ If fermentation activity is acceptable but the final wine shows a significant S-off-flavour, aeration and copper fining of the final wine may be necessary to eliminate volatile S-compounds. Pre-tests should be applied.	
Inputs			
Necessary: none	Necessary: ammonium salts Useful: thiamine, yeast hulls	Necessary:ammonium salts, copper sulphate/citrate Useful: thiamine, yeast hulls	
Regulatory framework: Di-Ammonium phosphate is allowed in most EU private standards The additive copper sulphate is allowed by most private standards.			**Fact sheets:** #: ammonium salts #: thiamine #: yeast hulls #: copper sulphate #: copper-citrate
Additional comments: The addition of di-ammonium hydrogen phosphate can be done by dissolving the salt in a small part of the fermenting wine, in order to avoid excessive foaming. The maximum legal dose of copper sulphate as additive is 1 g/hl. The maximum legal concentration of residual copper in wine is 1 mg/L (Cu^{++}). Pre tests of the fining should be applied and evaluated. Excessive copper in the wine can lead to a copper haze at a concentration of > 0.5 mg/L copper and also the oxidation risk of the wine is higher because copper acts as a catalyst for oxidation.			

Pre tests of the fining should be applied and evaluated. Excessive copper in the wine can lead to a copper haze at a concentration of > 0.5 mg/L copper and also the oxidation risk of the wine is higher because copper acts as a catalyst for oxidation.
This means that the use of copper sulphate / citrate as a fining agent for volatile sulphur compounds must be as low as possible for organic wine.

2.1.5.4. Oxygen Management

Principles
Oxygen is essential for yeast growth and activity. Only if oxygen is present can yeast produce sterols and unsaturated fatty acids which are needed to provide the required fluidity of cell membrane, tolerance to ethanol and, consequently, good cell activity throughout the fermentation.

Wine-making practices			Related documents
Pumping over with aeration *Oxygen is dissolved in fermenting juices by pumping over in an open system* Check volatile acidity ■ Pumping over with aeration of a volume of juice corresponding to the double of the volume of the container ■ Check volatile acidity and fermentation activity	**Macro-oxygenation** *Oxygen is added by bubbling pure oxygen or air inside the tank* Check volatile acidity ■ Sprinkle a measured amount of pure oxygen or air in order to add 8 mg O2/l ■ Check volatile acidity and fermentation activity		Technical note : Oxygen and wine
Inputs			
Necessary: none	Necessary: none Useful: pure oxygen		
Regulatory framework: No restriction in the use of these practices			
Additional comments:			

An addition of oxygen at the end of the exponential growth of yeast population (1/2 of sugar depleted) can re-establish cell membrane functionality. At this stage, due to the extremely quick oxygen uptake by the large yeast population, none of the added oxygen is made available for oxidation of wine components. This practice is advised in every wine, except in dessert wines where the oxygen addition could expedite alcoholic fermentation and reduce the final amount of residual sugars.

2.1.5.5. Enrichment

Principles
Adding sugar (in form of Saccharose from cane or beet, concentrated grape must or rectified concentrated grape must) on the top of what was originally contained in the grapes will result in an increase of final alcohol content in wine. This is a practice allowed in UE under certain restrictions.

Importand notes
According to Reg. CE 479/2008, the alcohol degree can be increased by a maximum of 3% in zone A, 2% in zone B and 1,5% in zone C.
The same regulation impose limits in the maximum level of degree alcohol (not more than 2%) and in volume reduction in case of self-enrichment (reverse osmosis, vacuum heating, cryo-concentration).
Beet and cane sugar addition is onlyallowed in zones A, B and part of C.
The other regions can use rectified concentrated must or concentrated must.

In organic wine-making sugar, concentrated and rectified concentrated must of organic origin are obligatory, if they are available. Otherwise a 'time-length' for exceptional use of conventional product has to be allowed.
An alternative technique is theself-enrichment of the wine. This is achieved by different physical methods eg. reverse osmosis subtracts water from juices. Vacuum-heating allows evaporation of a certain portion of water. Cryo-concentration freezes a part of the water in order to eliminate it.
Although these techniques are mainly of physical nature and without any danger for producers, consumers and environment, the organic sector aspires to a better management of the vineyard and its consequent yield and aiming to obtain grapes with higher natural sugar content. The enrichment is considered as a way to modify the original natural composition of juice.

Wine-making option			Related documents
No-Input Oenology	**Low-Input Oenology**	**High-Input Oenology**	
No enrichment	**Self-enrichment**	**Addition of sugars**	
A balanced composition of the grapes between sugar, acid, flavour/aroma can be obtained by an adapted vineyard management. e.g. Yield reduction, fertilisation and soil management, cover-crop management etc.	*The desired alcohol degree is reached by concentrating the juice by physical means (reversal osmosis, evaporation, cryo-concentration)* Precisely determine potential alcohol degree ▪ Run the treatment on the whole batch or on a portion of the juice ▪ To alcoholic fermentation	*Addition of dry sugar or of concentrated rectified must* Precisely determine potential alcohol degree and nitrogen availability for the whole fermentation ▪ Add sugar solution, preferably before the end of alcoholic fermentation. ▪ Check activity and volatile acidity till complete depletion of sugars	Technical note: Oxygen and wine
Inputs			
Necessary: none		Necessary: sugar Useful: ammonia salts	
Regulatory framework: Reg. UE 479/2008 - annexe V - defines precise rules for the enrichment practice Cryo-concentration is not allowed in some Member states by national wine laws			
Additional comments: Sugar from beet or cane is considered as high input, because it is raw-material which does not come from the grape itself; the production of rectified must requires a high energetic input and the use of ion exchange raisins. The high energetic input is also true for the self-enrichment techniques. Sugar, concentrated must and rectified concentrated must have to be of organic origin if available, otherwise a time-length for exceptional use of conventional product has to be allowed.			

2.1.6. Post - Fermentation

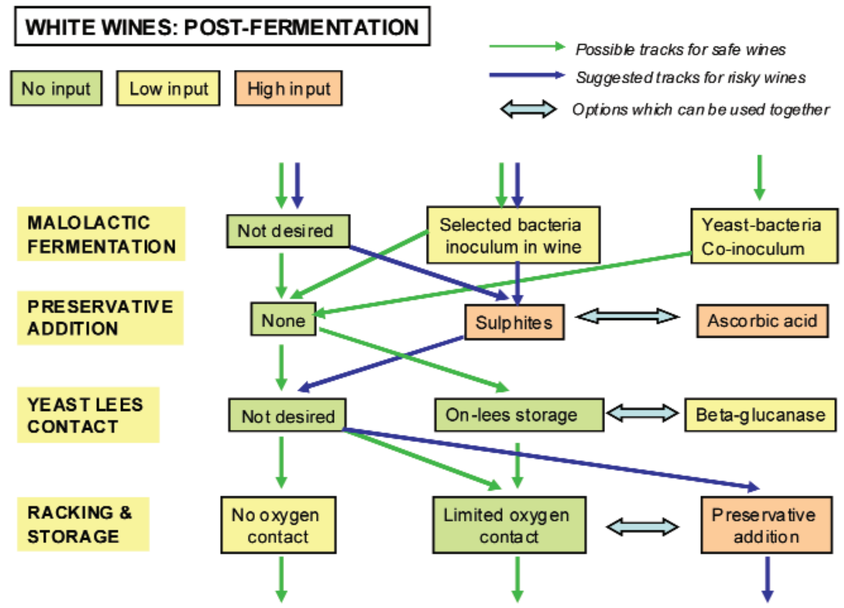

Fig. 47: White wines- post- fermentation options

General principles

The period between the end of alcoholic fermentation and the last fining and bottling of the white wine can last several months, long enough to lose quality because of oxidation or microbial spoilage even at low temperatures. Moreover, during this period several movements of the wine between containers occur, which in addition to some practices can increase the introductionof oxygen into the wine. In organic wine-making, where in the earlier phases the lowest possible amounts of preservative additives have been practiced, the possibilities of quality loss during this stage are quite high. Frequent analytical and sensory assessment of the wine, as well as care in any operation carried out in this period, are of capital importance to produce a high quality wine with low inputs.

2.1.6.1. Malolactic-Fermentation

Principles

Malolactic fermentation reduces titratable acidity of wine by transforming malic acid in lactic acid and CO_2, and modifiying the organoleptic profile of wine by adding typical flavours . Malolactic fermentation can be promoted or avoided in both white and red wines depending or the final style of wine required.

Uncontrolled growth of lactic acid bacteria can bring about the production of biogenic amines or off-flavours which may reduce the commercial value of the wine. Moreover, the wine-making prac-

tices which must be applied to promote spontaneous malolactic fermentation will encourage the development of other undesired micro-organisms like acetic bacteria and Brettanomyces.
In organic wine-making the control of malolactic fermentation is especially critical. The reduced use of additives and particularly the reduced use of SO_2 creates conditions for the development of bacteria at moderately high values of pH. Temperature control and filtration are the key tools to avoid malolactic fermentation.
It is even more difficult for organic wine producers to have a clean and safe malolactic fermentation by controlling the process. The fermentation has to develop and reach completion quickly and has to be carried out by a micro-organism with desired characteristics in order to avoid concurrent growth of spoilage yeasts and bacteria and the production of off-flavours.

Wine-making options			Related documents
Not desired	**Selected bacteria inoculation in wine**	**Yeast – bacteria co-inoculation**	
Growth of lactic bacteria must be limited by avoiding preservatives as much as possible	*The conditions which are limiting bacterial develop-ment are suspended for the time strictly necessary to run a quick MLF by seeding selected bacteria*	*Malolactic bacteria are inoculated during alcoholic fermentation*	Research results : yeast and lactic-bacteria co-inoculation
Temperature kept below 14-16°C during storage Add sulphite solution (min.50 ppm) or Lysozyme (500 g/l) and mix the liquid mass ▪ Frequent malic/lactic acid analysis ▪ In case of signals of bacte-rial activity, filter the wine avoiding contact with oxygen	If needed, warm-up wine to 18-24°C, increase the pH to minimum 3.2 ▪ Properly prepare the freeze-dried culture and seed the wine ▪ Frequent malic/lactic acid analysis ▪ As soon as malic acid has disappeared, filter and chill the wine	Properly prepare the freeze-dried culture ▪ Once alcoholic fermentation activity is evident (ca. 1/3 of sugar depleted) add the bacteria culture ▪ Check malic and lactic acids together with sugars during fermentation ▪ As soon as the two fermentation processes are com-pleted, chill down the wine and filter if no lees contact is desired	
Inputs			
Necessary: Lysozyme; K-metabisulphite, Gaseous SO_2	Necessary: selected bacteria	Necessary: selected bacteria	
Regulatory framework: Use of selected bacteria is allowed by the UE Reg. 834/2007 and by most private standards			Fact sheets: #: malolactic bacteria #: lysozyme
Additional comments: The practice of direct addition of bacteria on wine is preferred to co-inoculum for high pH white wines. The use of Lysozyme has to be labeled as an allergenic compound and the use increase the amount of Bentonite for protein-stabilisation.			

2.1.6.2. Addition of Preservatives

Principles
Once malolactic fermentation – when desired – has been completed, the wine has to be stored safely for months in the wineries. At this stage the wine is unprotected and very weak. No active antioxidants or antimicrobial activities are present, some carbon dioxide is still in solution but there is no active flow of gas from the liquid and therefore there is no protection against oxygen contamination. Nutrients for microbial developments are limited but largely sufficient to permit the growth of spoiling bacteria and yeasts.

Wine-making options			Related documents
No-Input Oenology	**Low-Input Oenology** **Other preservatives**	**High-Input Oenology** **Sulphites**	
Wines are protected from oxidation and microbial spoilage by other means. Not recommended on wines with high content of spoiling micro-organisms or poly-phenols.	*Ascorbic acid (vitamin C) is an antioxidant which can support SO_2 action.* ▪ Add together with SO_2	*Avoid oxidation of juice aroma and phenols; reduce development of bacteria and yeasts. Dosages ranging from 10 to 80 ppm depending on the wine type, the conditions and length of storage* Add sulphite solution and mix the liquid mass, or inject on line during wine movements ▪ Preferred sulphite form depends on dimension and equipment of the winery	Practical hint: reductive wine-making Technical note: Oxygen and wine Microbial contamination SO_2-Management Research results: Alternative additives to SO_2
Inputs			
Necessary: none	Necessary: ascorbic acid	Necessary: P-metabi-sulphite, Gaseous SO_2	
Regulatory framework:			**Fact sheets** #: SO_2 #: P-metabisulphite
Additional comments: SO_2: Several small additions in different steps of the process allow better efficacy using the same total dose.			

Quality losses can be avoided by storage at low temperatures, fully completing alcoholic and malolactic fermentations, filling tanks or barrels to the maximum, maintaining low pHs, and protecting against on-lees contact and inert gas production. If it is necessary to add SO_2 this is one of the best moments to completely profit of the properties of this preservative. Where addition is allowed in wine, ascorbic acid can also be used to reduce SO_2 addition.

2.1.6.3. Yeast Lees Contact

Principles

Yeast lees have several useful attributes particularly for organic wine-making.

They can release yeast wall components (e.g. mannoproteins) which are helpful for tartaric stabilisation as well as protein stabilisation and are believed to positively contribute to the (mouth feeling) of wine. The degradation of the yeast cells releases other amino acids, peptides and nucleic acids. These substances also contribute to the increase in complexity and taste intensity of the wine.

Yeast lees, even after the death of the yeast cells are very active oxygen scavengers and can reduce accumulation of dissolved oxygen into the wine. Glutathione and other sulphur peptides, normally contained in yeasts in significant amounts, are also released in the system and contribute to protect the wine against oxidation.

However yeast lees can also represent a danger. The released amino acids can become a nutrient for spoilage micro-organisms. The bread / nutty notes might not fit with the desired style of wine. When the yeast was stressed at the end of alcoholic fermentation, and depending on the yeast strain, the lees can also transfer to wine reduced notes from sulphurs and mercaptans.

The yeast lees contact is therefore a very powerful tool for organic wine-making. It can be applied only within a coherent strategy of juice preparation and fermentation (ie.suitable juice cleaning, low sulphur yeast strain dominance, correct nitrogen nutrition, oxygen supplementation during fermentation, frequent movement of the yeast deposit during the last stages of alcoholic fermentation, and early racking at the end of fermentation to eliminate solids of bigger dimension, etc.).

Wine-making practices			Related documents
Not desired	**On lees storage**	**Beta-glucanase treat-ment**	
When yeast lees contribute negatively to wine profile (undesired evolution of notes or off-flavours), they are eliminated from the system Make sure sugars are completely depleted ■ Rack the wine 2-3 times or filter the wine (avoid contact with oxygen)	*Lees are kept in contact with wine to protect against oxygen and to release desired sensory active compounds* *Rack the wine before end of fermentation to eliminate gross solids* ■ *Periodically move the wine to re-suspend fine lees* ■ *Check volatile acidity and malic acid during storage* ■ *Frequent wine tasting*	*A part of the wine with (all) yeast lees is treated separately to accelerate yeast autolysis* Concentrate fine lees into a portion of the wine. Tartaric acidification suggested. ■ Add beta-glucanase enzyme ■ Check volatile acidity and taste frequently during storage ■ Once the desired level of autolysis is reached (some weeks), filter the wine and use for blending	
Inputs			
Necessary: none	Necessary: none	Necessary: beta-glucanase enzyme	
Regulatory framework: Beta-glucanase is allowed by UE Reg. 834/2007 and by most EU private standards, the addition of tartaric acid is only allowed in the Mediterranean area winegrowing Zone C			**Fact sheet:** #: beta-glucanase #: tartaric acid

2.1.6.4. Racking and Storage

Principles

The storage of the wine in the winery and its movement among containers is a critical and often understated step of wine-making. All efforts carried out during the earlier stages can be rendered useless if the wine is not kept in good conditions before fining and bottling.

Oxygen and high temperatures are the major enemies of the wine. Both can accelerate oxidation reactions on aromatic compounds and polyphenols as well as the development of spoiling micro-organisms, especially when the wine has not been protected by the presence of additives. Perfect hygiene of containers and equipment is the basic standard. Temperature control is critical. Wine should never stay at more then 14°C for extended periods. When possible, even lower storage temperatures are suggested. It is important to ensure the complete filling of the containers. The avoidance of splashing of the wine during its movements between containers helps to reduce oxygen solubilisation. This is achieved by the use of the container bottom valves to transfer the wine. The use of pumps and other equipment with perfect sealing avoids the creation of 'Venturi' effects which can easily dissolve oxygen in the wine.

When a more critical protection against oxygen is required (i.e. in case of choosing "reductive wine-making" from the early stages or when the wine has a significant phenolic content which has not been previously eliminated), inert gases (nitrogen or argon) must be injected in container headspaces. CO_2 can be useful to fill up tubes and tank bottoms before each movement, and to protect the wine – air interface in the original tank.

Wine-making options			Related documents
No-Input Oenology **No oxygen contact**	**Low-Input Oenology** **Limited oxygen contact**	**High-Input Oenology** **Preservative addition**	
In each step, wine is never in contact with air Temperature control ■ Keep wine containers com-pletely filled and inject inert gases in the headspace ■ Fill tubes and tank bot-toms with CO_2 before any wine movement to avoid contact with air ■ Periodically check colour intensity and volatile acidity	Wine contact with oxygen is limited to the minimum. (Not to be applied on wines already clarified and/or with a high content of phenols, without antioxidant protection). Temperature control ■ Keep wine containers com-pletely filled ■ Avoid wine splashing and unsealed pumps to limit oxygen solubilisation ■ Periodically check colour intensity and volatile acidity	When it is not possible to avoid contact with air, the wine is protected by the means of additives Temperature control ■ Addition of SO_2, ascorbic acid, Lysozyme, tannins in dosage dependent on the alternative protection tools available ■ Check preservative content during storage and supple-ment if needed ■ Periodically check colour intensity and volatile acidity	Technical note: Temperature control Hygienic standards
Inputs			
Necessary: none Useful: SO_2	Necessary: none Useful: SO_2	Necessary: SO_2 Useful: ascorbic-acid, Lysozyme	**Fact sheets** #: ascorbic acid #: Lysozyme #: tannins
Regulatory framework:			
Additional comments: The use of Lysozyme has to be labeled as an allergenic compound and its use increa-ses the need for a higher dosage of Bentonite for protein-stabilisation.			

The cleaner and the cooler the wine is the more susceptible to oxygen solubilisation is the wine. In organic wine-making the strategy of high quality wine production with reduced or no input can be realized only with a perfect control of this stage.
Where it is not possible to manage storage temperature and oxygen contact, quality wines can be obtained by means of additives: SO_2 against micro-organisms and oxidation, Lysozyme to limit lac-tic acid bacteria development, ascorbic-acid to limit oxidation.

2.1.7. Fining

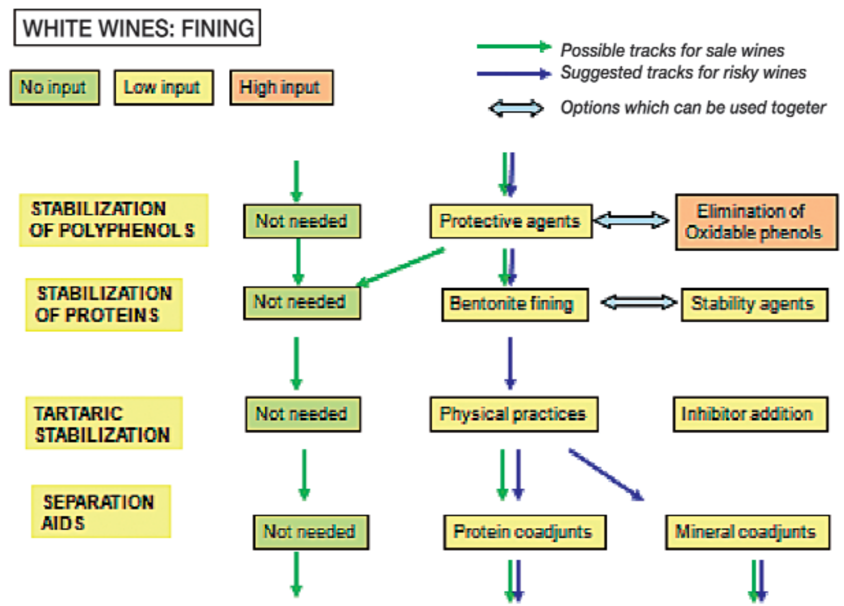

Fig. 48: White wines – fining options

General principles
At the end of the storage period and just before bottling is the last chance to treat the wine in order to guarantee the level of most commercial standards (wine stability and limpidity). The more strict and precise the management of the previous phases of wine-making has been the lower is the need of treatment at the end of the process although some fine adjustment can be needed. Conventional oenology has developed several tools to achieve stability and to make the wine-maker's work easier. Organic wine-making can choose from this palette the options which are more adapted to the prin-ciples of organic production.

2.1.7.1. Stabilisation of Phenols

Principles
Most of the problems concerning polyphenol stability should have been already solved at this stage of the wine-making process although a fine-tuning of the wine might be necessary.
There are at this stage two ways to solve the stability problems of phenols: to eliminate the most unstable ones or to add protective agents, which are avoiding or slowing down oxidation reactions.
To selectively eliminate part of the phenols, the same adjuvants utilised for the juice treatment phase are generally used viz. casein, ov-albumin, gelatine, plant protein, isinglass etc.

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Wine-making options			Related documents
No-Input Oenology *Wine is judged to have acceptable phenolic stability, or oxygen contact is completely avoided in the follow-ing steps of wine-making*	**Low-Input Oenology Protective agents** *Oenological tannins and yeast derivates are added for their antioxidant properties. Arabic gum prevents colloidal precipitation* Products are prepared according to producer instructions ■ Addition to wine avoiding oxygen contact	**Low-Input Oenology Elimination of oxidable phenols** *Adjuvants able to absorb unstable phenols are added to wine and eliminated by later racking and/or filtration* Properly prepare one or a combination of more of the following adjuvants: casein, ov-albumin, gelatine, plant protein, isinglass ■ Addition to wine avoiding oxygen contact	
Inputs			
Necessary: none	Necessary: tannins and/or yeast hulls, arabic gum	Necessary: one or more among casein, ov-albumin, gelatine, plant protein, isinglass	**Fact sheets** #: casein #: ov-albumin #: gelatine #: plant proteins #: isinglass #: arabic gum #: tannins
Regulatory framework:			
Additional comments: The use of casein, P-caseine, ov-albumin has to be labeled as an allergenic compound.			

Oenological tannins of different botanical origin are extracted by different ways and act as protective agents. They act as antioxidant producing free radicals on themselves before re-acting with the wine phenols.

These yeast preparations seem also to increase the polysaccharide content of wine, with positive effects on taste and stability; nevertheless, if added in too high amounts, they can adversely affect the sensory notes (e.g. cheese-like off-flavours), and even compromise wine colloidal stability. Polysaccharides such as Arabic gum can prevent the precipitation of colloids in wine.

2.1.7.2. Stabilisation of Proteins

Principles
Unstable wine proteins can precipitate in the final product giving origin to a deposit in the bottle which is not acceptable to certain markets.
Protein stability is not related with the overall amount of protein content in wine. Some protein fractions, whose nature and origin are only partially known, are more susceptible to precipitation than others. Protein stability of a wine is usually determined by heating the wine to provoke the appearance of precipitates.
Bentonite fining is a cheap and effective practice which allows protein stabilisation of all wines. The clay absorbs the proteins which are then eliminated from the wine. Unfortunately, the reaction is not specific, and other favourable components are removed along with the unstable proteins. The general tendency is then to reduce as much as possible the dosage of Bentonite. One suggested way is to restrict the Bentonite fining to the pre-fermentation phase in those wines which are typically unstable (i.e. Sauvignon blanc).
When Lysozyme is used as protection against lactic acid bacteria a higher dose of Bentonite for protein stabilisation is needed.
Contact with yeast lees is a good natural protein stabilisation technique.
Alternatively, for low level instability, the addition of polysaccharides such as Arabic gum can act against colloidal precipitation in wine, including protein haze.

Wine-making options			Related documents
No-Input Oenology **Not needed** *The formation of deposits in the bottle is accepted.* *No stabilisation treatment* *Natural stabilisation by yeast lees contact and yeast mannoproteins* Check protein instability ■ Check consumer attitude and implement educational actions	**Low-Input Oenology** **Bentonitfining** *Unstable proteins are eliminated by Bentonite treatment* Check protein instability ■ Add re-hydrated Bentonite and leave reacting with protein for some days, keeping the solids suspended ■ Clean the wine by racking and/or filtration	**Low-Input Oenology** **Stability agents** *Arabic gum is added to avoid colloid precipitation* Check protein instability ■ Add arabic gum before or after final filtration	
Inputs			
Necessary: none	Necessary: none		**Fact sheets :** #: bentonite #: Arabic gum
Regulatory framework:			
Additional comments: The quality of Arabic gum has to be very good. It has to be of natural origin, not produced synthetically. Only gum from Acacia should be authorized. Furthermore there are doubts about the ecological value of Arabic gum.			

2.1.7.3. Tartaric Stabilisation

Principles

Many wines have a bitartrate content that is above the saturation point, and are then susceptible to tartrate precipitation, if stored at low temperatures.

Wine consumers generally do not appreciate the presence of crystals at the bottom of the bottle and associate them with something chemical (although they come from a truly natural phenomenon). Nevertheless, some producers decide to not stabilize their wine against tartaric precipitation and to educate their clients about the presence of these crystals.

When a stable wine is planned, there are two main ways to reach the goal. To eliminate from the wine someof the ions (tartrate and potassium) which will bring concentrations to below the saturation point or to add substances which can inhibit the formation or the growth of the tartrate crystals.

Refrigeration of the wine (in batch or continuous) is the most common practice: additives are not needed, but it is very energy intensive. In regions with low winter temperature it is possible to store the wine for a while outside the cellar. Electro-dialysis eliminates some of the excess ions and it is probably the most environment friendly option, although the equipment is expensive and not affordable for every winery.

Metatartaric acid, Arabic gum or, more recently allowed, yeast mannoproteins can inhibit the formation and the growth of crystals, and are alternatives to the physical treatments for the less unstable wines with short shelf-life or higher price

Wine-making options			Related documents
No-Input Oenology *The formation of crystals in the bottle is accepted.* *No stabilisation treatment* *Natural stabilisation by yeast lees contact and yeast mannoproteins* Check tartaric stability ■ Check consumer attitude and implement educational actions	**Low-Input Oenology** **Physical treatments** *Excess ions are eliminated from the wine* Determine wine instability ■ Apply the most suitable technology for the specific winery (refrigeration, electrodialysis) ■ Avoid contact with oxygen	**High-Input Oenology** **Inhibitor addition** *Stability is reach through the addition of compounds inhibiting crystallisation* Determine wine instability ■ Add the most appropriate additive (metatartaric acid, arabic gum, mannoproteins, ■ Avoid contact with oxygen	
Inputs			
Necessary: none	Necessary: none	Necessary: metatartaric acid, Arabic gum, mannoproteins	**Fact sheets** #: metatartaric acid, #: arabic gum #: yeast mannoproteins

2.1.7.4. Separation Aids

Principles

The residual cloudiness of the wine or the hazes formed during fining treatments must be eliminated from wine by simple racking or by physical means.

To speed up this step and to assure a limpidity of the final wine, some adjuvants can be used.

Amongst adjuvants able to promote a better separation of solids from wine there is a list as previously outlined viz. bentonite, silica gel, kaolin with mineral origin, tannins, casein, ov-albumin, gelatine, plant protein and isinglass with natural origin.

Most of these products have multiple effects. Bentonite, for example, absorbs unstable proteins but also creates a heavy flock that settles quickly. Tannins have an antioxidant function but also help in the cleaning of the wine. Casein absorbs phenols but also allows, in conjunction with bentonite, a very efficient clarification.

These products are commercially available as mixtures of different adjuvants balanced according to the specific application.

Some wines may require additional enzyme treatment before the final membrane filtration as is in the case of pressed wines rich in pectin's or wines obtained from grapes heavily infected with botrytis and rich in glucane.

Wine-making options			Related documents
No-Input oenology *Wine viscosity and limpidity targets prevents the use of adjuvant*	**Low-Input Oenology Adjuvant of natural ori-gin** *Adjuvants are added to help create larger solids* Prepare the product according to producer instruction ▪ Add to wine and homogenize the mass.	**low-Input Oenology Adjuvant of mineral origin** *Additives are added to help creating larger solids and higher density* Prepare the product according to producer instruction ▪ Add to wine and homogenize the mass	
Inputs			
Necessary: none	Necessary: one or more among tannins, casein, ov-albumin, gelatine, plant protein, isinglass, pectolytic enzymes, beta-glucanase	Necessary: one or more among Bentonite, silica gel, kaolin	**Fact sheets:** #: Bentonite #: kaolin #: tannins #: casein #: ov-albumin #: gelatin #: isinglass #: plant protein #: silica gel #: pectolytic enzymes #: beta-glucanase
Regulatory framework:			
Additional comments: Time of contact and order of treatment can be of high significance. Be careful with casein or gelatines of animal origin, as they "denature" the vegetal product "wine". Casein, Potassium-caseinate, egg-white and ov-albumin has to be labeled as allergenic compounds.			

2.1.8. Filtration and Bottling

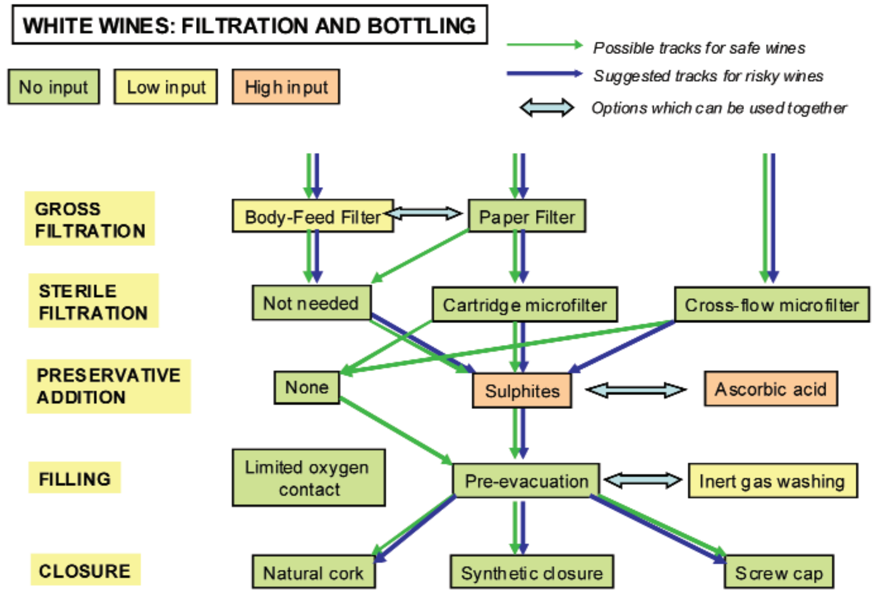


Fig. 49: White wines- filtration and filling options

General principles

Filtration and bottling are the final steps of the wine-making process and they must receive the same attention as previous phases.

It is very easy to solubilise oxygen into wine during these operations, and the contact with several items of equipment can be a source of microbial contamination. This is of particular importance in the filtering and bottling of sweet wines.

An accident during this phase is even more problematic as it will not be possible for the wine-maker to fix the problem later.

In organic wine-making the final wines are less protected by additives and less stripped of potentially dangerous components compared to conventional wines. It is advisable therefore to pay specific attention to the control of these last steps to give the wine a shelf-life adapted to its distribution and consumption destiny.

2.1.8.1. Filtration

Principles

Consumers generally appreciate limpid and brilliant wines. This commercial trend involves the necessity to eliminate from wine any visible particles or colloidal turbid aggregates. This is the goal of the clarification and stabilisation of wines.
All "oenological good practices" are necessary to achieve this goal viz. wine-ageing, wine-fining, racking and filtration.
The filtration of grape must or wine eliminates particles through a porous surface, whereas racking and centrifuging eliminate particles through gravity.
Wines with low preservatives – especially sweet ones – must be bottled free from significant microbial population. Even a very low level of contaminants can grow in the bottle during distribution and storage, often under uncontrolled conditions, and develop haziness, off-flavours or simply cloudiness none accepted by consumers.

It is a common belief that a too tight filtration – as for sterile and brightening ones – can eliminate from wine some positive components like macromolecules which are contributing to wine body and structure. Although, some scientific results doubt this.

Organic wines might be consumed by a segment of people who are less sensitive to cloudiness or presence of haze in wine. Nevertheless, off-flavours must be avoided in any consumer experience and organic wines arrive with more weakness at the bottling. For this reason sterile filtration should be seriously considered as an option for sweet but also dry white organic wines.

The use of cartridges with membranes of different porosity has been the most popular practice for many years, and is still very common in small facilities. Recently cross-flow filtration has been widely used due to its advantages viz. the possibility of cutting out a previous over-all filtration, a better filtration capacity and the absence of waste material. The major limit of this technology is the equipment cost.

Wine-making practices / type of filter for wine				Related documents
Earth filter	**Membrane plate filter or Lenticular filter**	**Membrane filter**	**Cross flow filter**	
Use earth, available in different particle sizes Even with the pink finest earth, a sterile filtration is not possible 2 or 3 earth filtrations are necessary to obtain limpid wine	Some filtration possible with earth filter Different threshold cut-off sizes are available. A sterile filtration is possible 2 or 3 filtrations are necessary to obtain limpid wine	These membranes can clog up if the wine had a too high turbidity Different threshold cut-off sizes are available. A sterile filtration is possible A pre-filtration is advisable with earth filtration for example	Cross flow method prevents the filter clogging One filtration is sufficient to obtain a "sterile" wine	Technical note: Hygienic standards
Inputs				
Necessary: diatomaceous earth, cellulose or perlite	Necessary: Diatomaceous earth, cellulose or perlite	Necessary: None	Necessary: None	**Fact sheets:** #:Diatomaceo-us earth #: cellulose #: perlite
Regulatory framework: No general recommendation / alimentary contact materials / membranes (classic or cross-flow) are obtained by organic synthesis.				
Additional comments: Centrifugation can be used / Some cross-flow membranes are mineral.				

Fig. 50: Cross-flow filtration

Fig. 51: Cellulose or diatomaceous earth filter

2.1.8.2. Addition of Preservative

Principles

A further addition of sulphites and, if required, ascorbic acid might be needed if the residual level of preservative in the wine is too low and / or if the technology available doesn't guarantee satisfactory protection of the wine against oxygen during bottling. Also the choice of the closure is a factor to be considered at this stage as well as the microbial stability of the wine.

Wine-making options			Related documents
No-Input Oenology	**Low-Input Oenology Other preservatives**	**High-Input Oenology-Sulphites**	
Wines are protected from oxidation and microbial spoilage. Not recommended on wines with high content of spoilage micro-organisms or polyphenols.	*Ascorbic acid (vitamin C) is an antioxidant which can support SO_2 action.* ■ Add together with SO_2	*Avoid oxidation of wine aroma and phenols; reduce development of bacteria and yeasts.* *Dosages ranging from 10 to 50 ppm depending on the conditions of bottling, the shelf life targeted and the closure chosen* *A level of min. 30 mg of free SO_2 at bottling is recommended* Preferably inject on line during wine movements	Practical hint: reductive wine-making Technical note: Oxygen and wine Microbial contamination SO_2-Management Research result: Alternative additives to SO_2
Inputs			
Necessary: none	Necessary: ascorbic acid	Necessary: P-metabisulphite, Gaseous SO_2	
Regulatory framework:			
Additional comments: The use of ascorbic-acid is only recommended in combination with an appropriate amount of SO_2, otherwise it increases an early and intensive oxidation of the wine.			

2.1.8.3. Filling

Principles

The wine can be totally or partially saturated in oxygen after an uncontrolled filling process. The oxygen present in the head-space of the bottle (especially when screw caps are used) can be enough to completely consume SO_2 contained in wine. The filling taps are among the most common source of microbial contaminants due to the difficulties encountered when cleaning them.

In the context of organic wine production, the filling step must be carried out with well maintained and modern machinery. The hygiene procedure for detergent use and hygienic procedures must be strictly followed.

To avoid oxygen solubilisation in the wine during this step, various items of equipment offer interesting options. For example the option to remove the air from the empty bottle by means of inert gas flushing or systems which aspirate the air from the empty bottle and/or the head-space by creating a partial vacuum before closure insertion, or a combination of both principles.

Wine-making practices			Related documents
Limited oxygen contact *Exposure of the wine to air is avoided during wine movements through the equipment. Time taken in filling stage and wine temperature are controlled to minimize oxygen solubilisa-tion.*	**Pre-evacuation** *Air contained in the bottle is aspirated before filling. Head space air is aspirated before closure insertion* Follow the procedures suggested by filling machine producers ■ Strictly respect maintenance programs of the equipment	**Inert gas washing** *The empty bottle is flushed with inert gas in order to flush out the air before filling. The head-space is flushed with inert gas before closure application* Follow the procedures suggested by filling machine producers ■ Strictly respect maintenance programs of the equipment	Practical hint: reductive wine-making Technical note: Oxygen and wine Hygienic standards
Inputs			
Necessary: none	Necessary: N_2, CO_2	Necessary: N_2, CO_2	**Fact sheets** #: CO_2 #: N_2

2.1.8.4. Closure

Principles

Though cork has been the only option for hundreds of years, other options have recently seen a wide usage and an increasing acceptance by consumers.

Synthetic closures are constituted by plastic polymers, and can have an apperance very similar to natural cork.

Screw caps have seen a new life: after having been used for decades on very short shelf-life products, new developments in the material used and in the bottling procedures have allowed their use also for premium and super-premium wines.

Different factors are driving the decision of the producer toward one or the other closure viz. cost, consumer acceptance, image of wine, commercial shelf-life, tradition and appellation rules. The most relevant factor for organic wine-maker is probably the OTR (oxygen transfer rate), which measures the permeability of a closure to oxygen and consequently the time a specific wine has before appearance of oxidised traits.

Wine-making practices			Related documents
Natural cork	**Synthetic closure**	**Screw-cap Glass-closure**	
Natural cork is chosen for a combination of technical, economical and commercial reasons. Check closing machine functioning ▪ Expect some inconsistency within bottles of the same lot after aging	*Synthetic closure can be cheaper than corks and offer an acceptable per-formance for young and short-drinkable wines* Adapts closing machine to the closure chosen ▪ Pre-evacuation necessary for some types	*Some screw caps assure an almost perfect impermeability to oxygen. Marketing concern in some countries* Specific closing machine and bottles are needed ▪ Head-space is significantly bigger than with other closures ▪ Specific procedures must be followed	Practical hint: reductive wine-making Technical note: Oxygen and wine
Inputs			
Necessary: none	Necessary: none Useful: N_2, CO_2	Necessary: none Useful: N_2, CO_2	**Fact Sheets** #: CO_2 #: N_2

According to some experts, screw caps with metal liners have an OTR close to zero. They are so impermeable to oxygen that in some cases the wine develops reduced taints with time. Synthetic closures usually show a high consistency in OTR values. Depending on the plastic polymer and on the production system used, they can be very permeable to oxygen with extremely low OTR. Closures made of grinded or powdered cork are similar. Natural cork shows a lower consistency in OTR value and on average it can be more impermeable than synthetic closures.

It is clear that the choice of the closure must be coherent with the rest of the decision taken during the process of production of an organic wine. If a strategy of maximum protection from oxygen and lowest possible levels of sulphite input have been followed, the closure used must guarantee a degree of permeability compatible with the required commercial shelf-life.

Fig. 60 : Different closures (natural cork with or without cap; glass-closure and screw-cap)

2.2. RED WINE PRODUCTION

(Trioli, G. with contribiution of: Cottereau, P.; Hofmann, U.; v.d. Meer, M.; Levite, D.)

2.2.1. Introduction

It is easier to produce a low-input red wine than a white one. Red wines often have higher alcohol than whites and their tannins play a double role of antimicrobial and antioxidant agents.
The modern consumer is looking for red wines with a smooth palate, low astringency and ripe fruit aroma and the presence of off-flavours can drastically reduce competitiveness of the wines in the market. These consumer demands are pushing wine-makers to look for a full maturity of grape in order to obtain intense varietal fruitiness, absence of vegetal notes and softer tannins. A side effect of this trend is the general increase of pHs in red wines, which requires more attention to the management of spoilage micro-organisms.

In white wines, the major danger is **microbial spoilage** resulting in off-flavours due to development of bacteria and non-*Saccharomyces* yeasts in juice and wine. The most common practices against microbial contamination are careful hygiene, temperature control, physical treatments to reduce microbial populations and addition of antimicrobial substances.

Oxidation is less of a worry in red wines than in whites. Tannins consume significant amounts of oxygen which are required in the polymerisation which results in more stable pigments and soft polyphenols. Dissolution of oxygen also reduces the appearance of reduced odors.
This oxygen presence must be controlled as an excess can cause a loss of colour and aroma. In some varieties, poor in red pigments, oxygen can cause significant loss of colour and consequent depreciation of the wine. Moreover, dissolved oxygen can greatly stimulate the growth of acetic bacteria and non-*Saccharomyces* yeasts, among which *Brettanomyces* is considered by far the most dangerous.

Good **prevention** and **control** is possible which greatly reduces the use of additives and adjuvants. The initial use of healthy and balanced grapes is the key to success in organic red wine-making. The presence of mould or bacteria infections makes it impossible to produce low input red wines of outstanding quality and can necessitate physical or chemical interventions to reach an acceptable quality level.
In addition to the oenological quality of the grapes, the choice of suitable equipment and procedures during grape processing and maceration are all essential to minimize the production of wines with defects like astringency, vegetal and reduced notes whose later elimination might require further treatments.

In the following chapters different options are described for each step of red wine-making. No-input options are included (green colour); as well low input ones (yellow colour) together with practices making use of all additives and adjuvants allowed by wine regulations.
Organic wine-making requires the limitation of the use of external inputs, and the choice of the lowest input option in each step of wine-making can expose the producer to an unacceptable **level of risk**.
A good knowledge of the health of the delivered grape and composition as well as a constant tasting and analytical control of the wine can help the wine-maker to follow the best track to succeed in producing a quality wine which is safe for consumer and is environment friendly.

2.2.2. Harvesting

The most important prerequisite for high quality organic red wine is the harvesting of healthy and physiologically mature grapes. The grapes should be protected from fungi or insect attack and free from contamination from Botrytis-sour rot, Oidium etc., right up to the harvest. If there are visible infections of sour rot, Oidium or other fungi infection, the rotted grapes should be sorted by hand picking at harvest. Only healthy grapes reaching the desired ma-turity level are picked. Infected, not full coloured or immature grapes are eliminated in the vineyard; this is the most effective sorting method.

Red grapes can be harvested during the higher day temperatures .
An important prerequisite for optimal wine quality is the optimal physiological maturation of the grapes which is dictated by the grape variety, the environmental and climatic conditions as well as the type of wine that the wine-maker wants to produce. Thus a perfect knowledge of véraison conditions (the optimal relationship between sugar, acid content and pH of the juice as well as the colour of the berries, the smell and taste of the grapes and juice) will al-low the vine-grower to organise the harvest according to the various grape maturity periods.
The grape crop should be harvested by hand or mechanically under favorable climatic conditions, all at once or in several stages, with sorting in the vineyards or on sorting tables in the winery. Thanks to its speed and ease of use, the mechanical harvester permits a rapid harvest of grapes at their optimal quality level and at the most favorable moments, but manual grape-picking can be even more selective and qualitative. Unfavorable climatic conditions at harvest can lead to quality and yield loss in a very short time. Under poor conditions mechanical harvesting can be recommended without selection of the grapes by hand picking.
In certain regions or appellations and vineyards, quality concerns prohibit mechanical harvesting.

The harvest transport is determined by the organisation of harvest work (harvesting by hand or mechanical) and the winery's equipment. From the quality and wine-making viewpoint the grapes should arrive at the winery rapidly and intact. If necessary the grapes and the must should be protected from oxygen and microbial infection by using SO_2, carbon dioxide or dry ice.
Exaggerated brushing and crushing of grapes has to be avoided by:

- using shallow transport containers, vats or bins;
- using easily cleaned material to ensure proper hygiene;
- dumping the grapes into the destemmer, crusher or press directly.

2.2.3. Grape Processing

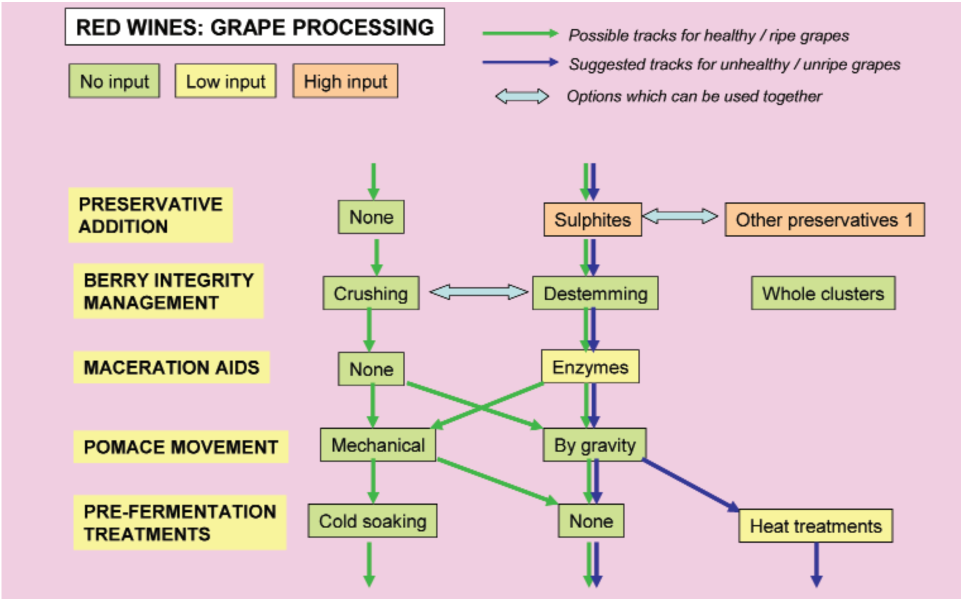


Fig. 61: Red wines – grape processing options

General principles
The healthy state of the grapes, coupled with the knowledge of its varietal traits, defines the strategy to be used in grape processing.
In case of limited mouldiness and hand picking, preliminary sorting of the grapes is an expensive but very useful practice. In case of mechanical harvest or reception of grapes from third parties, a careful selection of grape lots can be very valuable. There are some chemical, spectrophotometric and sensory tools available or under development to assist in the determination of grape quality
Grapes affected by *Botrytis* or Powdery Mildew have weak skins which are easily fragmented by mechanical actions. These skins contain oxidative enzymes and off-flavour precursors whose presence in must and wine has to be avoided.
Skins of unripe grapes as well as grapes infected by downy mildew or bacterial diseases contain astringent and aggressive tannins and can be source of unpleasant herbaceous aroma.
For the above reason, grape processing must proceed with caution and with limited me-chanical action on unhealthy and/or unripe grapes. The extraction must be as selective as possible in order to dissolve pigments but retain the required compounds.

With healthy and ripe grapes an opposite strategy can be followed by trying to extract as much juice as possible from the skins in order to increase the wine structure and its varietal identity. According to some, it is better to accelerate the dissolution of good elements during the early stages (before alcohol extraction occurs) by using enzymes and applying cold soaking.

Grape integrity and consequent microbial contamination is another parameter which influences the choice of time and condition of grape processing.

2.2.3.1. Addition of Preservatives

Principles
The addition of preservatives depends on the health of the grapes and on the overall technological level of the winery.
Healthy grapes can be quickly processed without preservative addition. The presence of moulds (especially from *Botrytis*), the loss of berry integrity with consequent juice release, a long duration of transport and/or processing steps, the lack of temperature control etc. necessitates the requirement to protect the juice against microbial contamination through preservative addition. This dosage will be proportional to the level of damage of the grapes.
The following planned steps will also define the need of preservatives. If thermal treatment (short-time high heating, flash-pasteurisation) of grapes is planned, it can result in a lower need for preservatives.

Wine-making options			Related documents
No-Input Oenology	High-Input Oenology Sulphites	High-Input Oenology Other preservatives	
Grapes are protected from microbial spoilage by other means. Not possible on disease contaminated grapes or which have lost their integrity during harvest and transport.	*Reduce development of bacteria and yeasts; limit the damages from laccase enzyme, increases extraction rate. Dosages ranging from 10 to 40 ppm depending on the state of grapes.* Distribute sulphites on grapes as soon as berry integrity is lost. ▪ Preferred sulphite form depends on when the addition is done (powder on trucks or receivers, solution of gas on-line).	***Oenological tannins** limit the negative effect of laccase in mouldy grapes* ▪ Add to must after crushing and destemming. *Lysozyme* limit the growth of lactic bacteria in contaminated grapes of high pH ▪ Add to must after crushing-destemming	Technical note: Microbial contamina-tion SO_2 Management Research results: Alternative additives to SO_2
Inputs			
Necessary: none	Necessary: P-metabi-sulphite, Gaseous SO_2	Necessary: tannins, Lysozyme	
Regulatory framework:			**Fact sheets** #: SO_2 #: P-metabisulphite #: tannins #: Lysozyme
Additional comments: The use of Lysozyme has to be labeled as an allergenic compound and its use increases a need for higher amounts of Bentonite for protein-stabilisation.			

2.2.3.2. Berry Integrity Management

Principles

In whole berries, enzymes and substrates are kept separated in different vegetal organs; oxygen is not present; micro-organism presence is limited to berry surface and no significant development occurs. As soon as the berry integrity is lost (mould attack, mechanical damage, grape harvesting and delivery etc.) chemical and enzymatic reactions starts, oxygen gets in touch with substrates, and micro-organisms start feeding on juice sugar and nutrients.

In red wine-making the time between harvest and onset of fermentation is much more limited than for white wines. Nevertheless, it is important to keep under control the harvest and transportation conditions: delays of partially crushed grapes for several hours at high temperatures can initiate significant development of micro-organisms. Frequent and efficient cleaning of grape bins and harvesting equipment is an important and often neglected rule.

Apart from carbonic maceration of whole clusters, the grapes are usually immediately crushed and/or destemmed and transferred to the maceration tank. According to the desired wine style and with the equipment available in the winery, the order of the two operations can change. Some prefer to avoid complete crushing and deliver a certain percentage of whole destemmed berries to fermentation.

The use of suitable equipment for the movement of the solids can avoid maceration of the skins and consequent release of grassy or astringent compounds into the wine. Soft technology and the use of gravity as the sole force to move crushed grapes are becoming popular.

Wine-making practices			Related documents
Crushing	**Destemming**	**Whole clusters (carbonic maceration)**	
Berries are at totally or partially pressed to increase the rate of extraction during maceration. *The stalks should preferably be eliminated before skin maceration* Hand or mechanical harvest ■ Quick transportation to winery ■ Crushing machine	*The elimination of stems and leaves eliminate a potential source of astringent tannins, grassy aromas and minerals.* Hand or mechanical harvest ■ Quick transportation to winery ■ Destemming machine	*Red grape clusters are stored in close containers for some days in order to promote carbonic maceration.* Keep clusters under saturated CO_2 atmosphere for few days ■ Press the grapes and proceed as for whites	
Inputs			
Necessary: none	Necessary: none	Necessary: none	

Fig. 62: Red wine fermentation tanks on the top to fill the horizontal tank-press without pumping.

2.2.3.3. Maceration Aids

Principles

The processing of grapes for the production of some type of wines may take advantage from the use of pectolytic enzymes with high percentages of cellulase, hemicellulase, lipase and protease. This technique can accelerate the release of colour and tannins from the skin and allow a shorter maceration length for the preferential extraction of desired compounds. These enzymes are mainly used to increase the colour intensity in young wines or to obtain a softer tannin profile in well structured wines. Enzymatic activity greatly depends on temperature: if cold pre-fermentative maceration is planned, their action will result slower and subsequent thermal treatment can deactivated them.

Sulphur dioxide dissolves skin pigments into the liquid phase and its presence during maceration is sometimes desired when scarcely coloured grapes are processed. Alcohol and temperature play a synergic role with SO_2.

Wine-making options			Related documents
No-Input Oenology **None** *The grape characteristics and the type of wine to be produced don't require maceration aids.* Destemmed and/or crushed grapes ■ Temperature control ■ To maceration tank	**Low-Input Oenology** **Maceration enzymes** *Special enzymes are added to crushed grapes to accelerate and modify extraction activities* Destemmed and crushed grapes ■ Addition of enzymes (0,5 – 3 g/hl) ■ Temperature control ■ To maceration tank		
Inputs			
Necessary: none	Necessary: none		
Regulatory framework:			**Fact sheet:** #: pectolytic enzymes
Additional comments:			

2.2.3.4. Pomace Movement

Principles

Most grapes don't achieve complete skin ripeness and are processed when the unripe part of the grapes can be a source of herbaceous aromas or astringent tannins.
Mechanical laceration of the skin exposes a higher surface of vegetal skin tissues to extraction and can significantly increase the appearance of defects in the final wine. For the same reasons, seed integrity must be carefully respected.
In addition with the caution taken during destemming and crushing, the movement of pomaces in the winery must be done with minimal mechanical friction of the grape solids against the equipment and amongst themselves.
The type of pump used plays a very important role. Centrifuge pump use must be avoided even for the movement of low solid masses, as they can grind seeds and skins into fine fragments of easy extraction. Piston pumps are still popular, but helicoidal and peristaltic pumps of different types and materials, with reduced mechanical impact, are available on the market.

To minimize damage to the pomace some wineries rely only on gravity for pomace movement. After crushing it is gathered into bins which are lifted to the top of the maceration tanks for downloading.

Wine-making practices		
Mechanical *Crushed grapes are moved to maceration tank by mean of pumps.* *The choice of the pump used greatly affects the degree of mechanical soliciting of the grapes.*	**Gravity** *Crushed grapes are gathered into bins which are lifted to the top of maceration tanks for downloading.* *Grapes are moved without causing laceration of skins and seeds.*	
Inputs		
Necessary: none	Necessary: none	

Fig. 63: Mush pump to transport the fermented mush to the press.

2.2.3.5. Pre-Fermentation Treatments

Principles

Some grapes may benefit from practices aimed to increase the extraction of skin components before the beginning of the alcoholic fermentation.

Cold soaking is practiced on grapes in good sanitary conditions and without microbial contamination, with or without enzyme addition. This will increase the dissolution of components into an aqueous phase and generate more complex and fruity aromas in the wines. In some cases, the pomace is protected with CO_2 to preserve maximum varietal aromas and anthocyanins.

Wine-making practices			Related documents
Cold soaking	**Minimal practice**	**Heat treatments**	
Crushed grapes are refrigerated and left soaking before the beginning of alcoholic fermentation to obtain more complex aroma profiles and improved colour in some grapes. Only on ripe and healthy grapes.	*Crushed grapes are directly sent to maceration tank with or without temperature adjustment.*	*Grapes are heated at high temperature for short times – in case also high temperatures are applied – to increase extraction and to reduce microbial contamination.*	
Destemmed and crushed grapes ■ Refrigeration. To maceration tank ■ Storage at 6-10°C for 1-5 days ■ Increase temperature and fermentation onset	Destemmed and crushed grapes ■ Refrigeration when needed ■ To maceration tank and immediate onset of fermentation	Destemmed and crushed grapes ■ Heating at high temperatures for short time (i.e. 70°-75°C for 2 minutes) ■ High pressure and sudden release (optional) ■ To maceration tank (optional)	
Inputs			
Necessary: none	Necessary: none	Necessary: none Useful: enzymes, tannins	**Fact sheets:** #: enzymes #: tannins
Additional comments: The short time high heating is a very energy-intensive system			

Mouldy or unripe grapes management can be improved by heating at temperatures and for time periods sufficient to inactivate oxidative enzymes and extract colour without compromising wine aroma profile. When thermo-vinification is used to extract colour from red grapes which avoids the maceration step, the resulting wine has low colour stability and clarification difficulties. The addition of pectolytic enzymes and an at least a brief maceration to increase stabilizing tannins can be useful additional treatments to the thermo-vinification technology.

In some cases heating is coupled with high pressure applied to the pomace and followed by sudden releases, which can crack and fissure the skin structure allowing a faster extraction during the subsequent maceration (i.e. flash deténte; flash-pasteurization).

The technologies based on heating can also cause a partial reduction of microbial population.

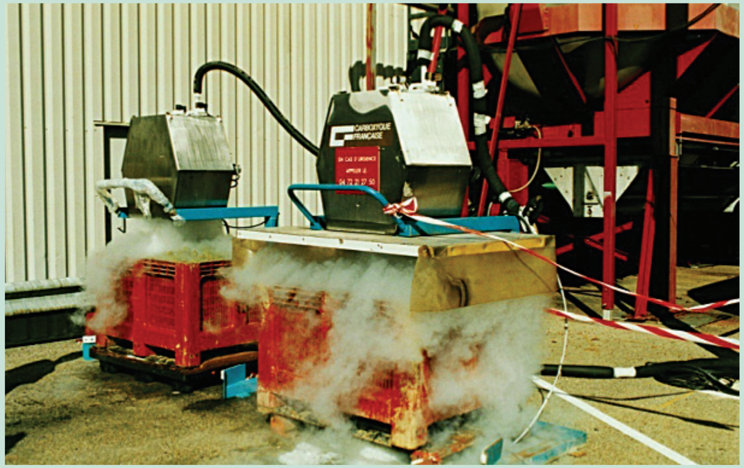


Fig. 64: Technical equipment for cold soaking with dry ice. Production of carbonic snow.

Fig. 65: Technical equipment for cooling and heating – turbular heat exchanger (Röhrenwärmetauscher; Echangeur de temperature tubulaire).

2.2.3. Fermentation

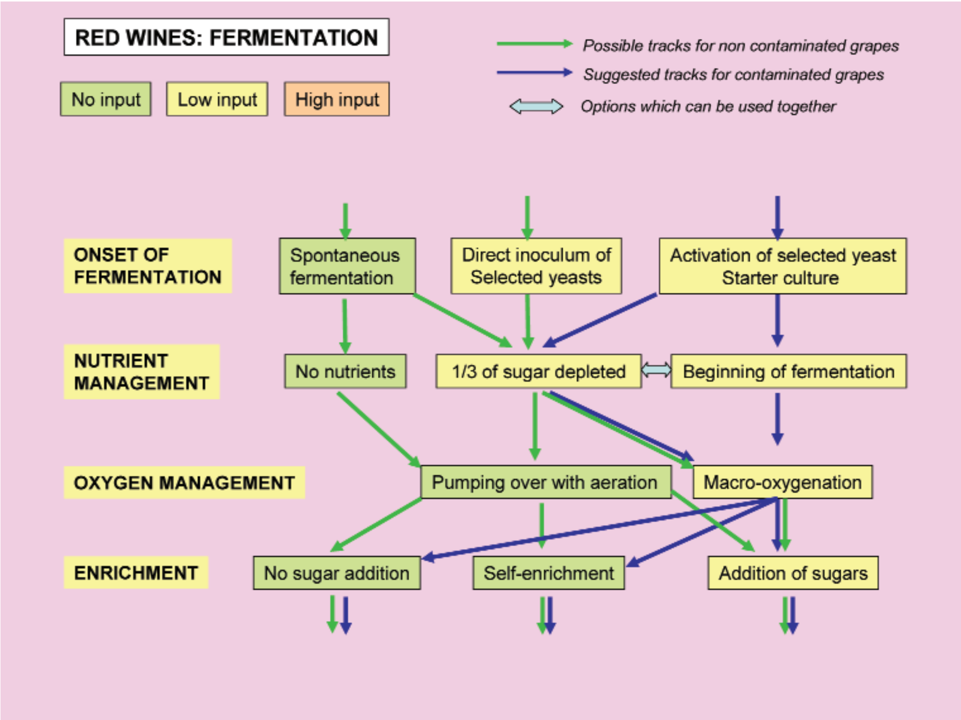


Fig. 66: Red wines – fermentation options

General principles

Good management of alcoholic fermentation can represent a powerful tool which limits the use of inputs and treatments.

During fermentation the wine is perfectly protected against oxidation and spoilage. Wine yeasts quickly utilize all oxygen present and so compete against contaminant micro-organisms. The protection against oxygen continues even after the complete depletion of sugars until yeast lees are present in the system.

In organic wine-making it is important to quickly promote the start of fermentation, to ensure that the process is dominated at the start by strains of suitable quality yeasts (by avoiding strains high producers of SO_2 or H_2S). It is important to ensure good nutrition and health of the yeasts in order to be able to use yeast lees without appearance of off-flavor and to avoid risks connected with stuck or sluggish fermentation.

The use of selected yeasts and nutrients for fermentation management can be easily counterbalanced by a much lower need of additives and adjuvants in the later phases of wine-making

2.2.3.1. On-set of Fermentation

Principles:

Alcoholic fermentation is an important step of the wine-making process. Complete sugar depletion without intervention of undesired micro-organisms and without metabolic interventions is the basis for the production of a quality wine.

In organic wine-making yeast fermentation can assume a key role. The promotion of a healthy and fast development of good wine yeasts drastically reduces the risks of oxidation and microbial contamination without the addition of inputs. The early dominance by yeast strain(s) with desired characteristics controls by competition on nutrients the development of contaminants.

A healthy and suitable yeast population at the end of alcoholic fermentation offers different options of “on lees” practices, with direct favorable effects on wine quality and indirect advantages in terms of protection from oxygen.

The main factor which defines the strategy of fermentation management is the microbial contamination level of the must to be fermented. High microbial contamination (total population > 10E5 UFC/ml) typically originates from: mouldy grapes which have lost integrity during harvest and transportation, crushed grapes which have been kept too long in absence of antimicrobial additives, lack of temperature control in some phases, spoilage by winery equipment with weak sanitation practices.

Low microbial contamination of juices (total population < 10E5 UFC/ml) can be obtained by: processing healthy and sound grapes, speeding up all phases of grape processing, controlling temperature at every step.

Contaminated musts treated by physical treatments (flash-déténte, thermo-vinification etc.) can have very low microbial populations at fermentation onset however these musts may have lost a major proportion of natural constituents (i.e. assimilable nitrogen and micronutrients) which require special attention in the management of fermentation.

The choice of a known yeast strain to dominate fermentation can be of critical importance. Some strains can produce up to 100 mg/l SO_2 or more, making useless all efforts to reduce preservative addition during wine-making. Some strains can also produce high amounts of volatile acidity and/or hydrogen sulphide which can compromise the final quality of the wine.

Hundreds of selected yeast wine strains are now commercially available in a dry form. After proper rehydration and seeding, these products allow a quick onset of fermentation and assure the dominance of strains with good characteristics. Activation of the culture – inoculation of the whole dry yeast dose in a portion of the juice 24 hours before - allows an even more rapid start of fermentation and dominance of the right strain on the indigenous unknown microflora.

Wine-making options			Related documents
No-Input Oenology Spontaneous fer-menta-tion *(only for minimally contaminated musts)* *Leave yeast population naturally present in grapes to develop and dominate fermentation* Temperature control ■ Check for volatile acidity and off-flavor development	**Low-Input Oenology Direct inoculum of selected yeasts** *Seed the must with a significant population of selected wine yeasts* Properly rehydrate dry yeasts in suitable dosage (15-25 g/hl) ■ Integrated rehydrated yeast suspension to the must to be fermented ■ Temperature control	**Low-Input Oenology Activation of selected yeasts starter culture** *Activate yeast development 24 hours in advance in a portion of must, to accelerate fermentation onset and to guarantee dominance of desired micro-organisms* Prepare 12-24 hours in advance a portion of must equivalent to 5-10% of the final volume ■ After proper dry yeast rehydration, seed this portion with 200-400 g/hl of dry yeasts ■ After 12-24 hours, use the fermenting portion to seed the whole volume of must ■ Temperature control	Technical note: yeast nutrients and their different functions Practical hint: Yeast seeding with activation
Inputs			
Necessary: none	Necessary: selected yeasts	Necessary: selected yeasts	
Regulatory framework: Use of selected yeasts is allowed by most private standard			**Fact sheet:** #: selected yeasts
Additional comments: The short time high heating is a very energy-intensive system			

Those who don't want to use commercial cultures of yeasts can always rely on spontaneous fermentation. Given that the dominant strain is of unknown characteristics, this practice can give uncertain qualitative results. If indigenous population is low – positive conditions – the fermentation can need some days before a real start.
To partially avoid these problems, some wine-makers promote spontaneous fermentation by testing several small volumes of must coming from different vineyards and to choose the one to be used as starting culture on the basis of sensorial and analytical results. Modern technologies make rather inexpensive this in-house yeast strain selection with the goal of obtaining pure indigenous cultures for inoculation of their wines instead of commercial preparations.

2.2.3.2. Nitrogen Management

Principles

Organic musts have, in general, a lower content of YAN (Yeast Assimilable Nitrogen) compared to those produced by conventional viticulture. In addition, a reduced use of preservatives like SO_2 in the pre-fermentation phases can induce a higher microbial contamination of the juice which reduces nitrogen availability for Saccharomyces cerevisiae.
129 As a general rule, the yeasts need 200-300 mg /l YAN to comfortably complete fer-mentation (nitrogen needs increases with sugar content). As well as the quantity of YAN, the timing of availability is also important. Yeasts need a minimum of YAN at the beginning of fermentation
Nitrogen nutrient addition at the beginning of fermentation is then recommended only for very low YAN juices (< 150 mg /l). An addition of YAN at the depletion of 1/3 – 1/2 of sugars is required in most cases. Later additions are useless or dangerous. The addition of 30 g/hl of ammonium salts increases YAN by 60 mg /l.

Wine-making options			Related documents
No-Input Oenology No nutrient addition *Yeasts are left developing on the natural reserve of YAN of the must – if sufficient.* Check the YAN availability of the must ■ Check fermentation activity, volatile acidity and sulphur compound production	**Low-Input Oenology Addition at beginning of fermentation** *In very low YAN musts, nitrogen is supplemented to allow a sufficient growth of yeast population* Check the YAN availability of the must ■ Add nitrogen nutrients ■ Check fermentation activity, volatile acidity and sulphur compound production	**Low-Input Oenology Addition at 1/3 – 1/2 of sugar depleted** *Nitrogen available at this stage is used by yeasts to produce enzymes which maintain themselve active untill the end of fermentation* Check the YAN availability of the must ■ Follow sugar depletion ■ Add nitrogen nutrients ■ Check fermentation activity, volatile acidity and sulphur compound production	Technical note: Yeast nutrients and their different functions
Inputs			
Necessary: none	Necessary: ammonia salts Useful: thiamine, yeast hulls	Necessary: ammonia salts Useful: thiamine, yeast hulls	
Regulatory framework: Di-Ammonium phosphates are allowed in most EU private standards			**Fact sheets:** #: Di-Ammonium phosphate #: thiamine #: yeast hulls
Additional comments:			

2.2.3.3. Oxygen Management

Principles

Oxygen is essential for yeast growth and activity. It is only if oxygen is present can the yeast produce sterols and unsaturated fatty acids which are needed to provide the required fluidity of cell membrane and, consequently, a good cell activity. The first generation of yeasts can usually find oxygen dissolved in the juice, but enzymatic and yeast activity quickly exhausts this reserve. The final yeast generations (those who must complete alcoholic fermentation) can then starve for oxygen.
An addition of oxygen at the end of the exponential growth of yeast population (1/2 of sugar depleted) can re-establish cell membrane functionality. At this stage, due to the extremely quick oxygen uptake by the large yeast population, none of the added oxygen is left available for oxidation of wine components.
In the production of some red wines which have high pigment content which needs to be stabilized, the addition of oxygen must be much higher than what required by the yeasts (8-10 mg/l). In these cases, several pumping over with air or controlled input of air or oxygen might be beneficial.

Wine-making practices			Related documents
Pumping over with aeration *Oxygen is dissolved in fermenting must by pumping over in an open system* *Check volatile acidity* ■ *Pumping over with aeration of a volume of liquid correspon-ding to the double of the volume of the container* ■ *Repeat operation accord*ingly with wine requirements ■ Check volatile acidity, sul-phur odours and fermenta-tion activity	**Micro- and Macro-oxygenation** *Oxygen is added by bubbling pure oxygen or air inside the tank* *Check volatile acidity* ■ *Sprinkle a measured amount of pure oxygen or air* ■ *Quantify oxygen addition on the basis of wine characteristics* ■ *Check volatile acidity, sulphur* odours and fermentation activity		Technical note: Oxygen and wine Practical hint: Hyperoxigenation
Inputs			
Necessary: none	Necessary: none Useful: pure oxygen		
Regulatory framework: No restriction in the use of these practices			
Additional comments: The micro- and macro-oxygenation techniques require frequent wine-tasting, in order to not oxidise the sensitive components too much.			

2.2.3.4. Enrichment

Principles

The increase of final alcohol content in wine, by adding sugar on the top of what was originally contained in the grapes, is a practise allowed in the EU under certain limits.

Importand notes

According to Reg. CE 479/2008, the alcohol degree can be increased by a maximum of 3% in zone A, 2% in zone B and 1,5% in zone C.
The same regulation impose limits in the maximum level of degree alcohol (not more than 2%) and in volume reduction in case of self-enrichment (reverse osmosis, vacuum heating, cryo-concentration).
Beet and cane sugar addition is onlyallowed in zones A, B and part of C.
The other regions can use rectified concentrated must or concentrated must.

In organic wine-making sugars and concentrated must as well as rectified concentrated must should be of organic origin and are obligatory if available. If not a 'time period' for exceptional use of a conventional product is allowed.

An alternative approach is self-enrichment which can be achieved by different physical means; reverse osmosis subtracts water from juices or vacuum-heating which results in the evaporation of a certain portion of water. 'Cryo-concentrations' make it possible to freeze part of the water to be eliminated in order to increase sugar concentration.
Although these techniques are mainly of a physical nature and without any danger for producers, consumers and environment, in the organic sector there is a tendency to prefer a better management of the vineyard yield to obtain higher quality grapes. The enrichment is considered a way to modify the original natural composition of juice.

Wine-making practices			Related documents
No-Input Oenology **No enrichment** *A balanced composition of the grapes is obtained through a better management of the vineyard*	**Low-Input Oenology** **Self-enrichment** *The desired alcohol degree is reached by concentrating the juice by physical means (revers-osmosis, evaporation, cryo-concentration)* Precisely determine potential alcohol degree ▪ Run the treatment on a portion of the juice drained from the maceration tank ▪ Reintegrate the concentrated portion to the rest of the mass	**High-Input Oenology** **Addition of sugars** *Addition of dry sugar or concentrated rectified must is done according to the rules and limits of Reg. CE 479/2008* Precisely determine potential alcohol degree and nitrogen availability for the whole fermentation ▪ Add sugar solution, preferably before the end of alcoholic fermentation. ▪ Check activity and volatile acidity till complete depletion of sugars	
Inputs			
Necessary: none	Necessary: none	Necessary: organic sugar, concentrated must Useful: ammonia salts	
Regulatory framework: Reg. UE 479/2008 - annexe V - defines precise rules for the enrichment practice			Reg. UE 479/2008,
Additional comments: Sugar from beet or cane is considered as high input, because it is raw-material which does not come from the grape itself; the production of rectified must (sugar from grapes) requires a high energetic input and the use of ion exchange raisins. The high energetic input is also true for the self-enrichment techniques incl. concentrated must. Sugar, concentrated must and rectified concentrated must have to be organic origin if available, otherwise a time period for exceptional use of conventional product has to be allowed.			

2.2.4. Maceration

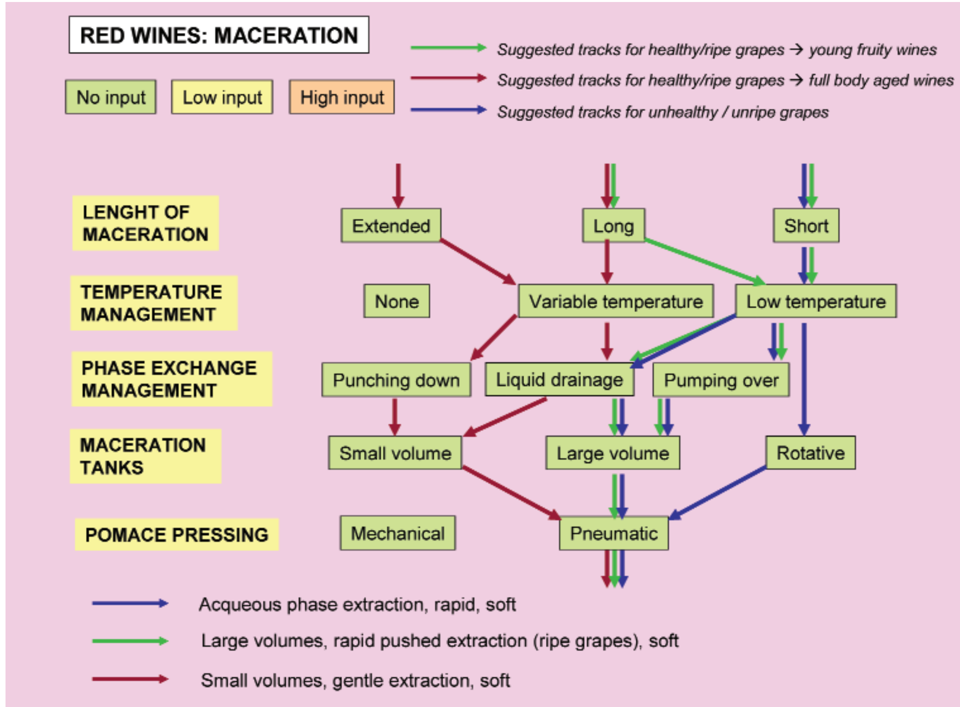


Fig. 67: Red wines – maceration options

General principles

Maceration is needed in red wine production to extract colour and structure from the skins. Whatever maceration strategy is used, the amount of skin component dissolving into the wine will only represent a fraction of the total potential.

The main concern in maceration is to be as selective as possible. In addition to positive compounds like anthocyanins, polysaccharides, aromas and some minerals, unripe skins can also release harsh tannins, herbaceous notes, abnormal acidity and mouldy grapes can be source of oxidative enzymes, glucans and unpleasant aromas.

Thus the maceration strategy depends on the specific traits of the grapes to be processed and which should be characterized in detail by chemical and sensory analysis.

When processing unripe or mouldy grapes, the aim is to limit the dissolution of harmful compounds while promoting an acceptable presence in the wine of colour and structure. This is achieved by increasing the dissolution during the aqueous phase by the use of enzymes by reducing the mechanical actions in order to limit laceration of the skin and formation of lees, and by avoiding contact with oxygen in order to preserve the small content in colour and varietal aromas.

Ripe grapes permit a wider range of options. If a full bodied wine to be aged is planned, maceration can be more intense and its duration can be extended after the end of the alcoholic fermentation. Alternatively if a young fruity and easy-to-drink wine is aimed at the maceration strategy is closer to what is used for unripe and mouldy grapes.

2.2.4.1. Length of Maceration

Principles

The length of the maceration process is one of the main factors which define the final result, not only in terms of total quantity of the solutes but also in relation to their quality.
In the first 1 to 3 days of maceration, before fermentation starts, the pomace is soaked in an aqueous solution during which small and charged compounds like anthocyanins, acids, minerals and small aroma molecules are released. Tannins which are not linked to cell structures will be released more quickly.
In the second phase, during the bulk of fermentation, the alcohol concentration in the system increases and apolar and more complex molecules will finally be dissolved. Most of the tannins, aromas and polysaccharides pass into solution during this phase.
Extended maceration describes the practice of leaving the pomace soaking in the wine after the end of yeast activity, usually after having completely filled the tank in order to avoid development of acetic bacteria, and by regularly moving the system. It can last few weeks but is not uncommon to hear about periods of months. During this period the extraction has almost reached the plateau and the development is mainly due to chemical reactions with pigments and polysaccharides combining together to give more stable and desirable molecules. This practice, though, must be avoided when the grapes are not perfectly ripe.

Wine-making practices			Related documents
Extended	**Long**	**Short**	
Pomace is left soaking in wine for weeks after the end of fermentation At sugar dryness fill up the tank with wine or inert gas ■ Regularly submerge the pomace and add oxygen if needed. Frequently check evolution by chemical and sensory analysis (critical parameters: VA, vegetable and reduced notes, astringency) ■ Drain off and pressing	*Length of maceration (7-15 days) approaches the duration of alcoholic fermentation, till when the desired extent of extraction is reached.* Follow extraction from skin by chemical and sensory analysis (critical parameters: colour, vegetable and reduced notes, astringency) ■ Drain off and pomace pressing at the desired level of extraction	*Extraction is roughly limited to aqueous phase (1-4 days) to avoid appearance of negative notes in the wine* Follow extraction from skin by chemical and sensory analysis (critical parameters: colour, vegetable and reduced notes, astringency) ■ Drain off and pomace pressing at the desired level of extraction	Technical note: Oxygen and wine Practical hint: Hyperoxygenation
Inputs			
Necessary: none Useful: enzymes, O_2	Necessary: none Useful: enzymes, O_2	Necessary: none Useful: enzymes	**Fact sheets:** #: enzymes #: O_2

2.2.4.2. Temperature Management

Principles

The higher the temperature, the faster will be dissolution of compounds from the skin and the more intense will be yeast fermentation. But also chemical and enzymatic oxidative reactions and yeast alcohol stress will increase.
Low temperatures (20 to 25°C) are preferred when it is the varietal fruity aromas that are needed to be preserved and when colour stabilisation is not the main concern nor when potential alcohol degree is high enough to represent a risk of stuck or sluggish fermentation.
High temperatures (25 to 30°C) tend to increase pigment combination and stabilisation and to accelerate extraction. If the temperature is too high it can also determine heat degradation of anthocyanins and colour loss.
However it is strongly recommended not to exceed 30°C, especially toward the end of fermentation when the presence of alcohol can be very harmful for yeast activity and survival.

Wine-making practices		
None	**Variable temperature**	**Low temperature**
No temperature control is applied	*Temperature is controlled and modified during maceration to promote colour stabilisation in low colour varieties*	*Slow extraction of colour and aroma is promoted; oxidation of extracted compounds is limited.*
Temperature in the tank increases as a consequence of fermentation ■ Avoid nitrogen addition at beginning of fermentation unless in cases of strong N deficiency ■ Regularly check temperature and fermentation activity (critical parameters: sugar, VA)	Control temperature during fermentation ■ Close the maceration tank at the end of alcoholic fermentation ■ Gradually increase temperature (1°C per day, up to 30 - 32°C)	Keep temperature at 20 -25°C ■ Regularly check colour, aroma and fermentation activity ■ If yeast activity is too low, or toward the end of fermentation, increase temperature by 1 - 2°C
Inputs		
Necessary: none	Necessary: none	Necessary: none

2.2.4.3. Phase exchange Management

Principles

The extraction of compounds from skins is essentially a diffusion process. The efficiency of the extraction process depends on contact length and temperature. It also depends on the concentration difference for each compound between the solid phase (skins or seeds) and the liquid one (must or wine).

Punching down, pumping over, déléstage, remontage, rotative systems, rainfall devices, CO_2 bubbling all these systems have the same aim - to wash out the skin cap in order to substitute the concentrated must with a less concentrated surface to keep extracting from the skins.

It is also necessary to consider mechanical actions which tend to produce fragments of skin which suspend into the must. This creates more lees and, in case of skins with negative traits, accelerates extraction. Therefore, most recent developments in maceration techniques have been driven by the attempt of increasing the washing of the cap while reducing mechanical action on skins.

Finally, the variable proportion of water and alcohol in the must during maceration should be considered, as these two components have different extraction power on different categories of substances.

Wine-making practices		
Punching down	**Liquid drainage**	**Pumping over**
Once created, the skin cap is regularly submerged into the liquid both manually or automatically, by mechanical means or using pressurized gases. Easy but expensive practice.	*A portion of liquid is drained out from the maceration tank and brought back on the top of the cap with high flows, in order to totally submerge the cap (i.e. déléstage, Selector system)*	*By using a pump, the must or wine is taken from the bottom of the tank and pumped over the cap, in order to create a flow of fresh liquid through the cap.*
Wait till when fermentation onset creates a skin cap on the top of the liquid ■ Set up the parameters: frequency and operation length ■ Monitor colour, astringency, aroma profile and VA	Depending on the system used, wait till when the cap is formed or start the operation at beginning maceration ■ Set up the parameters: frequency and volume of liquid involved ■ Monitor colour, astringency, aroma profile and VA	Critical point: the type of pump used (must respect solids going through) ■ Set up the parameters: frequency, flow rate and volume of liquid involved ■ Monitor colour, astringency, aroma profile and VA
Inputs		
Useful: CO_2 / N_2 gases	Necessary: none	Necessary: none
Regulatory framework:		

Fig. 68: Illustration of a pumping over (remontage) with aeration.

Principles

The shape of the maceration tank, the global volume and the integrated devices or functions are often a major concern, as these parameters can greatly affect equipment and production costs.
Small volumes, short and large tanks are considered more suitable for high quality wines, as the relationship between liquid and solid phase during maceration allows a softer and manageable extraction.
Big, tall and narrow tanks can allow space optimisation in the winery and usually have significantly lower costs compared to the previous shape but the higher relative thickness of the cap and the high liquid pressure on the bottom lees requires stronger mechanical actions and is considered one of the causes of off-flavours.
Horizontal, rotating tanks are interesting options for wineries which need to process a large volume of grapes and/or to minimize maceration length. A slow but frequent movement of the whole tank allows a complete mixing of the phases and a quick and early extraction from the skin. Although, depending on the type of grape and on the system used, heavy mechanical action can adversely affect wine quality.

Producers have enriched their maceration tanks with a large range of devices in order to improve management and control of the different operations using timers, valves, gas injectors, heating a cooling options, automatic pomace discharge, etc. The usefulness of these options greatly depends on the size of the winery and on the availability of personnel during the harvest period.

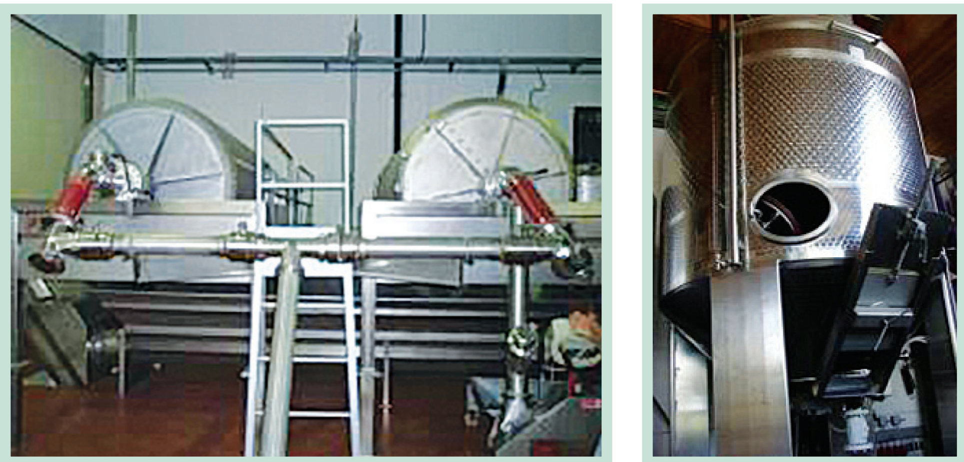
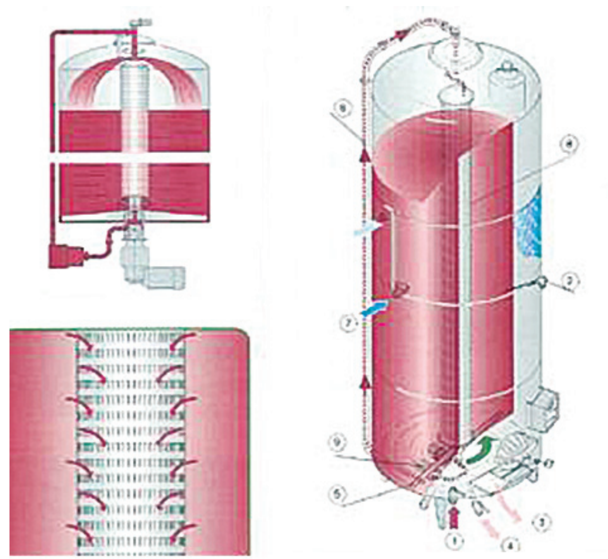


Fig. 69: Red-wine maceration tanks: Rotation tanks, stainless steal cup plunching fermenter

Wine-making practices		
Small volume	**Large volume**	**Rotating systems**
Use of tanks with ratio height / diameter close to 1 Preferred for the production of high quality wines. Allow any kind of pomace management, including manual punching down. Pomace cap it thin and large, then extraction results faster and softer.	*Use of tanks with ratio height / diameter around 2 or higher.* *Low investment costs, suitable for the production of wines with short maceration length.* Warning: a thick layer of seed accumulates at the bottom of the tank. Be careful to avoid seed crushing while pumping. ■ Keep bottom less suspended ■ Frequently monitor astringency and sulphur off-flavours	*Maceration happens in horizontal tanks rotating on their main axis to mix their content.* *Allow fast turn-over of the maceration tank, higher extraction from low colour potential grapes and easy discharge of the pomace.* Avoid fast rotation and consequent less formation, Very slow and frequent programs are preferred. ■ Frequently monitor astringency and vegetable character
Inputs		
Necessary: none	Necessary: none	Necessary: none



1 pumped grape inlet
2 temperature probe
3 pomace discharge hatch
4 draining valve
5 automatic pomace discharger
6 must remounting piping
7 cooling / heating fluid
8 central column filter
9 must remounting piping

Fig. 70: High quality pumping-over fermenter for reds, white and rosé (with permission of Fa. Defranceschi, Bolzano)

2.2.4.5. Pomace Pressing

Principles

At the end of maceration the pomace is pressed to recover a significant volume of wine.
This pressed wine is more concentrated than the wine collected while draining off and often it's total or partial integration into the main mass positively contributes to the overall quality of the final wine. Although the pressed wine can contain undesired compounds and cause defaults like excessive astringency, vegetal character etc. they also contain a large amount of lees that are a potential source of sulphur off-flavours.

In organic wine-making and in low input technology it is important to manage this phase in a way which is coherent with the rest of the process in order to avoid as far as possible the need for later additions or treatments.
The use of fast or high pressures should be avoided. A golden rule is to keep pressed wine separated. When different fractions are to be selected so that wine treatments are limited (i.e. addition of pectolytic enzymes, protein fining etc.) a limited portion of the whole wine volume is kept separated. The separation of presses is especially interesting in "assemblages", if the aim is a full-bodied, stout wine.

Wine-making practices	
Mechanical pressing	**Pneumatic pressing**
Pressing is done by applying mechanical pressure on to pomace (vertical, dish or continuous presses) Avoid complete filling of the press tank ▪ Reduce friction between equipment and pomace ▪ Preferably use more cycles and steps at lower pressure	*The pressure is applied by a membrane progressively filled with air or water. Absence of friction between pomace and equipment.* Avoid complete filling of the press tank ▪ Preferably use more cycles and steps at lower pressure
Inputs	
Necessary: none	Necessary: none

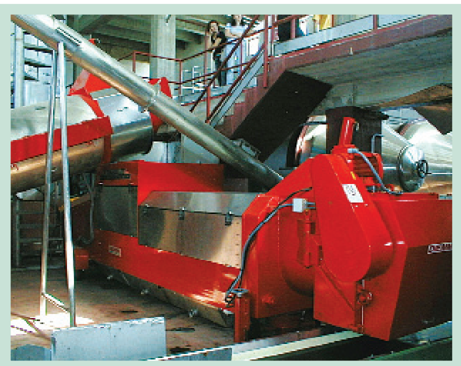


Fig. 71: Red wine Pomace pressing system.

2.2.5. Post-Fermentation

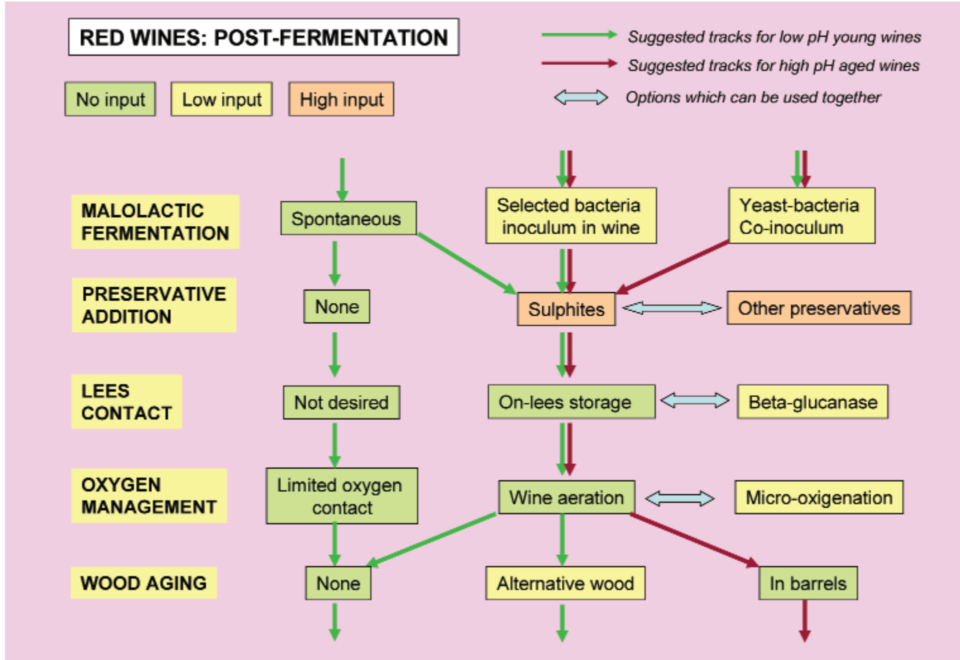


Fig. 72: Red wines post- fermentation options

General principles

The post-fermentation phase in red wine-making has a role which is of much higher significance than for white wines. In most white wines there is only a relatively short period during which the wine must be fined thus avoiding the occurrence of any oxidation and spoilage. In most red wines however the must undergoes a malolactic fermentation and does need a presence of dissolved oxygen to start polyphenol reactions and colour stabilisation. Although microbial spoilage and excess of oxidation must be avoided, it can be difficult to find the right balance between the opposing needs of this phase.

Over and above the use of additives or treatments, the wine-maker is successful by following the recommended sequence of post-fermentation steps, by avoiding delays and by carefully monitoring the development of the significant indicators. A quick and complete malolactic fermentation will quickly protect the wine from microbial spoilage by decreasing the temperature and adding SO_2. Good management of the oxygen dissolution in the early stages prevents the appearance of sulphur off-flavours and the reduction of other negative notes like vegetal and the induction of polyphenol reactions. In addition good management at this stage will avoid the need to expose the wine to oxygen for too long with all related microbial and chemical dangers. A periodical monitoring of the volatile acidity content and of the specific yeast and bacteria population allows the wine-maker to stop any adverse microbial contamination at the beginning.

Good timing, good hygiene and frequent control are then, for the producers of organic wines, the best weapons available in this phase of wine-making.

2.2.5.1. Malolactic Fermentation

Principles

Malolactic fermentation reduces titratable acidity of wine by transforming malic acid into lactic acid and CO_2 and modifies the sensory profile of wine by adding typical notes. For these reasons it is very often desired in red wines.

In organic wine-making, due to the reduced use of SO_2 during grape processing and maceration, spontaneous malolactic fermentation occurs readily even before the complete depletion of sugars. Nevertheless, uncontrolled growth of lactic acid bacteria brings the risk of production of biological amines or off-flavours which might reduce the commercial value of the wine.

In some cases, lactic bacteria growth might be slow and malolactic fermentation might not occur for some time after alcoholic fermentation. This situation exposes the wine to defects in quality as the conditions at this stage and the wine-making practices which must be applied to promote a spontaneous malolactic fermentation (no SO_2, warm temperatures) are favourable for the development of undesired micro-organisms like acetic bacteria and Brettanomyces.

To reduce risks, the use of selected cultures of Oenococcus oenii has been proposed. They can be added to wine after the end of alcoholic fermentation – with or without acclimatization, depending on the commercial product – at a concentration high enough to immediately start malolactic degradation. More recently in Europe, there is an increasing tendency to anticipate the bacteria seeding in the early stages of alcoholic fermentation, to ensure that the growth of other undesired bacteria strains or species is avoided.

Wine-making options			Related documents
No-Input Oenology Spontaneous	**Low-Input Oenology Selected bacteria in-oculum in wine**	**Low-Input Oenology Yeast – bacteria co-inoculation**	
The growth of indigenous bacteria is promoted.	*Selected bacteria are seeded immediately after the complete depletion of sugars to quickly carry out MLF*	*Malolactic bacteria are inoculated during alcoholic fermentation*	Research results: Yeast and bacteria co-inoculation
No or minimum use of SO_2 at grape processing ■ Frequent malic/lactic acid analysis since the last phases of alcoholic fermentation ■ As soon as malic acid has disappeared, protect the wine from further microbial activity	Avoid wine temperatures below 18°C ■ Properly prepare the freeze-dried culture and seed the wine ■ Frequent malic/lactic acid analysis ■ As soon as malic acid has disappeared, protect the wine from further microbial activity	Properly prepare the freeze-dried culture ■ Once alcoholic fermentation activity is evident (ab. 1/3 of sugar depleted) add the bacteria culture ■ Check malic and lactic acids together with sugars during fermentation ■ As soon as the two fermentation processes are completed, protect the wine from further microbial activity	
Inputs			
Necessary: none	Necessary: selected bacteria	Necessary: selected bacteria	
Regulatory framework: Use of selected bacteria is allowed by most private standard			**Fact sheet:** #: malolactic bacteria
Additional comments:			

2.2.5.2. Addition of Preservative

Principles

Once malolactic fermentation has been completed, the wine must be safely aged and stored for months in the wineries.

At this stage the wine is very weak and unprotected: no active antimicrobials are present, nutrients for microbial developments are limited but are sufficient for the growth of spoilage bacteria and yeasts. Lowering the temperatures of wines after fully completed alcoholic and malolactic fermentations can greatly reduce the growth of undesired micro-organisms. Filtration can also be a means to reduce microbial population, but for many red wines an early elimination of fine lees is not wanted. The addition of SO_2 is necessary and this is one of the best moments to make full use of the properties of this preservative. Alternatively Lysozyme can be used to avoid lactic bacteria growth although it must be remembered that this preservative is not ac-tive against acetic bacteria and yeasts.

Wine-making options			Related documents
No-Input Oenology	**High-Input Oenology Sulphites**	**High-Input Oenology Other preservatives**	
Microbial population is estimated low enough. The risk of microbial spoilage is judged low.	*Reduces development of bacteria and yeasts. Dosages ranging from 10 to 50 ppm depending on the conditions and length of storage*	*Lysozyme limit the growth of lactic bacteria in wines of high pH*	Technical note: Microbial contamination SO_2-Management Research result: Alternative additives to SO_2
Frequently monitor for volatile acidity content, appearance of off-flavours and for Brettanomyces population	Add sulphite solution and mix the liquid mass, or inject on line during wine movements ▪ Preferred sulphite form depends on dimension and equipment of the winery	Add to wine after MLF completion	
Inputs			
Necessary: none	Necessary: P- metabisulphite, Gaseous SO_2	Necessary: Lysozyme	
Regulatory framework:			**Fact sheets:** #: SO_2 #: P- metabisulphite #: Lysozyme

Additional comments: SO_2 fractioning (several small additions in different steps of the process) is more effective at the same final doses.
The use of Lysozyme has to be labeled as an allergenic compound and the use increases the need for higher amount of Bentonite for protein-stabilisation.

2.2.5.3. Lees Contact

Principles

Yeast lees can release yeast wall components (i.e. mannoproteins) which are believed to positively contribute to the taste of wine by helping the softening of tannins.

Yeast lees, even after yeast death, are also very active oxygen scavengers, and can avoid excessive accumulation of dissolved oxygen into the wine.

Nevertheless, yeast lees can also represent a danger, as the released amino acids can become a nutrient for spoilage micro-organisms.

The lees contact is thus an important tool for organic wine-making, and it can be applied by seeking the right balance between the opposite effects that are related with the practice.

Wine-making practices			Related documents
No-Input Oenology Not desired	**No-Input Oenology On less storage**	**Low-Input Oenology Beta-glucanase treatment**	
When yeast lees are negatively contributing to wine profile (undesired evolution notes or off-flavours), they are eliminated from the system	*Lees are kept in contact with wine to release desired sensory active compounds*	*A part of the wine with (all) yeast less is treated separately to accelerate yeast autolysis*	
Make sure sugars are completely depleted ▪ Rack the wine 2-3 times within a couple of weeks, or filter the wine	Rack the wine before end of fermentation to eliminate gross solids ▪ Periodically move the wine to re-suspend fine lees ▪ Check volatile acidity and malic acid during storage ▪ Frequent wine tasting	Concentrate fine lees into a portion of the wine. Tartaric acidification is suggested. ▪ Add beta-glucanase enzyme ▪ Check volatile acidity and taste frequently during storage ▪ Once the desired level of autolysis is reached (some weeks), filter the wine and use for blending	
Inputs			
Necessary: none	Necessary: none	Necessary: beta-glucanase enzyme	
Regulatory framework: Beta-glucanase is allowed by UE Reg. 834/2007 and in most EU private standards			**Fact sheet:** #: beta-glucanase

Additional comments:

2.2.5.4. Oxygen Management

Principles

Oxygen is needed in red wine-making mainly for two reasons: to avoid appearance of sulphur compound and consequent off-flavours (more frequent in some varieties than others) and to promote binding reactions between anthocyanins and tannins and between tannins and tannins which lead toward a more stable red colour and a softer mouth feeling. Excess of oxygen causes oxidation of the wine and development of oxidative bacteria and yeasts.

Raking by splashing wine in the open air is still a very common practice and if well managed can help in adding amounts of oxygen close to saturation. Chemical and enzymatic reactions, as well as yeast lees very quickly consume large amounts of oxygen.

The micro-oxygenation technique has become very popular in the last decade. The principle is to constantly add small amounts of oxygen, enough to advance desired reactions, but not more than what the wine can consume which avoids accumulation of dissolved oxygen in the wine. This practice can be applied to wine whilst in tanks, in some way reflecting the slow aeration typical of barrel aging.

As these practices aim to promote certain processes whilst avoiding dangerous excesses, temperature control, perfect hygiene of containers and equipment and frequent monitoring are to be considered the golden rules.

Wine-making practices			Related documents
No-Input Oenology Limited oxygen contact	**Low-Input Oenology Wine aeration**	**Low-Input Oenology Microxygenation**	
In some red wines a reduced contact with oxygen is preferred to preserve maximum of freshness and fruitiness	*Wine contact with oxygen is promoted during racking and wine movement*	*Small amounts of air or oxygen are constantly sprinkled into wine through special equipment*	Technical hints: Oxygen and wine Practical hint: Hyperoxygenation
Keep wine containers completely full ■ Avoid splashing of wine during rakings ■ Periodically check sulphur compounds appearance	Rack by splashing wine and fill tanks from the top: oxygen solubilisation varies from 3 to 7 mg/l ■ Chemical reactions in wine consume oxygen. ■ Periodically check colour tonality and volatile acidity, avoid excess oxidation	Temperature control ■ Modulate the input of oxygen on the basis of sensory analysis and acetaldehyde accumulation ■ Periodically check colour tonality and volatile acidity, avoid excess of oxidation	
Inputs			
Necessary: SO_2	Necessary: none Useful: SO_2	Necessary: none Useful: oxygen	**Fact sheets** #: oxygen

2.2.5.5. Wood Aging

Principles

Wood containers have been used for wine during centuries, and its presence has become part of the wine identity in many regions.

Nowadays the use of wood barrels is practiced for different reasons:

i) micro-aeration of wine - as oxygen enters the wine through stave junctions and at every headspace filling by promoting chemical stabilisation reactions
ii) tannin increase - as wood tannins are dissolved in wine and contribute to increased structure and body (toasted wood is used to avoid excess of tannin release),
iii) aroma contribution - due to the presence in the wood of compounds with vanilla and toasted notes they can be transmitted into the wine increasing its complexity.

As the three functions cannot be separated, the practice has to be limited to red and white wines of suitable original composition.

Alternative use of wood has become popular in the last decades: chips, cubes or staves are added to wine for a limited time in order to replace the functions ii) and iii) and to add aroma and tannins typical of wood aging without oxidation and costs linked to barrel aging. If used together with micro-oxygenation, the use of wood chips, cubes or staves can partially replace even the function of the traditional use of wood.

Tannin addition can usefully improve the body and structure of wine and in some cases can also provide some aromatic contribution.

Fig. 73: Big Wood barrels for fermentation and storage, barrique´s for storage.

Fig. 74: Traditional measures to observe the fulfilling of the barrels.

Fig. 75: Traditional measures to preserve the barrels by burning sulphur-strips.

Wine-making practices			Related documents
None *No barrel or alternative woods are used, to preserve freshness and fruiti-ness in wine.* *Tannins can be used to reinforce structure when needed.* Keep wine in stainless steel containers ▪ Periodically check sulphur compounds appearance	**Alternative wood** *Wood chips, cubes of staves are put in contact with wine for some weeks* Preliminary tests with high dosages are advisable to simulate effects in advance ▪ Add alternative woods at dosage chosen, periodically taste wine to monitor compound releases from wood ▪ Eliminate the wood from wine by racking and filtration as soon as the desired effect has been reached It is not advisable to use wood chips in too small pieces or in powdered form to avoid the occurrence of excessively strong wood-like aroma	**In barrels** *Wine is kept into wood containers of various size for periods ranging from 3 to 18 months* Fill barrels with wine and keep them in a cellar with suitable temperature and relative humidity ▪ Periodically fill up the barrels and monitor volatile acidity, colour tonality, microbial contaminants and sensory profile. Rack and aerate if sulphur defects appear ▪ Take out wine from barrels as soon as the desired effect is reached	
Inputs			
Necessary: SO_2 Useful: tannins	Necessary: wood chips, cubes or staves Useful: SO_2	Necessary: none Useful: SO_2	**Fact sheets** #: SO_2 #: P-metabisulphite #: wood chips

2.2.6. Fining and Stabilisation

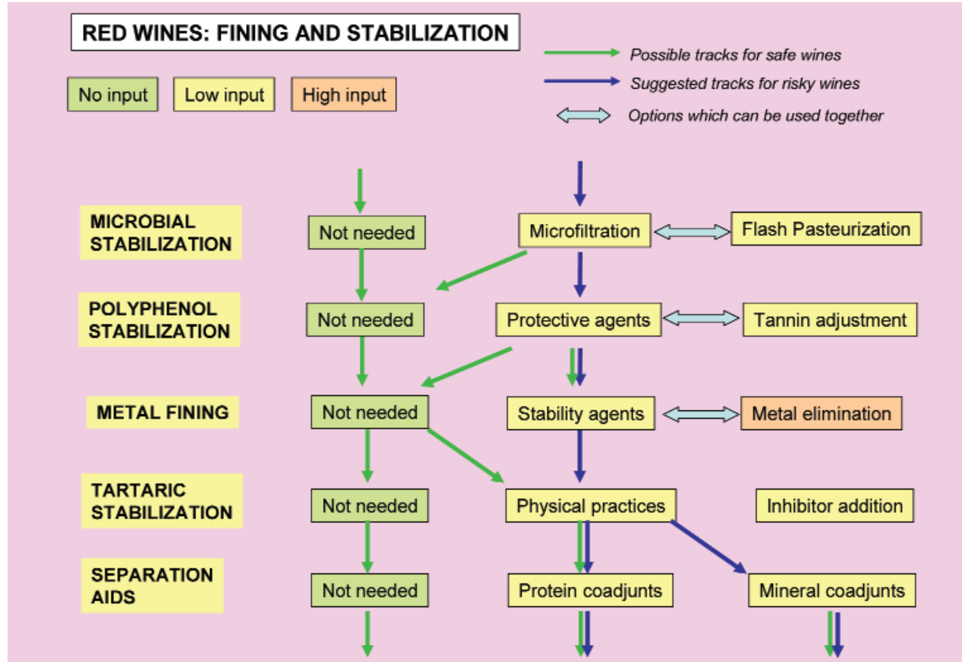


Fig. 76: Red wines – fining and stabilisation options

General principles

The end of the storage period and just before bottling is the last chance to treat the wine in order to guarantee commercial standards (wine stability and limpidity).
The more strict and precise the management of the previous phases of wine-making has been the lower is the need of treatment at the end of the process. Although, some fine adjustment can be beneficial.
Conventional oenology has developed several tools to reach stability and to make the wine-maker's work easier.
Organic wine-making can choose from these tools the options which are more adapted to the principles of organic production.

2.2.6.1. Microbial Stabilisation

Principles

During storage an ageing red wine can be the object of development of undesired micro-organisms, more commonly Brettanomyces and lactic acid bacteria.
Brettanomyces is a yeast which develops in conditions of high pH, low SO_2 and in the presence of

some oxygen it is a typical contaminant of wooden barrels. It produces ethyl-phenols and is the origin of unpleasant animal/chemical like off-flavours.
Lactic acid bacteria, mainly Pediococcus and Lactobacillus spp., can grow in wine after malolactic fermentation using the small quantities of nutrients that are still available. They can produce metabolites which are the origin of mousy and animal off-flavour (meat, leather taints) in the wine.
In wines stored with low SO_2 and in presence of oxygen it is not uncommon to observe a development of acetic bacteria and oxidative yeasts.
Temperature control and oxygen management, together with frequent microbiological analysis of the wine, are important tools to avoid such problems.
When unexpected microbial development has occurred, it is advisable to rapidly eliminate the micro-organisms from the wine through physical treatments, to add some preservatives and to avoid the usage of contaminated containers.

Wine-making practices		
Not needed *Population of contaminant micro-organisms in wine is below the threshold of risk*	**Micro filtration** *Low porosity filtration is used to quickly reduce spoilage micro-organism presence. Cross-flow micro filtration represents the preferable option* Wine is filtered at porosity below 0,5 μm	**Flash Pasteurisation** *Heating the wine at high temperatures for few seconds kills the majority of the micro-organisms with minimal effects on wine sensory characters* Treat the wine at 75°C for 10 to 20 seconds
Inputs		
Necessary: none	Necessary: none	Necessary: none

2.2.6.2. Stabilisation of Phenols

Principles
Polyphenols in red wines have been extracted during maceration, softened and stabilized during aging. A fine-tuning might be necessary at the end of the process to reach the best balance.
At this stage there are two ways to solve the stability problems of phenols either to eliminate the most unstable ones or to add protective agents which are avoiding or slowing down oxidative reactions and precipitation.
To selectively eliminate part of the phenols, different adjuvants can be used viz. casein, ov-albumin, gelatine, plant protein, isinglass etc.
Protective agents can be used such as oenological tannins of different botanical origin and extracted by different ways. These act as antioxidants producing the radicals before they react with wine phenols.
Yeast preparations also seem to increase the polysaccharide content of wine with positive effects on taste and stability.
Finally, polysaccharides such as arabic gum can restrict the precipitation of colloids in wine.

Wine-making options			Related documents
No-Input Oenology *Wine is judged to have acceptable phenol stability and taste balance*	**Low-Input Oenology Protective agents** *Addition of yeast derivates and/or arabic gum to reduce colloidal precipitation. Tannins of different origins are used to fine-tune structure and taste characters, as well to eliminate sulphur off-flavours and increase protection from oxidation* Products are prepared according to producer instructions ▪ Addition to wine	**High-Input Oenology Tannin adjustment** *To reduce the presence of unstable or astringent tannins, the wine is treated with different adjuvants able to bind polyphenols* Properly prepare one or a combination of more of the following adjuvant: casein, ov-albumin, gelatine, plant protein, isinglass ▪ Addition to wine	
Inputs			
Necessary: none	Necessary: tannins and/or yeast hulls, arabic gum	Necessary: one or more among casein, ov-albumin, gelatine, plant protein, isinglass	**Fact sheets** #: casein #: ov-albumin #: gelatine #: plant proteins #: isinglass #: arabic gum #: tannins #: yeast hulls

2.2.6.3. Tartaric Stabilisation

Principles
Many wines have bitartrate content above the saturation point, and are then susceptible to tartrate precipitation if stored at low temperatures. In red wines tartrate precipitation involves pigments and produces an evident and thick deposit in the bottle which is sometimes not appreciated by consumers.
Nevertheless, some producers decide to not stabilize their wine against tartaric precipitation and to educate their clients to the presence of deposits.
When a stable wine is sought there are two approaches. Either eliminate from the wine some of the tartrate and potassium to bring the concentration below the saturation point, or add substances which can inhibit the formation or the growth of the tartrate crystals.
Refrigeration of the wine (in batch or continuous) is the most common practice. Additives are not needed but the process is costly. Electro-dyalisis technology eliminates part of the excess ions and it is probably the most environment friendly option. However the equipment is expensive and not affordable for every winery.

Wine-making practices			Related documents
No-Input Oenology *The formation of crystals in the bottle is acceptable. No stabilisation treatment* Check tartaric stability ■ Check consumer attitude and implement educational	**Low-Input Oenology Physical treatments** *Excess ions are eliminated from the wine* Determine wine instability ■ Apply the most suitable technology for the specific winery (refrigeration, electrodyalisis)	**High-Input Oenology Inhibitor addition** *Stability is reach through the addition of compounds inhibiting crystallisation* Determine wine instability ■ Add the most appropriate additive (metatartaric acid, arabic gum, mannoproteins)	
Inputs			
Necessary: none	Necessary: none	Necessary: metatartaric acid, arabic gum, mannoproteins,	**Fact sheets** #: metatartaric acid, #: arabic gum #: mannoproteins

Metatartaric acid, arabic gum or, more recently allowed, yeast mannoproteins can inhibit the formation or the growth of crystals, but their effect might be not strong enough to stabilize young red wines.

2.2.6.4. Separation Aids

Principles

The residual cloudiness of the wine or the hazes formed during fining treatments must be eliminated from wine by simple racking or by physical means.

To speed up this step and to assure a more pushed limpidity of the final wine, some adjuvants can be used.

Wine-making options			Related documents
No-Input Oenology *Wine viscosity and limpidity targets do not allow the use of adjuvant*	**Low-Input Oenology Adjuvant of natural origin** *Adjuvants are added to help improved flocculation* Prepare the product according to producer instruction ■ Add to wine and homogenize the mass.	**Low-Input Oenology Adjuvant of mineral origin** *Adjuvants are added to help improved flocculation* Prepare the product according to producer instruction ■ Add to wine and homogenize the mass	
Inputs			
Necessary: none	Necessary: one or more among casein, ov-albumin, gelatine, plant protein, isinglass, pectolytic enzymes, beta-glucanase	Necessary: one or more among Bentonite, silica gel, kaolin	**Fact sheets:** #: casein #: ov-albumin #: gelatine #: plant proteins #: pectolytic enzymes #: bet-glucanase #: bentonite #: kaolin #: silica gel
Regulatory framework:			
Additional comments: Time of contact and order of treatment can be of high significance. The use of caseine, P-caseine, ov-albumin, egg-white or plant proteins has to be labeled as an allergenic compound.			

Among adjuvants able to promote a better separation of solids from wine are bentonite, silica gel, kaolin with mineral origin. They are used less than in white wines and essentially used to speed up precipitation and obtain a more compact lees. Bentonite must be used carefully as it eliminates red colour.

Casein, ov-albumin, egg whites, gelatine, plant protein, and isinglass are the main adjuvants used in red wine fining. This step in many cases coincides with the phenolic stabilisation one.

2.2.7.Filtration and Bottling

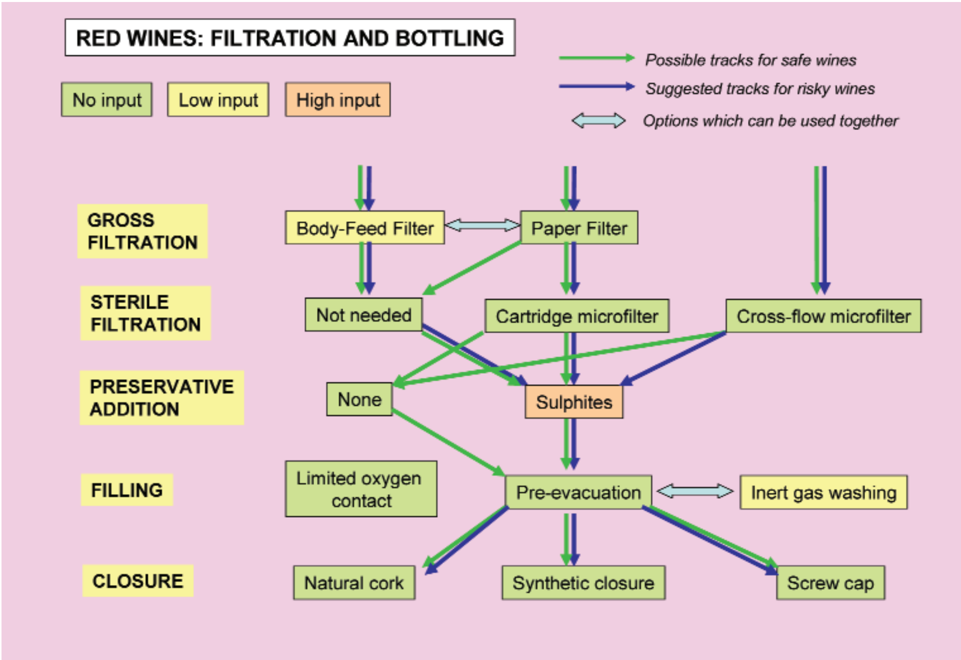


Fig. 77: Red wines – filtration and bottling options.

General principles

Filtration is not always carried out in red wine making. Wines which have been aged for a long time in barrels or tanks usually have fewer problems of cloudiness and stability once in the bottle. Moreover, oxygen entrance at bottling is a minor concern in red wines.

Most attention in these final phases is focused on microbial contamination. This can be a problem in bottled wine even after several months and sometimes can occur randomly in some bottles of the same lot. The development of yeasts and bacteria in bottle, a risk which is higher in sweet wines with low protection from sulfur dioxide, can lead to commercial problems.

In organic wine-making the final wines are less protected by additives compared to conventional wines. It is advisable then to control these last steps as far as possible to give the wine a shelf-life adapted to its distribution and consumption profile.

2.2.7.1. Gross Filtration

Principles

Sterile and brightening filtrations need a previous cleaning of the wine to increase the filtration capacity of the system.

The goal is usually reached with a body-feed filtration. The coating is done through the use of filter aids of variable porosity and characteristics, whose composition is often a balanced mix of perlite and cellulose or cotton fibres.

Filtration on paper sheets is also very popular.

Although none of these filter aids or sheets release substances to wine and therefore don't represent a concern for organic wine production regarding consumer health, their waste can have an impact on the environment but is mostly used for compost making.

Wine-making practices		
Body-feed filter *Wine is passed through a coat of perlite and cellulose which retains solids.* Choose the filter aid with suitable porosity ▪ Filter the wine by controlling oxygen contact	**Paper filter** *Wine is passed through a sheet of cellulose which retains solids* Choose the filter sheets with suitable porosity ▪ Filter the wine by controlling oxygen contact	
Inputs		
Necessary: perlite, cellulose	Necessary: paper sheets	

2.2.7.2. Sterile Filtration

Principles

Wines with low preservatives – especially sweet ones – must be bottled without a significant microbial population. Even a very low level of contaminants can grow in the bottle during distribution and storage, often under uncontrolled conditions, and develop haziness, off-flavours or simply cloudiness none of which is acceptable to consumers.

It is a common belief than too light a filtration – as for sterile and brightening ones – can eliminate from wine some positive components like macromolecules which are contributing to wine body and structure although some scientific results question this statement.

Organic wines might be consumed by a segment of people who are less sensitive to cloudiness or presence of haze in wine. Nevertheless, consumer requirements necessitate the need to avoid off-flavours and organic wines are more susceptible at the bottling stage. Thus sterile filtration should be seriously considered as an option, not only for sweet but also dry red organic wines.

Wine-making practices		
Not needed *Microbial presence and brightness of wine are considered acceptable.*	**Cartridge filter** *Wine is pushed through a cartridge containing a membrane with low porosity* Evaluate wine filterability before operation ▪ Check wine sterility after filtration	**Cross-flow filter** *Wine is put under pressure into a tubular membrane of defined porosity, through which the filtered wine permeates* Previous gross filtration can be avoided ▪ Check wine sterility after filtration
Inputs		
Necessary: none	Necessary: none (cartridges)	Necessary: none (membranes)

The use of cartridges with membranes of different porosity has been the most popular prac-tice for many years, and is still very common in small facilities. Recently cross-flow filtration has seen a wide acceptance thanks to its advantages viz. the possibility to avoid a previous gross filtration, a better filtration capacity and the absence of waste material. The major limit of this technology is the equipment cost.

Fig. 78: Membrane-filter for sterile filtration

2.2.7.3. Addition of Preservative

Principles

A further addition of sulphites at bottling should be considered. In red wines the main danger is that a presence of Brettanomyces and/or lactic acid bacteria there may be a development during bottle storage of off-flavours and formation of CO_2. Sulphur dioxide can inhibit the growth of these micro-organisms and can also avoid early oxidation of the wine during bottle aging. Many red wines however have high pH values which reduce SO_2 efficacy. A prevention strategy must then be preferred.
For long aging red wines the traditional cork closure remains by far the most popular choice. For young red wines the use of synthetic closures has seen a great expansion in the last decades. Screw caps and very low oxygen transfer rate closures are usually not employed on red wines, as the complete absence of oxygen is considered one of the causes of the appearance of sulfur off-flavours during bottle aging.

Wine-making options			Related documents
No-Input Oenology *Wines are protected from oxidation and microbial spoilage by other means. Not recommended on wines with some presence of spoilage microorganisms*		**High-Input Oenology Sulphites** *Slow down oxidation of wine aroma and phenols; reduce development of bacteria and yeasts.* *Dosages ranging from 10 to 30 ppm depending on wine pH, the conditions of bottling, the shelf life targeted* Preferably inject on line during wine movements	Technical note: Oxygen and wine Technical note: Microbial contamination SO_2-Management
Inputs			
Necessary: none		Necessary: P-metabisulphite, Gaseous SO_2	
Regulatory framework:			**Fact Sheets** # 1: SO_2 # 2: P- metabisulphite

2.2.7.4. Filling

Principles

The wine can be saturated with oxygen after an uncontrolled filling process. The oxygen present in the head-space of the bottle (especially when screw caps are used) can be enough to completely consume the SO_2 contained in the wine. The filling taps are among the most common source of microbial contaminants due to the difficulties encountered to clean them properly.

Thus in organic wine production the filling step must be carried out with well maintained and modern machines, and the procedures for cleaning and sterilization must be strictly followed.

There is a range of equipment that will avoid oxygen solubilisation of the wine during this step. Options include the possibility to wash out the air from the empty bottle by means of inert gas flushing or systems which aspirate the air from the empty bottle and/or the head-space to create a partial vacuum before closure insertion or a combination of both.

Wine-making practices			Related documents
Limited oxygen contact *Entrance of air in the wine is avoided during wine movements through the equipment. Time of the filling step and wine temperature are controlled to minimize oxygen solubilisation.*	**Pre-evacuation** *Air contained in the bottle is aspired before filling. Head space air is aspired before closure insertion* Follow the procedures suggested by filling machine producers ■ Strictly respect maintenance programs of the equipment	**Inert gas washing** *The empty bottle is flushed with inert gas in order to exit the air before filling. The head-space is flushed with inert gas before closure application* Follow the procedures suggested by filling machine producers ■ Strictly respect maintenance programs of the equipment	Technical note: Oxygen and wine
Inputs			
Necessary: none	Necessary: N_2, CO_2	Necessary: N_2, CO_2	
Regulatory framework:			**Fact Sheets** #: CO_2 #: N_2

Fig. 78: Bottling machine for a small family winery.

2.2.7.5. Closure

Principles

Though cork has been the only option for hundreds of years, other options have recently seen a wide usage and an increasing acceptance by consumers.

Synthetic closures are constituted by plastic polymers, and can have an appearance very similar to natural cork.

Screw caps have seen a new life after having been used for decades on very short shelf-life products. New developments in the material used and in the bottling procedures have now allowed their use for premium and super-premium wines.

Different factors are driving the decision of the producer toward one or the other closures viz. cost, consumer acceptance, image of wine, commercial shelf-life, tradition and appellation rules. The most relevant factor for organic wine-maker is probably the OTR (oxygen transfer rate) factor which measures the permeability of a closure to oxygen and consequently the time a specific wine has before appearance of oxidised traits.

According to some experts, screw caps with metal liners have an OTR close to zero. They are so impermeable to oxygen that in some cases the wine evolves reduced taints with time. Synthetic closures usually show a high consistency in OTR values. Depending on the plastic polymer and on the production system used, they can be very permeable to oxygen with an extremely low OTR. Closures made of grinded or powdered cork are the same. Natural cork shows a lower consistency in OTR value within the same lot. In general they can be more impermeable than synthetic closures.

It is clear then that the choice of the closure must be coherent with the rest of the decision taken during the process of production of an organic wine. If a strategy of lowest possible sulphites has been followed, the closure used must guarantee a degree of permeability compatible with the required commercial shelf-life.

Wine-making practices			Related documents
Natural cork	**Synthetic closure**	**Screw-cap**	
Natural cork is chosen for a combination of technical, economical and commercial reasons.	*Synthetic closure can be cheaper than corks and offer an acceptable performance for young wines*	*Some screw caps assure an almost perfect impermeability to oxygen. Marketing concern in some countries*	Technical note: Oxygen and wine
	Adapt closing machine to the closure chosen	Specific closing machine and bottles are needed	
Check closing machine operation	Pre-evacuation necessary for some types	Head-space is significantly bigger than with other closures	
Expect some inconsistency within bottles of the same lot after aging	Expect some inconsistency within bottles of the same lot after aging	Specific procedures must be followed	
Inputs			
Necessary: none Useful: N_2, CO_2	Necessary: none Useful: N_2, CO_2	Necessary: none Useful: N_2, CO_2	
Regulatory framework:			**Fact Sheets** #: CO_2 #: N_2

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3. TECHNICAL NOTES

3.1. Hygienic standards (Cottereau, P.)

General notions on hygiene

In order to offer consumers wholesome and acceptable food and drink certain hygiene rules need to be put in operation. These rules will determine the cleaning-disinfecting processes that should be carried out:

- Start off with a good quality raw material: the treatments that need to be applied to the raw material itself will largely depend on the considered foodstuff.
- Clean and disinfect the equipment and/or the surfaces:
  - For surfaces that come into or could come into direct contact with foodstuffs, the cleaning-disinfecting methods must meet precise criteria.
  - For surfaces that do not come into direct contact with foodstuffs (floors, walls, ceilings, etc.), they must be permanently kept clean in order to avoid any cross-contamination between poorly maintained zones and surfaces in direct contact with foodstuffs or even the foodstuff itself.
- Assure good hygienic conditions of the surroundings. For many industries, treating the surroundings and ambient air has become a necessary complement to conventional hygiene measures that are applied to surfaces. Micro-organisms are conveyed by dust in the ambient air and they might settle on surfaces that come into contact with foodstuffs after having been cleaned and disinfected.
- Do not neglect staff hygiene?
- Comply with standards for foodstuff transformation and preservation operations.

Hygiene is therefore a series of measures and actions that apply at all times. The better designed the premises in which one works, following a suitable process using the correct equipment along with trained and well-informed personnel, the easier it will be to maintain good hygiene.

Hygiene in oenology

Hygiene in oenology is different to other food processing industries where an insufficient level of hygiene or incorrectly applied hygiene measures can lead to outbreaks of food-borne diseases.

Wine, on account of its composition (low pH and high ethanol content), is a hostile medium to many pathogenic germs. Nevertheless, lack of hygiene in oenology can lead to the **alteration of the product** (growth of micro-organisms) or instead a development of unwanted micro-flora. These are mainly yeasts (oxidative and some fermentative yeasts), moulds, acetic acid bacteria and lactic acid bacteria.
In oenology, concern for hygiene means applying measures linked:

- **To existing regulations:**
  - Compliance with European Directive n° 93-43 CEE of the Council dated 14/06/1993 (known as the Hygiene Directive).
  - Compliance with analytical wine standards. Such standards may be issued by the OIV (International Organisation of Vine and Wine) in the form of recommendations, before being definitively adopted by the European Commission.
  - Compliance with the Directive knows as the Machinery Directive, n° 98/37/CEE, which concerns requirements in terms of hygiene for food processing machinery.
  - Respect of the environment.
  - Compliance with the procedures to follow concerning water intended for human con-sumption.
  - Compliance with labour code with respect to staff safety during the preparation and use of chemicals.

- **To the quality of the product:**
  - To limit chemical contamination (heavy metals, pesticides, etc.).
  - To limit oxidation of the must.
  - To favour micro-organisms which are useful during fermentations?
  - To contribute to attaining and maintaining low microbial populations during stabilisation and bottling.
  - To avoid or limit thermal treatments or additions of chemical stabilisers.

- **To commercial commitments:**
  - Elimination of possible contaminations, linked to aestheticism (particularly with respect to direct sales).
  - Compliance with standards or, more specifically, explicit requirements linked to commercial contracts.

Application of hygiene in wine cellars

The application of hygiene measures required in oenology depends on the fluctuating activity of the wine cellar or the wine storehouse over the course of the year (activity peak during grape harvesting), the diversity of the products that may be involved (red wines, white wines, sparkling wines, stabilised and non-stabilised wines, filtered or unfiltered wines, etc.) and the materials involved (wood, stainless steel, concrete, etc.).
For the wine industry, as in all food processing industries, a hygiene plan must be drawn up in order to optimise the planning of cleaning-disinfecting operations in terms of procedures, frequencies and controls. However, in oenology, it is conceivable and even reasonable to define hygiene levels (table n° 4) given that, in oenology, the closer the wine is to the bottling step, the stricter the hygiene conditions should be.

Table 4: Hygiene levels in oenology

Hygiene level	Why?	How?	Where?
Minimum	To eliminate heavy contamination: earth, leaves, marc	Pre-washing	Floors Harvesting equipment
Elementary	To eliminate contamination	Pre-washing Cleaning (with brush or detergent) Rinsing	Crushers Wine presses Wine-making and tank storage areas
Thorough	To eliminate contamination and to to limit the proliferation of micro-organisms	Pre-washing Cleaning Rinsing Disinfecting Rinsing	Collecting heads of grape harvesting machinery Surfaces in contact with the must and the wine Pipes, pumps, valves
Very thorough	To lower the population of germs below a predetermined threshold	Pre-washing Cleaning Rinsing Disinfecting Rinsing Control	Surfaces in contact with wine in the case of specific seeding Bottling chain

Source: Guide practique de l'hygiene en ?nologie (Practical guide to hygiene in oenology) – ITV, 1985

It is possible, in this way, to adapt a hygiene plan to each critical stage of the wine making process.

The means available to the oenological industry are chemical, physical and/or mechanical.

Chemical measures are approved cleaning-disinfecting products that are used to scale and decolourise materials in contact with the must or the wine.

Physical measures are heat, or more specifically steam (in the form of damp heat, steam or hot water), but also processes such as microwave treatments, ultraviolet treatments, etc.

Mechanical measures are mainly means that reinforce the action and/or facilitate the application of cleaning-disinfecting products (brushes, foam guns, scrapers, foam balls for closed circuits, etc.). Mechanical measures also include high pressure water, which also enables thorough pre-washing and efficient rinsing.

Hygiene aims to the elimination of contaminations. For this, both cleaning and disinfecting phases are indispensable and complementary:

- Cleaning removes visible or microscopic contamination adhering to surfaces, rendering them clean.
- The aim of disinfecting is to reduce in a significant but temporary manner the population of micro-organisms harmful to the quality of the wine. Since contamination can be favourable to micro-organisms, disinfecting must always be preceded by cleaning.

Whatever the type of contamination, nature and surface condition of the material, all hygiene procedures proceed in the following steps: pre-washing, cleaning, rinsing, disinfecting and, last but not least, final rinsing (sterile water).
The steps differ depending on whether two agents (a cleaning agent followed by a disinfecting agent) or a single mixed agent (cleaning and disinfectant) are used.

The choice of detergent or disinfectant must take into account the nature of the contamination, the properties of the surfaces to be cleaned, particularly the chemical, mechanical and thermal stability of the material, as well as the risks of corrosion.

Another parameter that is often neglected but which is very important is the quality of the water, particularly its hardness. It is worth recalling that water composition can vary widely from one region to another.

Hygiene and Environment

Nowadays, respecting the environment is a priority. Past incidences of pollution abuses now means that industrial or farming activity is closely monitored In the wine sector, the cleaning operations indispensable to maintaining the hygiene of wine storehouses and equipment may be the source of organic and chemical discharges. Before attempting to deal with such discharges, it is important to try to reduce at source the polluting load and to reduce the volume of discharges without having an adverse effect on hygiene, which should remain the priority concern of the wine-maker.

Cleaning with lower amounts of discharges and less polluting discharges is an imperative that can be attained by taking into account the work organisation, the choice of the cleaning products, the equipment and the design of the wine storehouses themselves.
The most relevant example is water management. Training and awareness of personnel, combined if necessary with regular readings of water meters, is an indispensable prerequisite to any water management policy. In parallel, the installation of automatic shut-off devices enables water losses to be kept to a minimum.
Thus, depending on the type of cleaning that needs to be carried out, it is possible to obtain an equivalent result by using less water and often by discharging less pollution. With regard to cleaning products, in-place cleaning (IPC) and recycling, already operational for soda scaling solutions, are in the development stage, especially for large production facilities.
Foam guns, by increasing contact time, particularly in the case of vertical surfaces, contribute to enhancing the performance of cleaning devices.
In the same way, the generalisation of hot water circuits helps to optimise cleaning operations using less water.
Operations linked to hygiene represent a prominent part of the pollution originating from wine cellars. Environmental issues are being developed within legislation and which reflect on the image of wines. These operations justify the development of cleaning technologies that are less polluting, that consume less water and that offer recycling possibilities. This imperative must also be taken into account in training and in the research orientations of the wine industry.

Extract from:

Hygiène en Œnologie – Nettoyage – Désinfection – HACCP (Hygiene in Oenology - Cleaning – Disin-fecting – HACCP)
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3.2. Temperature Control (Werner, M.; Rauhut, D.)

Effect of Temperature on Must and Wine

How can temperature control help to avoid additives?

Temperature control during the wine-making process is very important for the final quality of the wine. Even if it cannot replace all functions it can complement the effect of sulphur dioxide (SO_2) at certain points.

Temperature influences the activity of enzymes, which are present at various points during the whole production process of wine. Enzymes are already present in the grape and may affect the aroma through **oxidation** and influence the **degradation of the grape mash** during maceration. They are also responsible for the **metabolic processes in the living micro organisms**, such as bacteria,yeasts and fungi. The most relevant species that are affected during wine-making are: acetic acid bacteria, lactic acid bacteria, yeasts and the fungus Botrytis cinerea. Their activity is always influenced by the temperature. Thus, the wine-maker has the possibility to control these factors by controlling the temperature. An increase in temperature accelerates enzymatic processes. In biological systems reactions do not run at 0°C. Above 0°C the reactions start slowly and finally reach a maximum at around 37°C. Temperatures over 37°C change the structure of enzymes and finally lead to the decrease and elimination of enzymatic activity. Thus, every enzymatic process has its optimum and the wine-maker can choose between delaying and enhancing the activity of certain micro-organisms by controlling the temperature.

Grape harvest:

When harvesting and crushing grapes, temperatures should be as low as possible in order to minimize the activity of fungus (e.g. *Botrytis cinerea*, *Trichothecium roseum*), undesired bacteria (e.g. *Gluconobacter*, *Acetobacter*) and undesired yeast species (e.g. non-*Saccharomyces yeasts*), which can be present on the grapes. As soon as the grapes are injured, sugar is available for the metabolism of micro organisms. It is at this point that the addition of sulphur dioxide has the effect of inhibiting the activity of micro-organisms and inhibiting the activity of enzymes. Temperature control is an effective tool to control these reactions. Temperature should be low during the whole process: picking of grapes, transport, crushing and maceration (if applied). Only by avoiding the multiplication of undesired fungus, bacteria and yeast on the grapes can one avoid the formation of volatile acids, toxins and/or ethanol in this early stage of wine production. Crushed grapes, exposed to sunlight and warm temperatures al-ways lead to a loss in quality. Especially when grapes are injured or infected by fungus ethanol which can be developed from indigenous yeasts which are present on every grape. Acetic acid bacteria can then form acetic acid from ethanol. As the micro-organisms on grapes are always present in combination, the management of the crushed grapes will always affect many different factors. Ribéreau-Gayon et al (2006) recommend harvesting the grapes at a temperature below 20°C. Additionally they remark that the harvested grapes must be as intact as possible during the transport. This reduces not only the growth of micro-organisms but also must oxidation and stem maceration.

Must treatment:

When following a reductive way of must treatment, oxidation processes by enzymes should be avoided. Certain enzymes (peroxidase, polyphenol oxidase) are able to transfer oxygen from air to certain wine compounds, resulting in the decrease of the aromatic expression and browning effects in must. For this reason low concentrations of sulphur dioxide and also very low temperatures can inhibit this activity. In general white must is very sensitive to oxidation as the aroma of white must and wine is more fragile than for red mash or wine.

In must clarification, static sedimentation is a common low input treatment. As highly suspended solids in the must are often associated with a negative effect on the wine quality, it is recommended to clarify the must to a low turbidity level of around 200 NTU (Ribéreau-Gayon et al, 2006). Again low temperatures (<20°C) help to facilitate the sedimentation of solids in the must. The elimination of the sediment can also reduce the amount of oxidizing enzymes (oxidase activity). The reduction of the oxidase activity can be achieved by taking off the sediment or by inactivation by heat treatment. Heat leads to the denaturation of the enzyme and will also result in a lower need of sulphur dioxide at this point of wine-making (Troost, 1988).

Fermentation temperature:

As the activity of micro organisms always depends on the temperature of the surrounding environment the fermentation activity of the wine yeast *Saccharomyces cerevisiae* is influenced by the temperature of the must. Seen from a metabolic point of view a temperature range from 20-25°C is very favourable for the course of the alcoholic fermentation. But at that temperature one can run risk that the fermentation activity becomes too strong and also some aromatic compounds will be diminished. Thus in general alcoholic fermentations should be performed at a temperature range of 15-18°C in order to reach a complete fermentation without problems. If fermentations are cooled to 10°C or lower specific selected yeast strains should be used which are able to perform the alcoholic fermentation at this temperature. Spontaneous fermentations with indigenous yeasts usually take more time, especially at lower temperatures. Low temperatures inhibit the growth of the indigenous yeasts hence delaying the start of fermentation.

Stabilisation:

Even if the energy consumption is rather high, cold stabilisation is a common way of stabilising wine. There are two main types of precipitation caused by cooling the wine close to freezing point for a restricted period. First there is the precipitation of tartrate crystals. Second there is the precipitation of colloidal substances like unstable colouring matters and proteins. This effective treatment prevents the wine from later precipitation in the bottle assuming that the bottled wine will not be cooled to a lower temperature than that of the treatment. Microbial activity is not eliminated by cold stabilisation. Micro-organisms have to be eliminated by sterile filtration. Further stabilisation is achieved by an adequate dosage of sulphur dioxide before bottling, preventing the wine from aroma loss and colour change during the aging in the bottle. Permanent cool temperatures will slow down the aging process of the wine during storage.

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Troost, G. (1988): Technologie des Weines (Handbuch der Lebensmitteltechnologie), 6 Auflage, Ul-mer Verlag Stuttgart, p. 318

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A. (2006): Handbook of Enology Volume 1, John Wiley and Sons, England, p. 407-408

3.3. SO_2 – Management (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

Relevance of the use of sulphur dioxide in musts and wines

Sulphites are nowadays considered as fundamental additives in different stages of wine production for their antimicrobial, antioxidant and anti-oxidising activity.

In musts and wines sulphur dioxide inhibits the growth of bacteria and wild yeasts whilst selected yeasts (*Saccharomyces* spp.) show a certain tolerance toward the additive. This is very important from the technological point of view as it determines the predominance by the selected strains in the fermenting medium (selected just on the basis of their resistance to SO_2).

In addition to their activity in the selection of fermenting micro-organisms, sulphites have other important microbiological effects. Bacteria are very sensitive to sulphur dioxide and for this reason sulphiting is a good technique for avoiding malolactic fermentation (when not desired) as well as for reducing the risk of microbial pollutions (e.g. development of acetic bacteria or uncontrolled lactic fermentations).

The antioxidant action of SO_2 in musts consists mainly in the inhibition of enzymatic oxidations. The addition of sulphites stops the oxygen consumption in the must itself by the inhibition of the enzymes which catalyze the oxidation of phenolic compounds (polyphenol-oxydases). One of these enzymes, normally present in the grape (tyrosinase), is totally inactivated by a relatively low addition of sulphur dioxide (approx. 50 mg/L), whilst another enzyme, produced by Botrytis cinerea and derived from rotten grapes (laccase) is less sensitive to sulphur dioxide. Hence the risk of browning and oxidations is higher in the musts produced from botrytized grapes.

Another advantage related to the use of sulphites in the early steps of wine-making process is their ability to bring about a greater extraction of anthocyanins and phenolics during the maceration of red grapes. Sulphur dioxide can denature some proteins, located in the membranes of the grape skin cells, producing micro-leaks and improving the extraction of colouring matter. Moreover, sulphur dioxide can bind anthocyanins making them more soluble and extractable, especially in a water-alcoholic medium. The problem of this kind of interaction is the slight loss of wine colour that resultIng fact the resulting must formed by the interaction of sulphur dioxide with colour compounds (anthocyanins), is not coloured.

If antioxidising activity is mainly affecting the must and the inhibition of enzymes its use in the completed wine is based on its ability to directly react with oxygen in the presence of metallic catalysts (such as iron or copper). This kind of reaction reduces the oxygen availability in the medium and its ability to react with other substances (e.g. polyphenols).Thus sulphur dioxide is particularly important in the conservation of wine.

State of sulphur dioxide in musts and wines

In must and wine sulphur dioxide are in equilibrium between different forms viz. total SO_2, free SO_2 and molecular SO_2.

Different compounds (sugars, carbon compounds) are able to act as SO_2-binding molecules. Acetaldehyde (MeCHO) is the most reactive. The product formed by its interaction with the bisulphite ion is stable and its formation reduces the activity of the additive with regards to both its antimicrobial action and its antioxidant properties. The fraction of SO_2 bound by acetaldehyde and other compounds represents the combined fraction of the additive itself.

The following Figure 79, describes explain the equilibrium of sulphur dioxide in musts and wines.

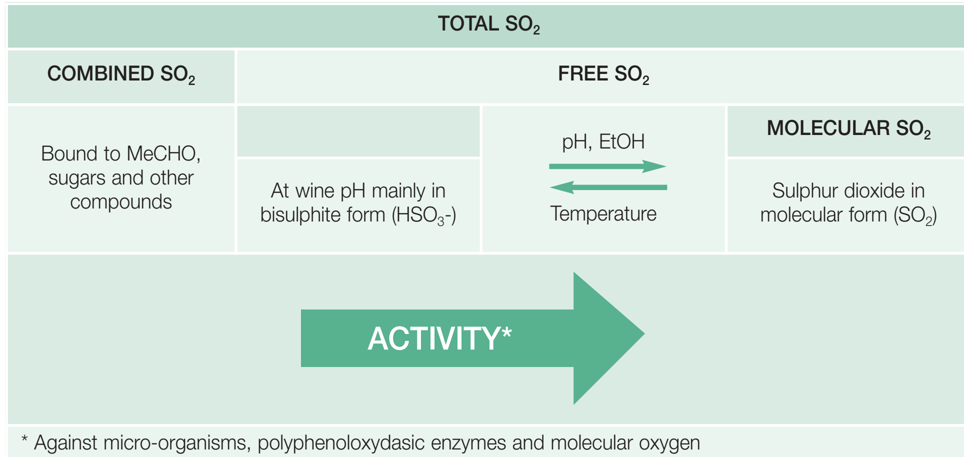


Fig. 79: Schematization of the equilibrium of sulphur dioxide in wine

At the pH of the wine, free sulphur dioxide is mainly present as the bisulphite ion (HSO_3^-); even though this form shows a good activity both against the microorganisms and against oxidation, the most active form of the additive is the molecular one (SO_2).

The percentage of free sulphur dioxide in molecular form depends on the pH, being higher when the pH is lower. Thus the effects of sulphites are more intense when the pH is low. Alcoholic degree and temperature also affect the equilibrium between bisulphite ions and molecular SO_2 the molecular fraction increases at higher alcoholic concentrations and temperatures.

As already mentioned acetaldehyde is the most important SO_2-binding compound in must and wine. Some yeasts strains can produce MeCHO as a reaction to the presence of high levels of sulphites in their growing medium; this means that when sulphur dioxide is added in high amounts to the must it can cause an increase of acetaldehyde production by the yeasts and, as a consequence, a lower ratio between free and total SO_2 at the end of alcoholic fermentation[7].

For this reason, wine-makers are inclined to limit the use of sulphites before alcoholic fermentation, with the advantage of reducing acetaldehyde production. This results in a more favorable ratio between free and total SO_2, and, consequently, to a wider margin of action as regards any subsequent addition of the additive.

Toxicity of sulphites

Despite the fundamental reactions outlined above sulphur dioxide is well known as a poisonous and allergenic substance (LD50: 0,7-2,5 mg/kg b.w. depending on animal species; maximum daily intake: 0,7 mg/kg b.w.[8]), and for this reason it could have a strong impact on the perception of the consumers as regards human health.

[7] Increase of sulphur dioxide in combined form; for example, 100 mg/L of total SO_2 added before alcoholic fermentation can become, at the end of sugar depletion, 60-70 mg/L, with less than 10 mg/L in the free form.

[8] Ribéreau-Gayon et al., 1998. Traité d'Œnologie. Microbiologie du vin, Vinifications. Vol. I. Dunod, Paris.

Based on EC Regulation 1991/2004, sulphites must be declared on the label if their overall content in wine is higher than 10 mg/L. This represents a serious problem for wine producers (when speaking about the opportunity to reduce the levels of SO_2) and it is an important issue particularly for the "organic sector". Even conventional wine-makers are oriented towards a lowering of the amount of SO_2 in their products and perhaps the consumers expect to find only minor levels of the additive in wines from organic viticulture. Moreover, some questions related to the use of sulphites in oenology are still undecided. For example: "How much is it possible to reduce sulphur dioxide levels without risking taste and quality degradation or increasing microbial contamination or oxidation during the vinification or the storage in barrels or bottles?"

3.4. Relevant wine-making practices to lower sulphur dioxide levels (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

Introduction

Nowadays different alternative practices and additives could be used in reducing the use of sulphites in wine-making, but the complete elimination of sulphites is, at the moment, still not possible.
The feasibility of sulphur dioxide reduction is possible during the vinification stage. There are not suitable technologies in all phases of the wine production process that are available to partially replace or reduce the sulphur dioxide.
In the following pages, a short review of the available alternatives to SO_2 (practices and additives) are presented in order to explain their usage

Correct management of selected yeasts inoculation

Performing alcoholic fermentation with no added sulphites means that the inoculation of selected yeasts takes place in a medium highly contaminated by wild micro-organisms. In these conditions, wild yeasts and lactic bacteria may grow and consume the assimilable nitrogen (YAN) which is a basic source of nutrition for Saccharomyces yeasts. This consumption occurs in the juice just in the first hours after pressing and generally leads unavoidably to a sluggish fermentation process.
In order to avoid this situation, when no SO_2 is used before alcoholic fermentation, a very early inoculation of the selected starter culture is strongly recommended. This practice allows Saccharomyces dominance during fermentation because the adaptation phase of the selected starter culture will be reduced. Obviously, the preparation of the starter should be done in strict accordance with the supplier instructions:

1. rehydration of the active dry yeast powder in warm water (35-40 °C) for 10-15 minutes;
2. eventual addition of nutrients during rehydration (e.g. yeast walls and thiamine, which are important growing factors for the yeasts);
3. careful addition of subsequent small aliquots of juice and agitation, to facilitate respectively the yeast acclimatization, and the production of fatty acids and sterols (fundamental factors for yeast metabolism);
4. Addition of the starter culture to the rest of the must.

When the fermentation is managed without sulphur dioxide, the control of yeast assimilable nitrogen is also recommended. Musts from organic grapes are generally not very rich in YAN and so need to be reintegrated, if possible, before yeast inoculation.
These actions (early inoculation of selected yeasts and control of YAN levels) reduce the risk of stuck or sluggish alcoholic fermentation and allow the complete transformation of the sugars even if no sulphites are added. Moreover, a lower addition of SO_2 before fermentation can reduce the production of acetaldehyde and so decrease the take up of the additive and so improving its potential activity in the later steps of the wine-making process.

Yeast – lactic bacteria co-inoculation

This recently introduced practice permits an effective and simultaneous management of both alcoholic and malolactic fermentation. For further details on this technology refer to thennexes on experimental results.

Lysozyme

Sulphur dioxide is able to affect bacteria metabolism, and for this reason, it represents one of the main tools in preventing microbial infection, as well as the behavior of malolactic fermentation when it is not desired.
From this point of view, according to different studies, a suitable alternative to sulphites is lysozyme (500 mg/L of this egg derived protein have the same effect on lactic bacteria[9] than 40 mg/L of SO_2 ; Gerbaux et al., 1997[10]).
In contrast to sulphites, this preservative is particularly active at high pH values and so it can be helpful in certain critical conditions which are propitious for microbial growth.
The use of lysozyme should be carefully considered as its protein nature may cause an interaction with phenolic compounds with the consequent loss in colour of the red wines. Furthermore it may cause protein instability in white wines.
Lysozyme is extracted from eggs, and for this reason it can be an allergen. The risk connected with its use in wine-making is due to the persistence of its activity for different times after application. According to Bartowsky and co-workers[11] (2004) 75-80 % of the initial activity is still detectable in white wines (Riesling) after six months, whilst no residual activity was detectable in red ones after only two days.

Hyper-oxygenation and hyper-reduction technologies

Hyper-oxygenation practice and hyper-reduction technologies can be also used to reduce levels of SO_2 in musts. The former consists in a massive addition of oxygen or air with the purpose of completely oxidising all the unstable substances. Hyper-reduction is based on the addition of ascorbic acid or other antioxidants to protect the must itself from oxidative reactions.
More detailed information of these techniques is available in thennexes related to "Oxygen and wine", "Practical Hints – Hyper-oxygenation" as well as in the Annex on Experimental Results.

9 Lysozyme is not active on acetic bacteria or yeasts; it acts only against lactic bacteria
10 Gerbaux et al., 1997. Use of lysozyme to inhibit malolactic fermentation and to stabilize wine after malolactic fermentation. Am. J. Enol. Vitic., 48: 49-54.
11 Bartowsky et al., 2004. The chemical and sensorial effects of lysozyme addition to red and white wines over six months cellar storage. Australian Journal of Grape and Wine Research, 10: 143-150.

Conservation under inert gases

The direct reaction between sulphites and molecular oxygen is slow and requires the presence of catalysts such as iron or copper. In must this kind of reaction is not really important, because of the faster oxidations catalyzed by polyphenoloxidases; on the contrary, in wine, despite its relative slowness, it can compromise the quality of the wine during aging.

Thus it is extremely important to maintain the containers (both steel tanks and wooden barrels) completely full during wine storage, to minimise the presence of atmospheric O_2 in the headspace of the tank itself. The use of inert gases such as nitrogen or argon can be useful in the management of the wine level inside steel tanks. These gases (as opposed to others such as carbon dioxide) show a low solubility in the wine itself and are able to significantly reduce the concentration of oxygen in the headspace, minimising the risk of oxidation.

3.5. Yeast nutrients and their different functions (Werner, M.; Rauhut, D.)

Good yeast activity is essential for a complete alcoholic fermentation in wine-making. The optimal biologic conditions for yeasts would be the presence of oxygen, a balanced amount of nutrients and an adequate temperature. Besides glucose and fructose, which are not limiting factors in grape must, yeasts require easily metabolisable nitrogen sources (ammonium, amino acids), growth factors (vitamins), micro-nutrients (minerals) and 'survival' factors (long-chain fatty acids and sterols). The nutritional composition of the natural grape juice can vary very much from year to year, depending on the soil fertility and climatic conditions. If the natural concentration is unbalanced, winemakers can add certain nutrients in order to optimize the nutrition of the yeast and avoid sluggish fermentations and the formation of undesired off-flavours during the fermentation.

Ammonium:

The addition of ammonium salts is the easiest assimilable source of nitrogen for the fermenting yeast. It should be added in the form of di-ammonium-hydrogenphosphate instead of ammonium sulphate in order to limit the sulphate concentration in the must. The availability of nitrogen at the start is essential for the setup of new cell walls, the production of enzymes and also for the production of membrane proteins which are located in the cell membrane and responsible for the transport of sugar and amino acids. So ammonium ions should be present during the multiplication phase and the start of fermentation. The addition must be done at the first half of fermentation as later additions cannot be ingested by yeasts due to the increased alcohol level. Excessive amounts of ammonium lead to problems with the uptake of amino acids which is why the dosage should be chosen according to the individual deficiency.

Thiamine:

The addition of the vitamin thiamine is recommended for grape must which has been heat treated or which has come from botrytized grapes and is to be used for the production of specialty wines. These factors reduce the natural concentration of this vitamin significantly. Thiamine especially plays an important role with regards the need for sulphur dioxide in the final wine. By reacting as a coenzyme of the pyruvate decarboxylase it is involved in the degradation of carbonyl compounds (which bind to SO_2) in the last steps of the sugar depletion. Additionally it has a general positive effect on the course of fermentation under difficult conditions. The addition of thiamine should be either by thiamine (hydro)-chloride or in combination with the addition of ammonium salts.

Yeast Cell Walls (Yeast Ghosts):

Another way of supporting the yeast at the beginning and during the fermentation is the addition of yeast cell walls. This preparation consists of natural yeast components which were produced by destroying the yeast cells and extracting the soluble parts. The result is a solution of clean cell walls which is not really a nutrient, but it can support the yeast in other functions. Cell walls are an important source of sterols, which are not consumed as energy source, but will be incorporated into the new cell walls of the multiplying yeast. A strong cell wall is important when ethanol levels increase in the must because the sterol production of the yeast itself is only taking place in the presence of oxygen and not under anaerobic fermentation conditions. In addition yeast cell walls can have an absorptive effect on substances which are toxic to yeast, which could have originated in the vineyard or have been produced by micro-organisms. Thus yeast cell walls can have an important contribution to the optimization of the alcoholic fermentation but they are not a complete substitute for nitrogen. They do not deliver pure ammonium to the yeast.

Inactive Yeasts:

Inactive yeasts are non-viable cells made from natural yeasts, although the composition of nutrients in the cells is comparable to that of active dry yeast cultures. Inactive yeasts represent a complex natural source of various nutrients such as micro-elements which are available in an easily assimilable form to the active yeast. Inactive yeasts cannot be used as a major source of nitrogen, as they do not contain pure ammonium as in case of ammonium salts (unless the commercial product is a blend). Every product may have a slightly different effect on the fermentation depending on the exact heat treatment of the yeast cells, the perforation of the cell walls and the release of soluble nutritional elements from the inside of the cell. Some products are even promoted with an additional or side effect like *antioxidative* or *fining characters*. These products also have in common the fact that they are sources of useful nutrients, including amino acids, micronutrients and vitamins.

References:

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. (2006) Handbook of Enology, Volume 1, John Wiley and Sons, England

3.6. Oxygen and wine (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

General Principles

Oxygen represents about 20 % of the air that we breathe thus it is everywhere. Thus wine-makers must be aware of it's important role in affecting different technological operations.

There are different wine-making theories with regards oxygen management in enology.

Some producers are convinced that O_2 is an "enemy" for the wine (oxidations, browning) whilst others think that a limited and controlled oxygenation is fundamental for correct wine development These opposite beliefs lead to the definition of two different strategies in the management of the interactions between oxygen and wine. Firstly the total protection of the wine itself from contact with air (e.g. in hyper-reductive technologies) or conversely the controlled oxygenation of the wine (such as in micro- or hyper-oxygenation).

Both these approaches are utilized in wine-making nowadays with different technological implications and different impacts on the characteristics of the products obtained.

Effects of oxygen dissolution in wine

Oxygen can play a double role in wine, affecting sometimes positively, sometimes negatively the wine characters. The equilibrium between these affects depends on the amount of the dissolved concentration of oxygen, on the moment of the dissolution, and on the characteristics of the wine itself (e.g. red wines are less sensitive to oxidation respect to white ones).
In particular, the effects of oxygen might be related to the following aspects:

1. Modification of phenolic compounds:

- Browning and modification of colour for both musts and wines, as a consequence of the oxidation of polyphenols.
- Positive effects on wine evolution and aging (e.g. reduction of astringency, stabilisation of phenolic fraction).

2. Modification of aromatic fraction:

- Evolution of wine aroma and formation of aging related compounds.
- Decrease in varietal notes and development of oxidation typical characters.

3. Effects on the multiplication and growth of micro-organisms.

As mentioned above the equilibrium between these positive and negative effects of O_2, depend on different factors:

- Variety

Some varieties (e.g. Sauvignon blanc, all Muscat varieties) are very sensitive to air contact. The resistance of a substrate to oxidation is related to its composition: a higher content of natural antioxidant compounds in the juice (polyphenols, glutathione, ascorbic acid) can improve such resistance, reducing the susceptibility to O_2.

- Temperature

This variable affects both the dissolution and the activity of O_2 in musts and wines. At 20-25°C, the maximum possible amount for the dissolution of oxygen is approximately 6-7 mg/L (concept of "saturation") but this level can increase at lower temperatures: approx. 10 mg/L at 5°C. On the contrary, the rate of oxidation reactions increases at higher temperature. For example the oxidation of the red wine colour compounds such as anthocyanins occurs faster at 30°C than at 20°C.

- Step of the wine-making process

The oxidation rates of musts are usually higher than those detectable in wine, because in musts oxidations are enzymatically catalyzed by polyphenoloxydases (PPO). These enzymes are derived from the grape (tyrosinase) or from moulds (laccase from *Botrytis cinerea*) and they are able to dramatically increase the oxidation reaction. Laccase, in particular, can induce damage on the composition of the must itself. This is why the vinification of grapes infected with botrytis is often problematic from the point of view of O_2 management, and higher levels of sulphites are needed.

- Time of air exposure

Oxygen is quickly consumed after its dissolution, and the effect of this utilisation is dependent on the composition of the wine. The uptake of O_2 entails the development of certain reactions. If the air contact is limited in time the affect of oxygenation will remain limited but if the dissolution is prolonged a continuous sequence of dissolution is observed. The final effects of this sequence will depend on the ability of the must - wine to resist oxidation. If the content of antioxidants is low, the wine will not be able to effectively resist the effects of O_2 consumption.

Redox equilibrium of the wine and antioxidant compounds

Many compounds in the must and wine coexist as mixtures of their oxidized and reduced forms, the so called "redox pairs". The reduction of one compound always causes automatically the oxidation of another one. In chemical terms these oxidation-reduction (redox) reactions continue until the "equilibrium point" is reached and neither reduction nor oxidation compound dominate.
In the wine-making-related reactions, this "redox" equilibrium reflects two groups of compounds: some of them can act as oxidizing agents whilst others are reducing agents.
The most important oxidizing agent in musts and wines is oxygen. Other chemicals can increase its action in wine by acting as powerful oxidants themselves. An important example of this is related to the heavy metals such as iron and copper. These compounds are normally present in wine and are powerful catalysts. They can strongly increase the action of oxygen and the rate of oxidation reactions. In addition some free radicals and peroxides (e.g. hydrogen peroxide – H_2O_2) are produced from the oxidation of phenolic compounds and can also be involved as oxidising compounds.
The most important reducing agents found in wine are sulfur dioxide (SO_2), ascorbic acid, phenolic compounds and glutathione.
Ascorbic acid (AA), known also as vitamin C, can be found in a wide range of concentrations in different fruits. This compound plays an important role in limiting enzymatic browning in musts, but in its action in wines it has been demonstrated that it can react with oxygen generating hydrogen peroxide (a powerful oxidizing compound). Ascorbic acid is normally used in wine in combination with SO_2 in order to scavenge H_2O_2 reducing the risk of "oxidative damage".
Glutathione (GSH) is a tripeptide (made by glutammic acid, glycine and cysteine) widely occurring in nature in plants and micro-organisms. It is active against free radicals and other oxygen reactive compounds. GSH can strongly reduce the process of must oxidation, reacting with some products of the enzymatic transformation (PPO) of caffeoyltartaric acid (one of the most oxidisable substances present in the grape juice). The result of this reaction is named 2-S-glutathionyl-*trans*-caffeoyltartaric acid, also known as "Grape Reaction Product" (GRP). In normal conditions (with healthy grapes) this compound is stable in successive oxidations and for this reason glutathione is able to stop the oxidation chain which can lead to must oxidation and browning.
The problem remains in the musts affected by *Botrytis*, because GRP can be a substrate for laccase enzyme; for this reason the vinification of botrytised grapes always has more browning reaction problems.
It is well known that polyphenols and tannins are powerful antioxidants. These compounds are one of the main oxygen reactive chemicals present in musts and wines. The results of their oxidation are browning and the loss of colour as well as the formation of polymers with their subsequent precipitation. The presence of polyphenols in greater quantities in red wines explains the higher resistance of such products to oxidation.

Oxygen reactions in the musts

The oxidation reactions in musts are mainly related to enzymatic activities (PPO), on phenolic acids (e.g. caffeoyltartaric acid).
In the case of healthy grapes, tyrosinase (from the grape itself) is the major enzyme involved in the browning reactions. The activity of this macromolecule is its ready reduction in the juice because it is quite sensitive to SO_2 and it is easily removable by some fining agents such as bentonite. On the contrary, laccase from Botrytis cinerea is poorly affected by bentonite treatments as well as by sulphites this being a greater problem for wine-makers.

The strong reactivity of the musts to oxidation can be used to stabilize the must itself. The concept of hyper-oxygenation is based on the saturising O_2 addition to the juice in such a way that all the oxidisable substances are eliminated by polymerization and precipitation with a simple racking .

Oxygen reactions in the wines

As opposed to the reactions in musts, wine oxidation is mainly related to chemical or non enzymatic reactions.
It is important to remember that O_2 is not always negative for wine evolution. Pasteur himself during his studies, observed that suitable aeration was important in the development of alcoholic fermentation.
A well managed oxygen supply can determine certain advantages to wine especially red ones viz:

- evolution and stabilisation of the colour by the reaction between anthocyanins and tannins;
- reduction of astringency by the evolution of tannins;
- Better development of alcoholic fermentation by the production of basic growth nutrients for the yeast.

These advantages (particularly the first two points) have been since the dawn of the wine-making by the technique of wood aging (limited and controlled O_2 dissolution throughout the wood) and nowadays through the modern application in micro-oxygenation technology (microox). It is also well known that the passage of a limited flow of oxygen through the bottle closures is beneficial for the correct development of a wine as well as for its conservation.
When the oxygen supply is too high the ability of the wine itself to resist the oxygen levels oxidation will automatically occur.
As reported for the musts, it is the phenolic compounds that react with the oxygen which re-sults in the browning and loss of the colour, together with the precipitation of the colouring matter.
These oxidation reactions may also cause the formation of different kinds of volatile compounds which are sometimes responsible for aromatic changes. Acetaldehyde (MeCHO) is the main volatile compound involved with to oxygen consumption. It does not derive from microbial metabolism but from the oxidation of ethanol which has been catalyzed by some heavy metals (iron and copper).
In wood aging or micro-ox, it is this acetaldehyde that is involved in some reactions related to colour and phenolic stabilization. If O_2 dissolution is concentrated or prolonged the higher amounts of MeCHO are formed which in turn can induce the production of other aroma compounds (acetyls) which are responsible of the typical sensory notes of the oxidized wines.

Effects of oxygen on yeasts growth

It is generally accepted that in must, yeasts are able to respire sugars in aerobic conditions whilst they perform alcoholic fermentation (AF) in anaerobiosis.

Important note

When speaking about the effects of aeration on aromatic compounds, it must be stated that in the early steps of the vinification process, volatile compounds are relatively protected against O_2 as they are present in the form of "precursors". For example, terpenes, an impor-tant family of compounds which characterize the aroma of Muscat grapes (but are practically present in all the fruits) are mainly present in the must as glycosides (bound to sugars). In this form such molecules are less sensitive to oxidation than in the free form.
The practice of hyper-oxygenation, which is based on a concentrated oxygen supply just after juice extraction will adversely affect the composition of the aroma of the final wine as the aroma is protected in the combined form of these precursors.
Due to the fact that glycosides are broken down during the vinification with the subsequent release of the volatile compounds in free form, the effects of O_2 on the aromatic fractions of the wine will negatively affect the varietal characters of the product. The aromas, in fact, be-ing in free form will be more sensitive to oxidations.
This is particularly true for some compounds produced from specific aromatic varieties, such as Sauvignon blanc or Muscat varieties. The varietal aroma of Sauvignon blanc wines is related to the presence of certain sulphur containing compounds which are very sensitive to air. In the must these molecules are relatively protected as precursors (bound to the amino acid cysteine), but in the wine the free form is very sensitive to O_2.

In fact the ability of wine yeasts to use the glucose through respiration is dependant on the sugar content of the must. If the sugar concentration is higher than 9 g/L, *Saccharomyces cerevisiae*, the main micro-organism involved in alcoholic fermentation, is unable to bring about the aerobic transformation of sugars. This means that under normal conditions in the must (sugar content approx. 180-220 g/L), the yeasts can only stimulate alcoholic fermentation. This phenomenon is known as "Crabtree effect".
It is clear that the aeration of the must after the inoculation of selected yeasts (or the oxygenation of the *pied de cuvee* before addition), benefits the development of the fermentation process. These benefits are not related to increased yeast populations obtained by the respiration process[12], but instead they are mainly related to the fact that the oxygenation itself leads to the production of rudimentary growth nutrients for the yeasts, such as some fatty acids and sterols. Similarly a slight air supply (e.g. by pumping over) at the middle of AF, is also useful in obtaining satisfactory development of the final steps of the fermentation process.

12 The utilization of sugars by respiration produces more energy than the fermentation process. Thus respiration is encouraged in order to obtain a rapid multiplication of yeast populations during the industrial production of selected yeasts

Important note

As well as its action on yeasts, oxygen can also affect the metabolism of other micro-organisms. For example acetic bacteria are responsible of the oxidation of sugars which occur in aerobic conditions. In extreme conditions glucose is completely oxidized by these micro-organisms to water and carbon dioxide.
Ethanol is also a potential substrate of these bacteria. It is transformed in acetic acid and then ethyl acetate, compounds that are responsible for the increase of volatile acidity and for the formation of the typical odor which occurs in wines affected by senescence. Thus the reduction of oxygen during wine storage is essential for the prevention of both chemically and microbiologically-related oxidations. Therefore producers should take care to completely fill all the containers avoiding extended exposure of the wine to the oxygen present in the headspace (e.g. leaving the tanks empty after a racking). The use of inert gases such as nitrogen or carbon dioxide, and the control and re-introduction of sulphur dioxide could be useful strategies to protect the wine during transfers and storage.

3.7. Microbial contamination (Trioli, G.)

Microbial contamination or spoilage occurs with the development of micro-organisms whose metabolism can negatively affect wine quality.
Grape juice, rich in sugars and nutrients, is a suitable substrate for the growth of many species of micro-organisms, including yeasts, bacteria and moulds. After alcoholic fermentation, the ethanol presence reduces the potential for development of many micro-organisms but even under final wine conditions some yeasts and bacteria can still be active.

Spoilage agents

The low pH of juice and wine does not permit the growth of human pathogens which are thus not a concern in the wine industry. Many micro-organisms can however adversely affect wine quality by producing unwanted chemicals resulting from the degradation of favourable ones.

Oxidative yeasts

This group includes yeasts from the genera *Hansaenula*, *Hanseniaspora*, *Pichia*, *Candida*. These yeasts have a predominant oxidative metabolism, but some species can survive quite high levels of alcohol. They can metabolise sugars and organic acids in the presence of oxygen. Unwanted by-products resulting from this activity are acetic acid, ethyl-acetate and acetaldehyde, together with many other compounds whose high presence can initiate faults and off flavours in wine. Oxidative yeasts are found on grapes, in the juices and in the wine.

Apiculata yeasts

The name of these yeasts refers to the lemon-shaped appearance of *Kloeckera apiculata*. This yeast is predominant in grape juice before the complete onset of alcoholic fermentation and can growth fast at low temperatures. Compared to *Saccharomyces cerevisiae* (the main agent of alcoholic fermentation in wine) *Kloeckera* produces higher amounts of volatile acidity and ethyl-acetate. Its metabolism produces other volatile compounds whose significance in relationship with wine quality is undecided. Most wine-makers aim to avoid their presence, while others look for a limited presence in order to add some complexity to their wine. In a typical spontaneous fermentation, *Kloeckera* is dominant at the very beginning of the process and is later overwhelmed by *Saccharomyces* as soon as the alcohol degree reaches 4-5 %. It is claimed that Kloeckera yeasts are the main reason for the depletion of assimilable nitrogen, vitamins and other micronutrients in the must.

Fermentative yeasts

This family is essentially, well known as *Saccharomyces* spp. The different species of this yeast are the most resistant to the combination of alcohol and acidity typical of wine, and it is these yeasts which carry out the alcoholic fermentation until the complete depletion of sugars. They are in general regarded positively, but wine-makers must take into account the existence of a large variability among strains. Some strains can produce excessive amounts of acetic acid, sulphur compounds, SO_2, urea and volatile substances which might be detrimental to wine quality. Some wild strains of *Saccharomyces cerevisiae* must be considered as spoilage micro-organisms. Spontaneous fermentations are typically carried out by a dozen or so different strains. Often the strains which are predominant at the beginning of fermentation are not the ones which complete sugar degradation.

In the same winery, different years see the presence of different yeast strains. This uncertainty is the reason for wine-makers to question the spontaneous fermentation approach in wine making

Acetic bacteria

Gluconobacter and *Acetobacter* are the main genera of oenological significance within this family. Gluconobacter which is mostly found on damaged grapes degraded sugars into acetic acid and other compounds but have a low resistance to alcohol. *Acetobacter* uses ethanol as a substrate and metabolises it to acetic acid. Both bacteria need oxygen for their activity.

Lactic bacteria

This group include malolactic bacteria like *Oenococcus oenii* as well as many other micro-organisms belonging to the genera *Lactobacillus*, *Pediococcus* and others. Many of the lactic bacteria found in wine are heterofermentative and therefore their development in the grapes and juices must be avoided as they can lead to the production of excessive amounts of volatile acidity. A very large presence of lactic bacteria in the juice released from damaged grape berries has been widely studied. Without any control these bacteria can grow very fast and consume sugars producing a large amount of lactic and acetic acids as by-products. During alcoholic fermentation lactic bacteria presence is usually reduced due to competition with *Saccharomyces cerevisiae*. However towards the end of fermentation the lactic bacteria population increases and initiates the malolactic fermentation. (The main agent in wine at low pH is *Oenococcus oenii* (formerly *Leuconostoc oenos*)). This second fermentation is normally desired in red wines, but often unwanted in white wines where acidity and freshness must be maintained. Several species of *Lactobacillus* and *Pediococcus* can grow in wine and these bacteria are often responsible for malate degradation in wines at high pHs. Moreover, they can also be active after malolactic fermentation in dry wines as only a few hundreds mg/l of sugars are enough to encourage a significant population. This late development of bacteria in wine is definitely a spoilage reaction as it produces an unpleasant odour.

Brettanomyces

Dekkera/Brettanomyces is yeast which can be found both in grape juices and in wine. Some strains, even at relatively low populations, can produce ethyl-phenols whose odour is described as manure, band aid and horse sweat. The presence of *Brettanomyces* in the winery can lead to significant economical damage. This yeast may contaminate wood barrels as well as concrete tanks requiring thorough cleaning treatments or the complete renovation of the containers. *Brettanomyces* can also develop in the bottle often giving inconsistent faults in the wine at consumption. The presence of this yeast is not easily detectable and careful prevention is the best way to avoid the spoilage. High pHs and low SO_2 presence are the main reasons for allowing the development of *Brettanomyces* in the wine.

Conditions

The ecology of these micro-organisms depends on various important factors such as time, temperature, pH and oxygen.

Time

Micro-organisms need time to grow and multiply. A generation time can vary from a few tens of minutes to weeks depending on the microbe, the conditions and the nutrient availability. In optimal conditions, as for instance on grape juice at summer air temperature, yeasts and bacteria can double their presence every 1-2 hours. It must be realised that in optimal conditions one single cell of yeast can produce a population of several thousands of cells within one day. The most critical phases in wine-making must then be speeded up as much as possible (i.e. transport and storage of grapes, juice clarification, period within the end of alcoholic fermentation and malolactic fermentation, etc.)

Temperature

Every micro-organism has a specific optimal temperature range for its activity. *Saccharomyces cerevisiae*, for instance, has none or a very low activity below 10-12°C and shows a maximum growth in grape juice at around 35°C. The presence of alcohol reduces the optimum to 26-28°C. *Kloeckera* is more active than *Saccharomyces cerevisiae* at temperatures of 4-10°C, used for instance during juice settling in cold maceration. Lactic bacteria require 16-18°C to grow at a significant speed. Acetic bacteria can stand high temperatures even in presence of alcohol.
Cooling is costly in energy but is an effective strategy in reducing the growth of spoilage micro-organisms both in juice and in wine. Nevertheless, low temperatures slow the growth and the activity of the micro-organisms but don't inactivate or eliminate them from the system. A subsequent rise in temperature will restart the contamination process.

Oxygen

Oxygen is essential for the existence of some spoilage micro-organisms. Acetic bacteria and oxidative yeasts need abundant availability of oxygen. Some lactic bacteria and *Brettanomyces* can take advantage of a small presence of oxygen. *Saccharomyces cerevisiae* don't need oxygen to develop and ferment even though it benefits from its availability at around the middle fermentation stage. The avoidance of air getting in contact with juice and wine, through reduced tank headspace and inert gas protection, is thus a powerful strategy to avoid development of a large proportion of spoilage micro-organisms.

pH

Acidity is a major factor affecting lactic acid bacteria. Only *Oenococcus oenii* can show some activity at pHs as low as 2.9; most cannot significantly grow if not above 3.2. All of them, though, greatly increase their activity as the pH increases. At pHs around 4.0, some lactic bacteria can grow so fast as to overwhelm yeasts. Among yeasts, only *Brettanomyces* is significantly affected by pH, and low acidity wines are more easily contaminated than low pH ones. *Saccharomyces cerevisiae*, *Kloeckera* and acetic bacteria are almost equally active in the whole range of wine pH.

Inhibitors

Wine regulation permits the use of a certain number of substances which can inhibit the growth of spoilage micro organisms

SO_2

Very effective, low cost and a wide spectrum of action make sulphites by far the most used antimicrobial compounds in wine-making
SO_2 is active against bacteria and yeasts. One of the main reasons for its preference in wine-making is that, among wine micro-organisms, the least sensitive to SO_2 is *Saccharomyces cerevisiae* which

is required for alcoholic fermentation.
The effectiveness of SO_2 when added to wine depends on the presence of binding compounds and on the wine pH.
Pyruvate, acetaldehyde, 2-chetoglutarate and other carbon compounds, mainly produced by yeasts during fermentation are able to combine sulphites into a form that is not harmful for most micro-organisms. Only bacteria are affected by sulphur dioxide.
Within the free SO_2, it is the molecular fraction (SO_2--) which is active against all spoilage micro-organisms, and its relevance depends on pH. The same amount of free SO_2 is 10 times more active against microbes at pH 3.0 than at pH 4.0.

Lysozyme
Lysozyme, extracted from egg white, is an preservative able to break bacteria cell walls causing their death. Largely used in the diary industry, has been recently authorised for wine-making. It has no actions against yeasts and acetic bacteria. Its effectiveness against Lacto-bacillus, *Pediococcus* and *Oenococcus* is greater when these micro-organisms are in the growing phase and therefore its use as a preventive enzyme is preferable.

Potassium sorbate
It is only active against yeasts. If present during bacterial development it can be metabolised into compounds responsible for a strong geranium-like odour. For this reason its use is re-stricted to the bottling phase after wine filtration but is not acceptable for organic wine-making.

Dimethyl-Dicarbonate (DMDC)
This has recently been allowed for wine-making in the EU for use in sweet wines at bottling. It is an alternative to potassium sorbate as it is effective only against yeasts. Due to its poor solubility, DMDC is injected on-line in the wine at bottling through a special device. It acts as an immediate steriliser of yeasts and, after few hours, decomposes into methanol and carbon dioxide but is not acceptable for organic wine-making.

4. PRACTICAL HINTS

4.1. Reductive Wine-making (Trioli, G.)

“Reductive wine-making” is the phrase used to identify a wine-making strategy which avoids contact with oxygen in each step from harvest to bottling. It was the traditional wine-making practices in Germany for all white wines and it was new developed in Australia and New Zealand on varieties like Sauvignon blanc and then successfully applied on aromatic grape varieties all over the world.
The goal of reductive wine-making is to preserve grape aromas and precursors (the majority of which are easily oxidized) and to allow the production of wines with intense fruity and varietal profile.
Wines obtained through this technology are much more sensitive to oxidation than those produced with traditional wine-making, and the protection strategy must be strictly followed right up to the very end of the process. The complete and original grouping of oxygen sensitive compounds is kept in solution in the wine, and any late accidental contact of the wine with air can provoke chain reactions which lead to sudden and strong oxidation of the product.
Strict protection from oxygen can greatly limit the need for sulphur dioxide and in this sense reductive wine-making concepts and techniques can be very useful in organic wine-making. However it should be remembered that the application of reductive wine-making processes with low or no SO_2 use (though possible even in small well equipped facilities) should be considered a high risk process to be considered only if the wine-maker has a complete and absolute control of each step.
Usually producers benefit from the oxygen protection practices at some critical phase of their wine-making process in order to reduce the need of antioxidant additives.

Basic principles

- Ascorbic acid (vitamin C) presence in must or wine should always be coupled with a suitable level of SO_2 or other antioxidants which are able to block its oxidative prod-ucts (hydrogen peroxide and others).
- It is sometimes mistakenly believed that a liquid or a space saturated with CO_2 would be protected from the ingress of oxygen In fact each gas is independent and O_2 can freely enter a system which is saturated by another gas. Inert gas (CO_2, N_2 or argon) protection against oxygen is given by the fact that inert gas release creates a flow from the surface of the liquid toward the exterior which washes out the surrounding air and the oxygen that is present. When no gas movement occurs, the diffusion of each gas proceeds at a rate proportional to the concentration gradient between gases
- Dry ice (solid CO_2) at atmospheric pressure has a temperature of –78°C and quickly passes from the solid state to the gaseous one. Roughly, 6 kg of dry ice lowers by 10°C the temperature of 100 kg of grapes. This data is approximate since one must consider the variety of the conditions of use and in particular of the material of the tank and therefore of its thermal degree of isolation.

Table 5: Wine-making practices – step by step – how to do it.

Step	Operation	Notes
Harvest	In the case of mechanical harvest or partial loose berry, use dry ice to chill the grapes and to create a CO_2 gas flow	*CO_2 outflow to take air oxygen away from the released juice. Temperature decrease slows down enzymatic reactions and the development of microbial contaminants*
Grape transport or storage	In case of long distance transport or long storage of the grapes before processing, it might be necessary to add additional amounts of dry ice	
Grape processing	Wash out air from grape receiver, pipelines and press by using dry ice or a carbonic snow generator before starting grape processing.	*Once the grapes have entered the winery facility, a cheap and convenient source of CO_2 is that produced by fermentation in other tanks, which can be easily conveyed with normal pipelines (no chilling effect)*
Grape pressing (white wines)	Avoid oxygen entrance into the system between pressing cycles. Create a CO_2 flow onto the juice receiver below the press.	*Some recently manufactured presses have a lung system which allows an economical recycling of inert gas throughout different pressing cycles*
Must transfer	Fill pipelines and the bottom of the receiving tank with CO_2 before starting transfer operation. Fill tank from the bottom valve.	*On-line addition of SO_2 and other antioxidant is most effective at this step. Check pump seals before use to avoid harmful air entrance by Venturi effects*
Juice cleaning (white wines)	Make sure the clarification tank headspace is kept filled with inert gas. Remember that low temperatures increase the solubility of oxygen into the juice.	
Fermentation	No operation needed once fermentation has started. The CO_2 produced by fermenting yeasts (and bacteria) is forcing oxygen out from the system. As yeasts rapidly use the dissolved oxygen to build up ethanol tolerance, an aeration at 1/3 of sugar depleted is beneficial	*Make sure fermentation starts as soon as possible. Activated yeast starter cultures are strongly advised. When desired, promote malolactic fermentation immediately after or even during alcoholic fermentation (coinoculum)*
Wine transfer	At each racking or wine transfer, careful application of the same rules as for must trans-fer	
Wine Storage	Keep storage tanks completely filled and carefully protect the headspace – if any – with inert gas	*If suitable for the wine style desired, keep wine on yeast lees to profit from their oxygen scavenging capability.*
Filtration	When paper sheet or diatomaceous earth filtration is applied, keep separate the first filtered wine as it had washed out air form the pores. In the case of membrane systems, previously wash the equipment with inert gas.	*On-line addition of SO_2 and other antioxidants is effective at this step. Check pump seals before use to avoid harmful air entrance by Venturi effects*
Bottling	Inert gas washing of the equipment, pipeline and empty bottles must be carefully applied.	
Closure	Low oxygen transfer rate closures must be used. Carefully wash out air from bottle headspace.	*Tin layer screw caps and some types of synthetic closures give the lower OTR. Natural cork can also be almost impermeable to oxygen, but the inconsistency issue must be considered.*

4.2. Yeast seeding with activation (Trioli, G.)

The activation step represents a simple and inexpensive method to assure the dominance of the selected yeast strain used and a correct development of alcoholic fermentation.
The principle is to build up in advance a starter culture with a loading of active dry yeasts and to use it to seed the main lot of must that is to be fermented.
The high fermentation rate of the yeasts from the start plays a protective role against microbial contamination and oxidation which permits a much reduced or omission of sulphur dioxide usage.

Table 6: Yeast seeding with activation.

Step	Operation	Example for 20 hl	Comments
Step 1	*Two days before the main harvest, collect and process a sample equivalent to 5-10 % of the total harvest depending on the number of available containers*	Prepare 1-2 hl of must in a container with volume of > 3 hl	*In some cases, it can be easier to use a sample for the activation which has not been taken from the same vineyards but which can be blen-*
Step 2	*Once the sample is ready for seeding, rehydrate the selected dry yeasts according to producers instructions*	Rehydrate 500 g of active dry yeast in 5 liters of water (40-42°C) for maximum 30 minutes	*It is important that t the water temperature and rehydration time are correct in order to guarantee good viability of the yeasts*
Step 3	*Inoculate the rehydrated yeasts and homogenate the sample must*	Add the yeast suspension to the must while stirring	*The must sample has been seeded with 250-500 g/hl (25 g/hl calculated on the final volume of must), equivalent to 50-100 million cells/ml*
Step 4	*Fermentation starts after few hours. Monitor temperature and foam formation*	Chill if temperature goes above 30°C (by dipping plastic containers full of ice or by using dry	ice) *Yeasts start to ferment and multiply. In two days they increase their number at least*
Step 5	*10 fold* *Two days later, prepare the main crop must for fermentation*	Run assimilable nitrogen analysis and add ammonia phosphate and thiamine according to	the needs *Oxygen dissolved in fresh must – if compatible with the winemaking strategy – is helpful for*
Step 6	*the yeast.* *Check temperature of main crop must and starter culture: if difference > 10°C, adjust the tempe-*	*rature of the starter culture* Add 1-2 hl of the cold fresh must to the starter culture, and wait 1-2 hours before adding	the rest *Thermal shock can be harmful for the yeasts*
Step 7	*Inoculate the main crop must with the starter culture*	Add the 2 hl starter culture to the 20 hl main lot and homoge-	nize the mass *The fresh must receives a yeast population of*

Note: in some facilities it can be more convenient to use the same tank for the starter culture as for the fermentation of the main must. In this case the fresh must is added directly to the active starter culture after two days.

Advantages:

- Using the same amount of dry yeasts (i.e. 25 g/hl) and no cost increase, the fresh juice is seeded with 10 times more selected yeasts
- The dominance of the chosen selected yeast strain is guaranteed. The high population of actively fermenting yeasts readily overwhelms any must contaminants
- All nutrients of the must (vitamins, microelements, ammonia, amino acids, sterols, unsaturated fatty acids…) are entirely used by selected yeasts
- Oxygen and added nutrients solubilised into the fresh must are made available to the yeasts at the best moment for their utilization (advanced multiplication phase of the yeasts).

4.3. Hyper-oxygenation (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

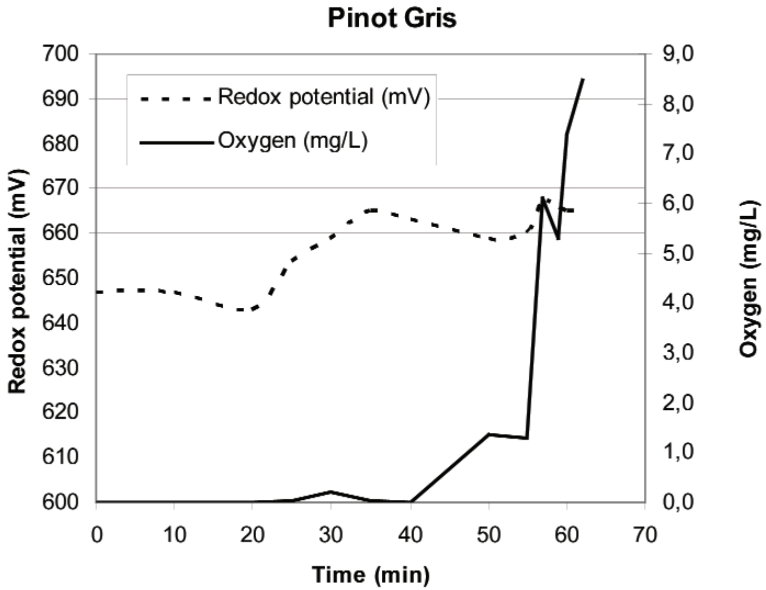
As mentioned elsewhere (see Experimental Results from WP3), hyper-oxygenation is based on the addition of saturating quantities of oxygen to the must, with the purpose of total oxidation and precipitation of unstable phenolic compounds and their subsequent elimination by racking.
If the O_2 supply is added early (e.g. just after pressing), the effects of the treatment on the varietal aroma are reduced at the minimum level (refer to the Annex on Experimental Results for further details).
The following table Nr. 8 is a summary of the main operations and critical points required for the practical application of hyper-oxygenation.
An oxygen sensor could be useful to monitor the behavior of hyper-oxygenation.When O_2 is no longer consumed and it starts to accumulate itself in the must (Figure 80), hyperox treatment can be stopped. Alternativly as already told, even the turn in colour (browning) is a suitable index from this point of view.

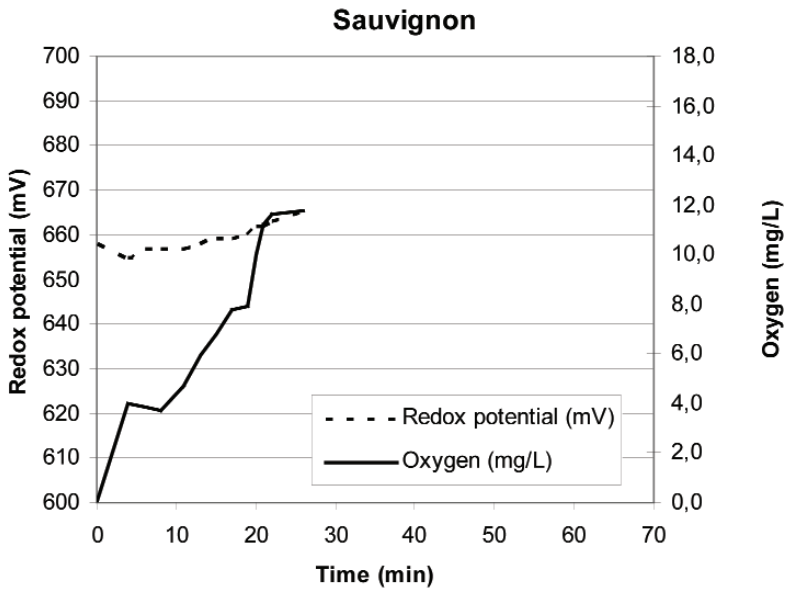
Important note

The producers must finally consider that different varieties have a different reactivity to O_2 and for this reason they need a different hyperoxygenation time. The richness in phenolic compounds can be a good indicator to decide the duration of oxygen supply.
In Figure 80, the must from Pinot gris grapes shows a high consumption of O_2 in the first 40 minutes of the treatment (all the supplied oxygen is consumed – no O_2 accumulation); after this time the level of dissolved O_2 starts to increase and the treatment is stopped after approx. 1 hour (saturation reached).
The must of Sauvignon blanc is demonstrated to be more sensitive to oxidation (less phenolic compounds) because O_2 levels start increase just at the beginning of the process and the duration of the treatment will be consequently lower.

Table 8: Practical operations during must hyper-oxygenation

Wine-making step		Treatment			Risk
1.	Must from the pressing plant (no SO_2 added)	O_2 addition	Air or oxygen from a cylinder	Use a microporous diffuser to uniformly distribute the gas inside the tank	None
			Pumping over	Let the air produced by a pump, bubbling inside the tank	
			Temperature should be not too low (15-20 °C), to avoid reducing the oxidation rate too much		
			Continue the O2 /air supply for 1-2 hours, until the must becomes brown		
2.	Hyperoxygenated must	Racking as fast as possible to eliminate the polymerized phenols Eventually, use of pectolytic enzymes to hasten the process			Development of wild yeasts
3.	Racked must	Inoculation of selected yeasts as fast as possible The use of an amount of unsedimented must produced from the pressing plant (step 1) could be useful for an early pied de cuvée preparation			Development of wild yeasts and consumption of yeast assimilable nitrogen (YAN)
4.	Alcoholic fermentation	YAN supply during both pied de cuvée preparation and addition Eventually, a small amount of di-ammonium-phosphate can be added just after pied de cuvée addition			YAN deficiencies and sluggish alcoholic fermentation





Pinot gris total phenols index (DO 280 nm): 12,2 - Sauvignon total phenols index (DO 280 nm): 5,6

Fig. 80: Examples of the behavior of different hyper-oxygenation treatments monitored by an oxygen sensor (redox potential is also recorded).

5. RESEARCH RESULTS FROM THE ORWINE PROJECT (WP 3)

5.1. Yeasts – lactic bacteria co-inoculation
(Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

General Principles

The fundamental roles that selected micro-organisms play in the behavior of both alcoholic and malolactic fermentation is well known.

Yeasts - lactic bacteria co-inoculation is a recent technique which is aimed at optimizing the management of the malolactic fermentation (MLF) by reducing the risks related to the incomplete transformation of malic acid as well as the production of toxic compounds, such as biogenic amines or ethyl-carbamate.

This practice consists of the simultaneous development in the must of both yeasts and lactic bacteria (MLB by adding a starter culture of selected MLB just few hours (e.g. 12 hours) after the inoculation of selected yeasts.

Co-inoculation and reduction of sulphur dioxide

Principles

According to Masqué and co-workers[13], the co-inoculation is not only useful in reducing the risk of incomplete malolactic fermentations or in avoiding the development of microbial alterations (formation of biogenic amines or other toxic compounds), but, due to the faster reaction of the MLF it means that the wine can be left without sulfur dioxide protection for long periods of time. Thus co-inoculation can be considered as a useful technique to optimize the management of SO_2 in wine-making.

This observation was also confirmed by the results obtained during the experimental trials that were performed during the first two years of ORWINE Project.

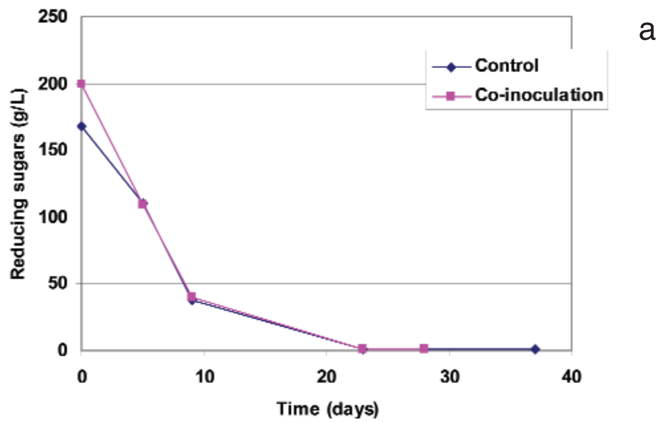
Description of the trials

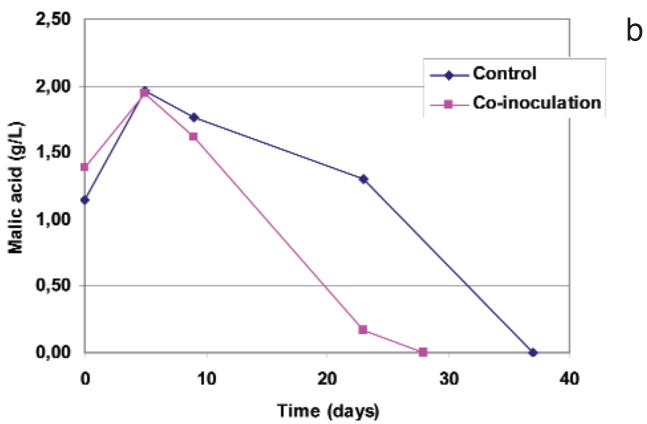
In different trials the co-inoculation technique was compared with the conventional useage of malolactic bacteria which is the late addition of MLB at the end of alcoholic fermentation. Sulphites were avoided when co-inoculation was used.

13 Masqué et al., 2008. Co-inoculation of yeasts and lactic bacteria for the organoleptic improvement of wines and for the reduction of biogenic amine production during the malolactic fermentation. Rivista Internet di Viticoltura ed Enologia (www.infowine.com)

Main results

The results confirmed that co-inoculation does not affect the behavior of alcoholic fermenta-tion (Figure 81a), but it can be helpful in reducing the time needed for MLF: the total consumption of malic acid was faster in the co-inoculated samples than in control wines, being malic acid almost totally consumed just at the end of alcoholic fermentation (Figure 81b).





Control: classic inoculation of MLB, in the final stages of alcoholic fermentation (12th day)
Co-inoculation: inoculation of MLB 12 hours after selected yeasts addition (2nd day)

Fig. 81: Effect of co-inoculation on the behavior of alcoholic (a) and malolactic (b) fermentations in Merlot wines (harvest 2007).

In 2007, the chemical composition of the final wines was very similar, with a very low volatile acidi-ty (0,21 g/L), and acetaldehyde levels (4-5 mg/L).
However the co-inoculated samples obtained in 2006 showed a remarkably lower level of volatile acidity (table 9) . Moreover, co-inoculation demonstrated the ability to control biogenic amine forma-tion even when sulphur dioxide was not used before alcoholic fermentation (table 10).

Table 9: Analytical parameters of some experimental Merlot wines from harvest 2006 (alcoholic de-gree: 12,00 % v/v)

MERLOT	Volatile acidity (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Acetaldehyde (mg/L)
Classic inoculation O_2	*0,51*	*0,08*	*1,60*	*3*	*14*	*2*
Co-inoculation NO SO_2	*0,31*	*0,06*	*2,04*	*n.d.*	*1*	*n.d.*

n.d. = not detectable
* 30 mg/L before alcoholic fermentation

Table 10: Biogenic amines in some experimental Merlot wines in different moments of the vinification process (harvest 2006)

MERLOT	Histamine (mg/L)	Tyramine (mg/L)	Putrescine (mg/L)
Classic inoculation O_2 *	*n.d.[a] – tr. [b]*	*0,2 [a] - 0,8 [b]*	*1,4 [a] - 1,9 [b]*
Co-inoculation NO SO_2	*n.d.[a] – tr. [b]*	*0,2 [a] - 0,8 [b]*	*1,2 [a] - 2,8 [b]*
Classic inoculation NO SO_2	*n.d.[a] – tr. [b]*	*0,2 [a] - 1,3 [b]*	*1,4 [a] - 5,2 [b]*

*a end of alcoholic fermentation (October 2006); b élevage sur lies (January 2007)
n.d. = not detectable; tr. = traces; * 30 mg/L before alcoholic fermentation

With regards to the sensory point of view, co-inoculation, in comparison with SO_2 addition before alcoholic fermentation, led to wines with less buttery, vegetal and volatile acidity notes. The analy-ses of aromatic compounds in these wines highlighted a higher level of volatile esters (basically con-nected to fruity and flowery sensations) in the samples obtained by co-inoculation.

Conclusions

The reduction of sulphur dioxide in the early stages of wine-making certainly is a sustainable prac-tice for both organic and conventional producers but its practicality is dependant on the particular care in the management of the fermentations.
With regards to red wines, some simple practices, such as yeasts - lactic bacteria co-inoculation can be helpful tools in managing MLF even when reduced SO_2 amounts are used.

5.2. Hyper-oxygenation (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

General Principles

The concept of hyper-oxygenation was introduced by Müller-Späth in 1977[14], and it is based on the treatment of the must with an excess of oxygen, with the aim to completely eliminate from the must itself all the oxidisable substances. The products of the oxidation of these compounds (particularly phenolic substances) are completely eliminated with a simple racking at the end of the hyper-oxygenation treatment.
Oxygen can be added as gaseous O_2 or air from a cylinder (with the aid of a microporous diffuser) or simply by pumping the oxygen over.
If the treatment is performed in the early phases of vinification (e.g. just after pressing), it is possible to obtain chemical stabilisation of the must by the elimination of the unstable phenolic substances (e.g. hydroxycinnamyltartaric acids) without damaging the volatile compounds which are at this moment protected in form of "precursors". In the fresh juice just after pressing, aromatic compounds are mainly present as glycosides, bound to sugars such as glucose. It is in this form that certain substances which are sensitive to oxidation, such as terpenols (Muscat-like aroma), are relatively stable and are poorly affected by the excessive injection of oxygen .

Hyper-oxygenation and reduction of sulphur dioxide

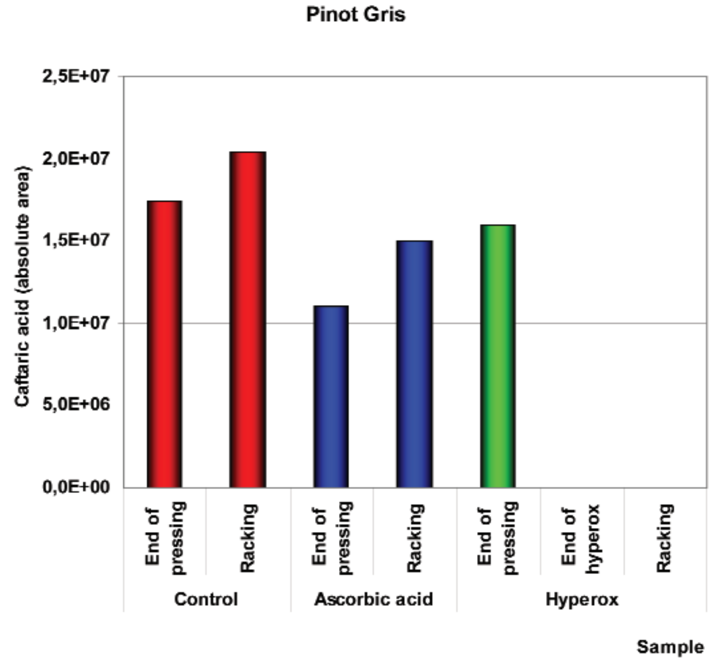
Principles

As outlined above the injection of oxygen eliminates by oxidation and polymerization the unstable phenolic fraction which adversely affects varietal aromatic compounds.
Sulphites must be avoided if hyperoxygenation is selected as a wine-making practice as due to its antioxidant activity, sulphur dioxide reacts strongly against O_2 activity.
Thus hyperoxygenation can have a role in the reduction of SO_2 as it requires the total elimination of sulphites before alcoholic fermentation hence the interest in this practice in organic wine-making.

Description of the trials

The application of hyper-oxygenation on organic musts was the subject of investigation during the three years of ORWINE Project.
The trials were at first related to the comparison between the traditional use of SO_2 during crushing and destemming (e.g. 30 mg/L addition), and its total replacement by using hyper-oxygenation.
Results demonstrated that hyper-oxygenation can give a good stabilisation of musts and wines, lowering the levels of oxidisable phenolic substances (Figure 83).

[14] H. Müller-Späth, 1977. Neueste Erkenntnisse über den Sauerstoffeinfluss bei der Weinbereitung – aus der sicht der Praxis. Weinwirtschaft, 113: 144-157.

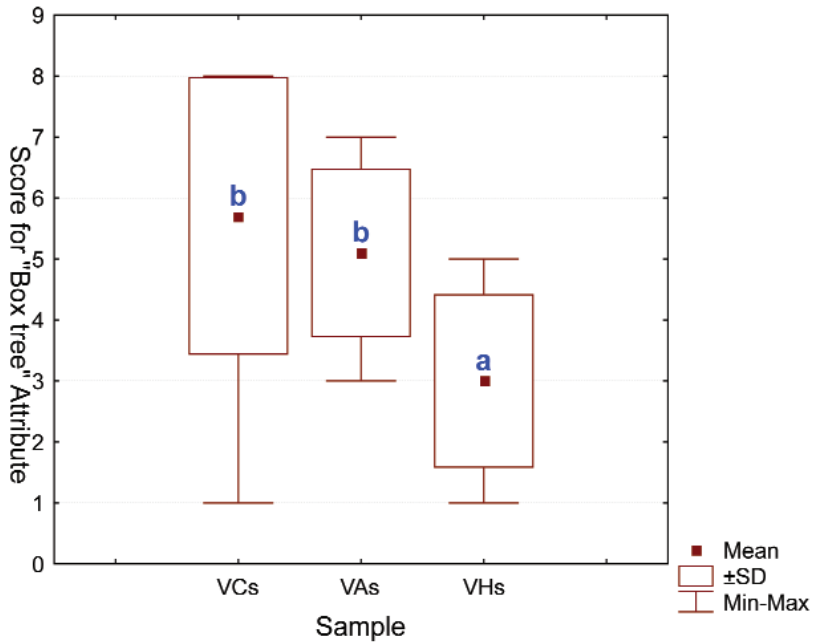


Control: conventional vinification (30 mg/L of SO_2 added during crushing - destemming)
Ascorbic acid: replacement of SO_2 with a mix of ascorbic acid (50 mg/L) and grape tannin (50 mg/L)
Hyperox: elimination of SO_2 using hyperoxygenation

Fig. 83: Caftaric acid[15] levels detected in different pre-fermentative steps. Three trials are compared (harvest 2006).

Nevertheless, this technique can be sometimes problematic for processing certain aromatic grape varieties whose aroma is particularly sensitive to oxidation (e.g. Sauvignon blanc). For such wines a significant loss in some varietal notes (e.g. "box tree" attributes) was high-lighted during sensory evaluation (Figure 84).

[15] Caftaric acid is one of the most oxidizable phenolics in must; it is the most important substrate for the enzymatic oxidations (polyphenoloxydases), and for this reason it is involved in the browning reactions of white wines. Caftaric acid disappears after hyper-oxygenation treatment.



VCs: conventional vinification (30 mg/L of SO_2 added during crushing - destemming)
VAs: replacement of SO_2 with a mix of ascorbic acid (50 mg/L) and grape tannin (50 mg/L)
VHs: elimination of SO_2 using hyper-oxygenation

Fig. 84: Results of a Sensory Attribute Difference Test carried out on Sauvignon blanc wines. Three trials are compared and the results of a Least Significant Difference Test, subsequent to a two factors (samples and panelists) ANOVA, are presented; different letters mark significant differences among samples at $p < 0,05$.

The use of hyper-oxygenation in some cases brought out a slower alcoholic fermentation and as a consequence a slight increase of wine volatile acidity resulted. This fact was related to an excessive delay between hyperoxygenation itself and the racking which normally follows the treatment. If the time between these two steps was too long, a rapid increase in the population of wild yeasts (non Saccharomyces spp.) was observed (table 11), and the development of these microorganisms led unavoidably to a rapid consumption of assimilable nitrogen (in table 11, almost the 80 % of the must original value).

Table 11: Development of Saccharomyces and non Saccharomyces populations before selected yeasts inoculation in a hyperoxygenated must; the levels of free amino acids are also reported.

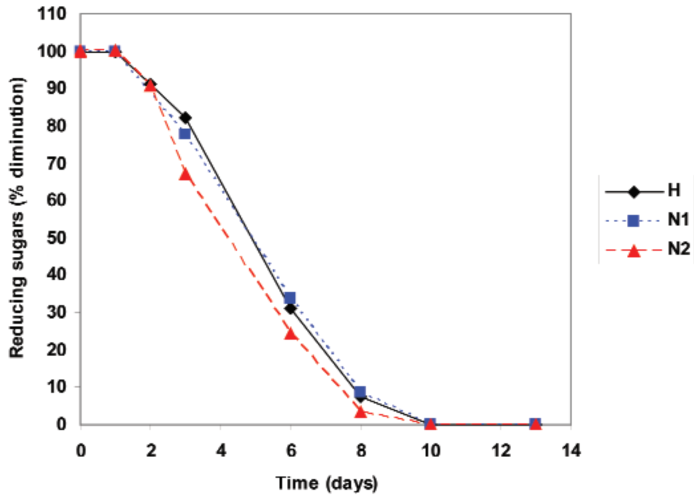
Sample	Date	Free amino acids (mg/L)	Saccharomyces (CFU/mL)	Non Saccharomyces (CFU/mL)
Must	03-set	94	$1,3 \times 10^6$	$3,7 \times 10^5$
After Hyperox	03-set	87	$1,1 \times 10^6$	$3,6 \times 10^5$
After Racking	04-set	21	< 10	$1,0 \times 10^6$
After SYI	04-set	20	$3,0 \times 10^5$	$1,9 \times 10^6$

SYI: Selected Yeasts Inoculation

This fact means that when the selected yeasts are added after the racking, they will find very little assimilable nitrogen in the must, and for this reason the behavior of alcoholic fermentation will be conditioned by this lack of nitrogen sources, with a higher risk of a stuck or sluggish fermentation.
To avoid these problems, the preparation of an active *pied de cuvée* (selected yeasts starter culture) is fundamental. This process must be carried out as early as possible even using some unsedimented must issuing from the pressing plant, instead of the racked must (as usually done). These precautions, together with a nitrogen supplementation (particularly ammonium salts, as di-ammonium phosphate) during *pied de cuvée* addition, are shown to be useful strategies to increase the fermentation rate and to avoid fermentation sluggishness (Figure 85).
Finally, to reduce the lag between hyper-oxygenation and racking, a treatment with pectolytic enzymes could be recommended.

Conclusions

In conclusion, the hyper-oxygenation of the must can be helpful to avoid the use of SO_2 in the pre-fermentation steps of wine-making process. Nevertheless the opportunity to use this technique should be carefully evaluated for the musts of certain grape varieties whose typical aroma is particularly sensitive to oxidation (e.g. Sauvignon blanc).
When using this practice, special precautions should be taken in the addition of selected yeasts and their management (e.g. nutrients supply, yeast acclimatization) as well as ensuring a rapid must clarification after the addition of oxygen These precautions are critical for the reduction of *non-Saccharomyces* growth, before selected yeasts addition, and in avoiding sluggish fermentations.



H: yeast walls (400 mg/L) and thiamine (0,6 mg/L) during yeast rehydration[16]
N1: yeast walls and thiamine during yeast rehydration (1/2) and after PdC inoculation (1/2); DAP (300 mg/L) at middle AF (6th day)[17]
N2: yeast walls and thiamine during yeast rehydration (1/2) and after PdC inoculation (1/2); DAP after PdC inoculation (1/2) and at middle AF (1/2 – 6th day)[18]

Fig.85: Behavior of alcoholic fermentation in hyperoxygenated musts treated in different ways with regards nitrogen supplementation and pied de cuvée preparation:
No fermentation problems were highlighted in musts from harvest 2008, but trial N2 showed a slightly higher fermentation rate.

[16] Yeast walls (400 mg/L) and thiamine (0,6 mg/L) during *PdC* preparation
[17] Yeast walls (400 mg/L) and thiamine (0,6 mg/L), a half on *PdC* at preparation, and a half on the whole lot at *PdC* addition
[18] Yeast walls (400 mg/L) and thiamine (0,6 mg/L), a half on *PdC* at preparation, and a half on the whole lot at *PdC* addition; *PdC* addition: di-ammonium phosphate (300 mg/L) also added on the must

5.3. Alternative additives to SO_2 (Zironi, R.; Comuzzo, P.; Tat, L.; Scobioala, S.)

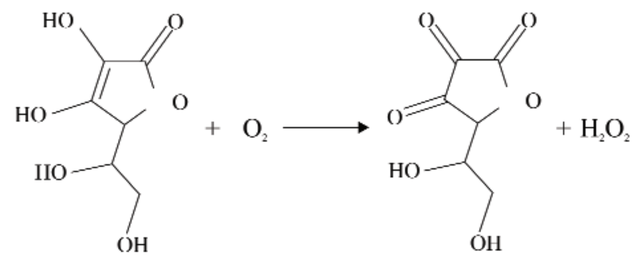
General Principles

The increase in knowledge which has characterized oenological sciences in the last decades has indicated that there are different additives and practices which can partially replace sulphites in some basic functions.
When considering alternatives to sulfur dioxide, it must be emphasised that, even today, the total elimination of SO_2 is still not possible without a risk of compromising wine quality. Nevertheless, the overall reduction in quality by using some alternative technologies or additives is definitely feasible and the concept of sulphite reduction is becoming particularly important not only for organic wine-making but also in the production of conventional wines.

Ascorbic acid and reduction of sulphur dioxide

Principles

Ascorbic acid (AA, vitamin C) is one of most important alternative additive to SO_2.
According to Rigaud and co-workers[19] it reduces the risk of enzymatic oxidations in the must (pre-servation of caftaric acid) and, for its antioxidant activity, it is able to scavenge oxygen and reactive oxygen molecules (e.g. some free radicals) even in wine and reducing the oxidation of phenolic com-pounds (Figure 86).



ascorbic acid   dehydroascorbic acid

Fig. 86: Oxidation of ascorbic acid to dehydroascorbic

With regards this last point of view, AA acts faster than sulfur dioxide thus being more useful in redu-cing the problems connected with a sharp oxygenation (e.g. during racking or bottling) For this reason it is often used on the wines just before bottling. Despite this faster reactivity, however, its action is less durable with respect to that of SO_2 so these two additives are mostly used in combination.
Another important reason why wine-makers mix SO_2 and AA, is the evidence reported in figure 86: the oxidation of ascorbic acid produces hydrogen peroxide (H_2O_2), which is itself a powerful oxidant; sul-phites are able to scavenge H_2O_2, giving an underlying contribution to the antioxidant properties of the mix itself.

[19] Rigaud et al., 1990. Mécanismes d'oxydation des polyphenols dans les moûts blancs. R.F.Œ, 124: 27-31.

This last consideration is an important concept. If the wine-maker wants to replace SO_2 by using ascorbic acid, it is not possible to limit the use of this additive and a suitable alternative must be used for this fundamental scavenging activity of sulphites against hydrogen peroxide.

Description of the trials

The approach of ORWINE programme to this problem consisted of using grape tannin as an "alternative scavenger". It is well known that tannins are able to reduce the activity of free radicals (such as superoxide or hydro peroxide)[20], and for this reason they can be used in combination with AA to replace one of the traditional uses of sulphites viz. their addition during crushing (in white wine-making).

The results obtained during the 2006 harvest showed that a mix of ascorbic acid and grape tannin was able to reduce the oxidation of phenolic compounds (in figure 83 the behavior was similar to that of the SO_2 added must). Thus this sort of hyper-reductive technology demonstrated its ability to stabilize the must on the basis of a principle which is opposite to that of hyper-oxygenation. ie. the protection of the must itself from oxidations (table 12).

Moreover, hyper-reduction was also able to preserve the typical smell of certain varietal wines such as Sauvignon blanc (figure 84). During the sensory evaluation of such wines, no significant differences were noted with regards the attributes relating to these varietal notes between the samples produced using sulphites and those obtained by adding the mix AA + tannins.

One of the problems related to the hyper-reduction technique is the higher susceptibility of the resulting wines to oxidation during storage. The POM Test, an index related to the susceptibility of the wine to oxidation was higher in the wines obtained by the mix AA + tannin as opposed to those obtained by hyperoxygenation or by the classic SO_2 addition during crushing.

[20] Vivas, 1997. Composition et propriétés des préparation commerciales de tanins à usage Œnologique. R.F.Œ, 84: 15-21.

Table 12: Summary of the mai aspects related to some alternative practices in the use of sulphur dioxide

	HYPEROXYGENATION	HYPER-REDUCTION
Basic principle	Total oxidation of the unstable substances	Total protection of oxidisable substances
Specific treatment	Massive oxygen addition on must after pressing	Ascorbic acid + tannins addition on must during crushing
Relationship with sulphites	No SO_2: alternative practice	No SO_2: alternative additives
Effects on O_2 sensitive phenolic compounds	Elimination by oxidation and precipitation	Preservation
Effects on O_2 sensitive volatile compounds	Partial loss	Preservation
Effects on the stability of the final wines	Higher stability to oxidation compared to that observed by the traditional use of SO_2 before alcoholic fermentation	Lower stability to oxidation compared to that observed by the traditional use of SO_2 before alcoholic fermentation
Effects on wine sensory characters	For certain varieties: partial loss of specific varietal notes	Preservation of specific varietal notes

For this reason, when hyper-reductive techniques are used, special care should be taken in the management of any operation which could affect the uptake of oxygen in the wine (e.g. racking, bottling, filtration, transfers of wine from one tank to another). Additional precautions, such as the saturation of tubing, tanks and connections with carbon dioxide, nitrogen or other inert gases can be useful to minimise reactions with these oxygen sensitive products and to avoid any further oxidation without the necessity of a massive use of sulphites.

In conclusion it is possible to question the use of grape tannin as alternative scavenger to replace sulphites as it can affect wine sensory characters causing wood-like notes in the sensory profile of the treated wines. However in the trials carried out in this ORWINE project and for the amounts used no evidence was found concerning any sensory effect of the added tannin.

Table. 13: Analytical parameters of some experimental wines obtained during harvest 2006; two varieties and three trials are compared

PINOT GRIS (FINAL WINE – JAN 07)						
Sample code	Date	DO 420	DO 320	DO 280	POM Test [21]	Catechins (mg/L)
VC	23-gen	0,1273	7,2	8,7	**3**	20
VA	23-gen	0,1545	7,1	8,4	**20**	14
VH	23-gen	0,1314	5,8	7,2	**0**	8

SAUVIGNON (FINAL WINE – JAN 07)						
Sample code	Date	DO 420	DO 320	DO 280	POM Test [9]	Catechins (mg/L)
VC	23-gen	0,0951	5,3	8,9	**36**	15
VA	23-gen	0,1078	6,4	10,4	**52**	13
VH	23-gen	0,1204	5,2	7,9	**0**	9

VC, conventional vinification; **VA**, use of AA + grape tannins; **VH**, hyperoxygenation

Conclusions

The use of ascorbic acid as an alternative additive to sulfur dioxide requires the replacement of SO_2 with other free radical scavengers. The use of a mix of AA and grape tannins gave good results in white musts, preserving oxygen-sensitive phenolic compounds as well as the typical notes of certain varietal wines whose aroma is susceptible to oxidation.

However when hyper-reduction technology is used special care is necessary to avoid massive oxygen application to the final wine which become more sensitive to oxidation with their higher content of phenolic compounds.

[21] The higher the POM Test value, the higher the susceptibility to oxidation of the wine

5.4. Natural production of SULPHITE (SO_2) by yeast during alcoholic fermentation (Werner, M.; Rauhut, D.)

During alcoholic fermentation yeasts naturally produce sulphur dioxide (SO_2) as a metabolic intermediate of the sulphate reduction pathway (Romano and Suzzi (1993), Ribéreau-Gayon et al., (2006)). Yeast strains can be categorized into **low SO_2 producers** (e.g. *Saccharomyces cerevisiae* var. *ellipsoideus*) and **high SO_2 producers** (e.g. *Saccharomyces bayanus* Sacardo). Certain yeast strains can produce up to 300 mg/L of sulphite during fermentation. Dott and Trüper (1976) described that the sulphite reductase of the sulphite-producing yeast strains might be altered. As a consequence sulphite (SO_2) will be accumulated in the cell and finally be released into the must. Former assumptions about mutations being the cause of the sulphite production could not be confirmed. Today producers of commercial dried yeast consider this important property of the yeast during the selection process. It is only when wine-makers wish to induce a spontaneous fermentation can the properties of the fermenting yeast strains not be guaranteed. The majority of today's commercial yeast strains are considered to be low SO_2 producers, showing a production up to 20 mg/L of total SO_2. Only few yeast strains appear to have a higher production (up to 80 mg/L SO_2).

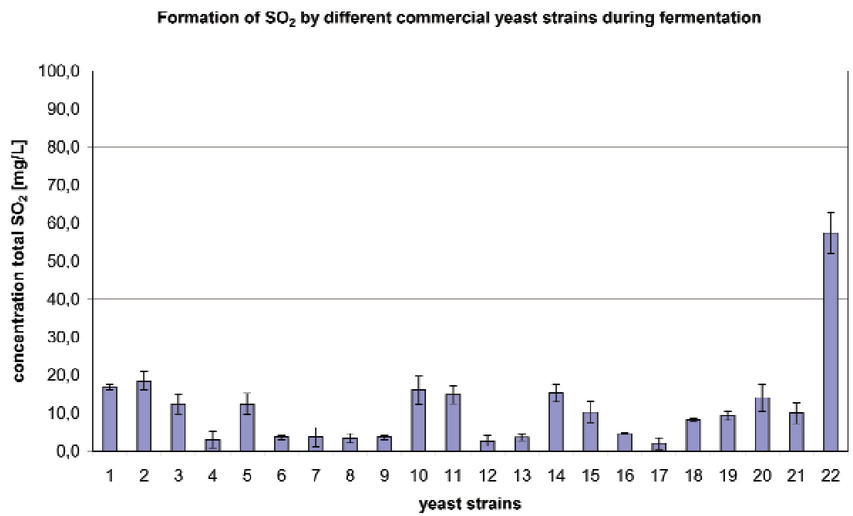


Fig. 87: Production of SO_2 by 22 commercial yeast strains during fermentation. Mean value of the triplicate. Bars show the standard deviation.

Figure 87 shows the SO_2 production of 22 commercial yeast strains used in Europe delivered from the main producers of yeast. The fermentations were performed with 2007 Riesling must, which was pasteurised in order to eliminate any undesired micro-organisms. The fermentation temperature was 18°C, the inoculation dosage was 30 g/hl pure dried yeast. Rehydration was done by water (35°C) for 25 minutes. The results show predominantly two groups of yeast strains. One group produces under 10 mg/L total SO_2, the other group produces between 10 and 20 mg/L total SO_2. Only one yeast strain reaches a concentration of 57 mg/L of total SO_2.

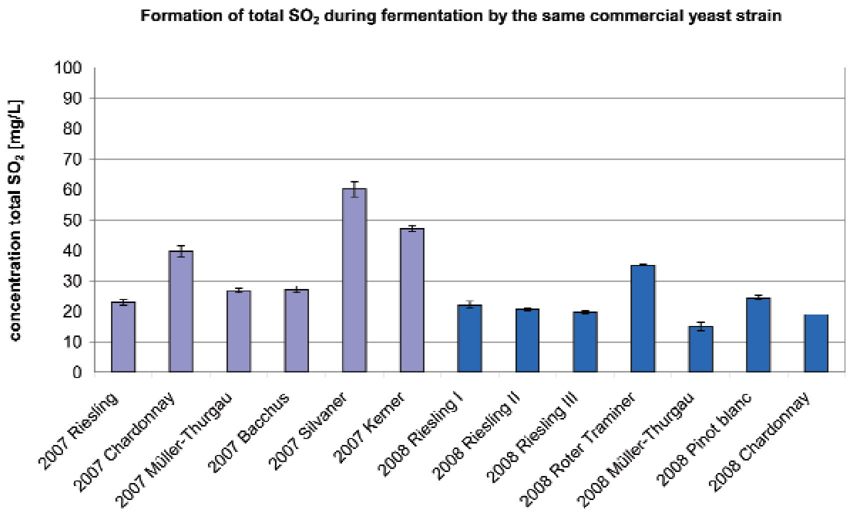


Fig. 88: Production of SO_2 by one commercial yeast strain during fermentation in must from different grape varieties. Mean value of the triplicate. Bars show the standard deviation.

Figure 88 shows the concentration of SO_2 after the alcoholic fermentation by the same commercial yeast strain in must from different grape varieties (vintage 2007 and 2008). Fermentation conditions were the same as for the comparison of yeast strains. All the different grape juices were pasteurised, in order to eliminate any undesired micro-organisms. The results show that the formation of SO_2 during fermentation depends also on the yeast variety and the composition of the grape juice. The grape juices in figure 88 were all fermented with the same commercial yeast strain, but the concentration of total SO_2 varies from 15 to 60 mg/L after the alcoholic fermentation. This indicates that even a yeast strain that is considered as a low SO_2 producer can produce higher concentrations in certain grape juices in certain years.

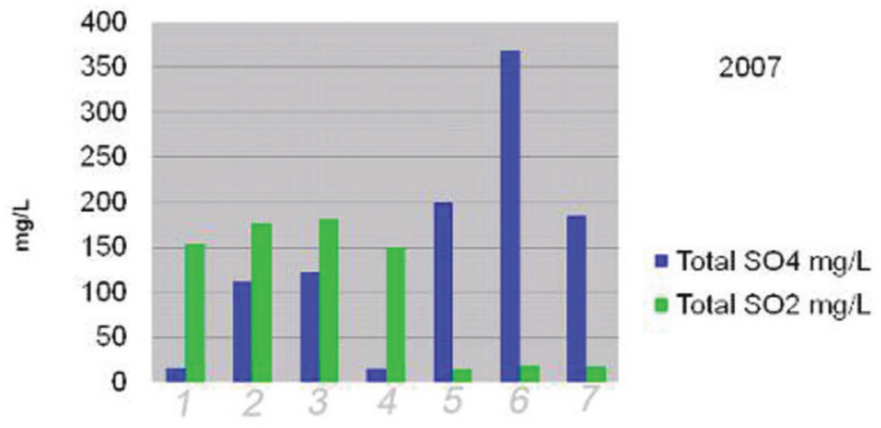


Fig. 89: Production of SO_2 by two different commercial yeast strains during alcoholic fermentation in must with the addition of ammonium sulphate and ammonium phosphate.
Variant 1-4: yeast strain 1; variant 5-7: yeast strain 2; variant 1 and 5: control; variant 2, 3 and 6: addition of ammonium sulphate, Variant 4 and 7: addition of ammonium phosphate. Source: partner ITV.

Figure 89, shows that the concentration of sulphate plays an important role in the SO_2 production during the alcoholic fermentation. Sulphate is present in the natural must or it can be introduced by the addition of ammonium sulphate, a nutrient. Alternatively ammonium can be added as ammonium phosphate. As the results in figure 89 show, not every yeast strain has the same ability to produce SO_2 on the basis of SO_4. Yeast strain 2 does not use sulphate, neither the natural nor the added sulphate in a relevant amount. This explains why this yeast strain can be considered as a low SO_2 producer. The yeast strain 1 shows a high ability to produce SO_2 on the basis of SO_4, even if it is only naturally present in the must. This yeast strain can be considered as a high producer of SO_2. The sulphur dioxide produced by the yeast will be bound to SO_2 binding compounds. Thus it will be included in the estimate of the amount of total SO_2 in the wine, which is limited by regulations, but it will not be available as active free SO_2. The final requirement for SO_2 by the specific wine is determined by many wine compounds, such as acetaldehyde, 2-keto-glutarate and pyruvate, but also the amount of sugar. Only by adding an adequate amount of sulphur dioxide will the wine be finally protected by a certain amount of active free SO_2.

References:

Dott, W. and Trüper, H. G. (1976): Sulphite Formation by Wine Yeasts, III. Properties of Sulphite Re-ductase, Archives of Microbiology 108, Springer Verlag, p. 99-104

Romano, P. and Suzzi, G. (1993): Sulphur dioxide and wine micro organisms. In: Wine Microbiology and Biotechnology. Edited by Fleet, G., Harwood Academic Publishers GmbH, Chur, Switzerland, p. 373-393

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2006) Handbook of Enology, Volume 2, John Wiley and Sons, England, p. 264

http://www.vignevin.com/outils-en-ligne/fiches-levures/levures-a-production-moyenne-a-elevee-de-so2.html

5.5. Influence of nutrients on the production of SO2–binding compounds by yeasts (Werner, M.; Rauhut, D.)

During alcoholic fermentation yeasts are able to produce certain by-products which bind to sulphur dioxide (SO_2). Acetaldehyde is probably the best known substance because its presence in a free form significantly influences the sensory character of a wine. If it is present in the free form, it causes an "oxidative note" which is often considered as an off-flavour. Only for specific wine types is it appreciated.
In addition to acetaldehyde there are many other carbonyl compounds which can act as binding partners for SO_2 in the wine. The higher the total concentration of binding compounds the lower the amount of active free SO_2 in the final wine at a given addition of sulphur dioxide (see also chapter about SO_2 management).

Table 14: Simplified general overview about relevant SO_2-binding carbonyl compounds present in wine and specialty wine. Under practical conditions their concentration varies from very low to high depending on the metabolic activity of yeast or other micro-organisms.

Carbonyl CompoundI	mpact on SO_2 binding	Origin
Acetaldehyde	High	Yeast metabolism
Pyruvate	High	Yeast metabolism
2-Ketoglutarate	High	Yeast metabolism
Reducing Sugars (Glucose, Fructose, …)	High, depending on concentration	Grape origin or addition
Gluconic acid	High	Microbial activity on grapes
5-Ketofructose	High	Microbial activity on grapes
Xyloson	High	Microbial activity on grapes
Propanal	Low	Microbial activity
Butanal	Low	Microbial activity
Glycerolaldehyde	Low	Microbial activity
Isobutylaldehyde	Low	Microbial activity
Diacetyl	Low	Microbial activity

Research trials have shown that the natural production of the three SO_2-binding compounds acetaldehyde, pyruvate and 2-ketoglutarate depend on the yeast strain and on the composition of the natural must. With regards to the nutritional composition of the must, thiamine plays a key role in the formation of SO_2-binding compounds. Thiamine acts as co-enzyme of pyruvate decarboxylase which lowers the concentration of the last intermediates in the sugar depletion pathway of the yeast. Certain factors like heat treatment of the must or Botrytis activity on the grapes can lower the natural concentration of thiamine in the must. Figure 90 shows the effect of the addition of nutrients (ammonium and thiamine) on the concentration of SO_2-binding compounds in a pasteurised Riesling must after alcoholic fermentation.

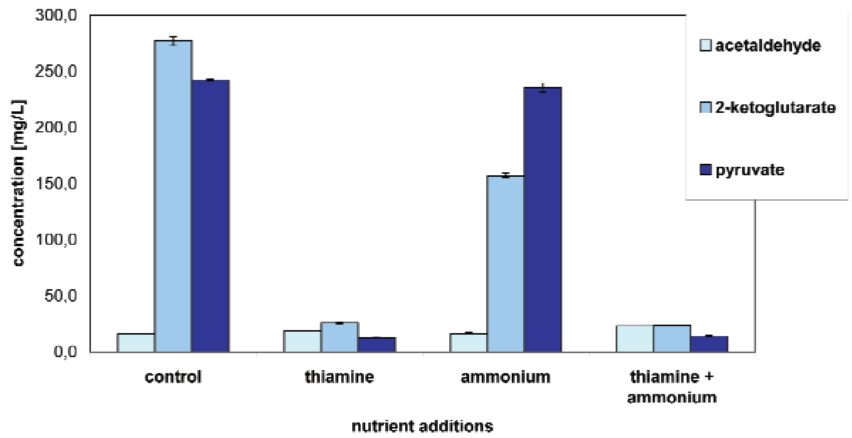


Fig. 90: Effect of the addition of di-ammonium-hydrogenphosphate (0.5 g/L) and thiamine (0.6 mg/L) on the concentration of acetaldehyde, pyruvate and 2-ketoglutarate in the final wine. Fermentation was performed by Saccharomyces cerevisiae in a pasteurised Riesling must. Mean value of the triplicate. Bars show standard deviation. Source: SRIG

The high concentration of the SO_2-binding compounds in the control wine can be explained by the pasteurisation of the juice, which was necessary to eliminate any undesired micro-organisms. The positive effect of ammonium and thiamine on the reduction of the SO_2–binding compounds can be demonstrated very clearly. The concentration of the substances could be reduced very much, even though the SO_2–binding substances could not be eliminated. Additionally the fermentation activity of the yeast could also be increased by both substances. According to the different concentrations of carbonyl compounds in the wine, each wine has a different "need" for SO_2 in order to guarantee consistent quality and stabilisation. Reducing sugars, such as glucose and fructose, which are present in sweet style wines, increase the binding potential significantly. Furthermore the pH-value and the temperature of the wine play an important role regarding the balance of free and bound sulphur dioxide, which is further described in the chapter about SO_2 management.

References:

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. (2006) Handbook of Enology, Volume 1, John Wiley and Sons, England

Wucherpfennig, K. (1985) Die schwefelige Säure im Wein – önologische und toxikologische Aspekte, Deutsches Weinbau Jahrbuch, 213-241

5.6. Wine Making Technologies and Practices (Cottereau, P.)

Improved Management Practices in Wine-making and experimental Testing
Implementation of new technologies

This task considers some physical technologies, which can be useful in reducing the risk of microbial contamination and wine oxidation as well as the use of SO_2. **Flash-pasteurisation** (FP), **Cross-Flow microfiltration** (CF-MF) and **Electro-dialysis bipolar membrane**) were evaluated to find out to what extent these technologies can be implemented in organic wine-making without affecting wine quality and production costs.
Electro-dialysis has been tested for acidification of red wine and flash-pasteurisation and cross-flow microfiltration for microbiological stabilisation against yeasts or bacteria.

5.5.1. Electro-dialysis with bipolar membranes / acidification

Grape acidity development as recorded during the last years shows a regular pH progression with very high levels in all the European countries. Very high pH has led to an increase in the amounts of SO_2 used.
INRA (in relation with EURODIA) have developed the use of electro-dialysis bipolar membranes. This technique allows pH regulation (acidification). This treatment can be automated and produces a required pH final value. Thus, controlled acidification allows the production of more favourable conditions for sulphur dioxide use (active SO_2).

Principles of the Electro dialysis bipolar membrane:

a) Bipolar membrane electro-dialysis efficiently converts aqueous salt solutions into acids and bases without chemical addition. It is an electro-dialysis process as ion exchange membranes are used to separate ionic species in solution with the force of an electrical field, but differs by the single water splitting capability of the bipolar membrane. The process also offers the capability to directly adjust the acidity of process streams without adding chemicals.
b) The wine can be acidified (Fig. 91) with the association of bipolar membrane and cationic exchange membrane. Hydrogen ions coming from the splitting of water which replace the potassium ions going out through the cationic membrane.

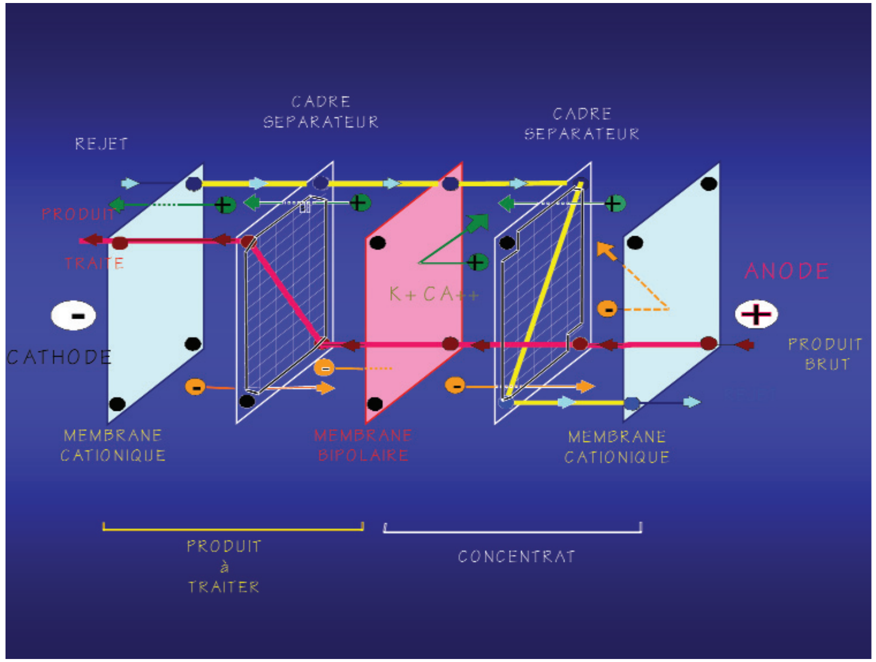


Fig. 91: Principe of electro-dialysis bipolar membrane

Experimental procedure:
The acidification by this bipolar process was performed on a red wine (Syrah) with a very high pH (about 4.15). Reference acidifications were performed with two levels of tartaric acid (1,5 and 3 g/L) added for comparison. After addition of tartaric acid the wines were cooled in a cold chamber for 15 days (0°C) and were racked to eliminate the tartaric precipitate.
The wine was treated by the bipolar process with a large pH range (from 3.25 to 4.15) and with the addition of SO_2 at bottling with two levels (no addition, and 1 g/hl). The activity of SO_2 /acidity was tested by growth of contaminant yeast (inoculation by *Brettanomyces*).

Results:
The bipolar process can accurately produce the required pH. As the theory indicates the variation of pH is linked to the substitution of K+ by H+. Tartaric acid concentrations are not different for all modalities. The acidity increased with the decrease of pH. After bottling, the differences between addition or no addition of SO_2 are very small (about 2 mg/L with addition of 1 g/hl). SO_2 added is therefore quickly bound in these wines.
The acidification with tartaric acid allowed small variations of pH; -0.15 for 1.5 g/L addition and –0.35 for 3g/L additions. In fact acidification allowed precipitation between tartaric acid and K+. The pH decrease is a consequence of the K+ concentration decrease. Tartaric acid concentrations were increased slightly. During a 35 day period the inoculated Brettanomyces population was followed (Figure 92).

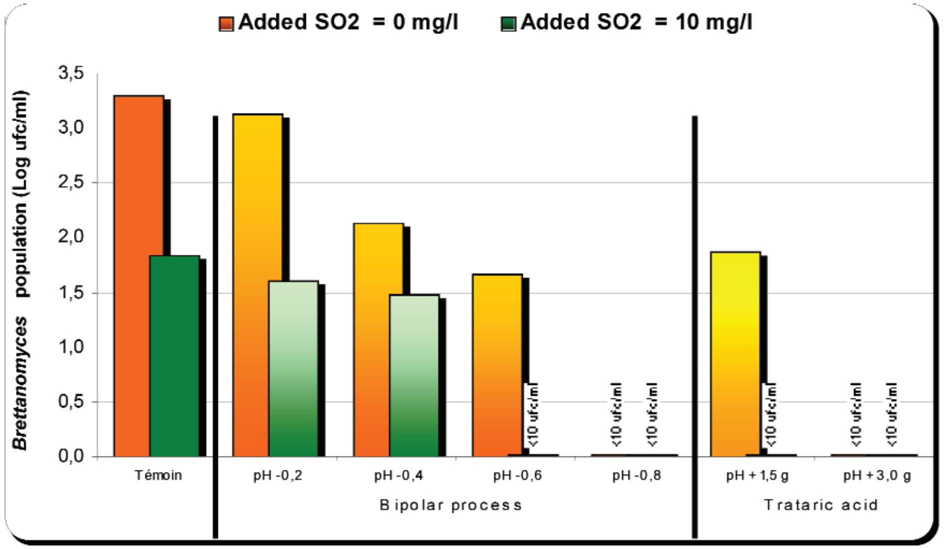


Fig. 92: Brettanomyces population in each treatment 3 days after contamination (average of two repetitions)

The decrease of Brettanomyces population is linked to the decrease of pH.
Acidification with tartaric acid is more efficient in the inhibition of *Brettanomyces* growth than bipolar process with the same pH variation with or without SO_2.
A very low SO_2 addition was much more efficient as the level of acidification was high.

The active SO_2 was directly linked to pH levels but this effect was identical in conventional or organic wines.
The present technique is not yet permitted in general wine regulations and can not be considered for at least 2 or 3 years.

5.5.2. Flash-pasteurisation (FP), Cross-Flow microfiltration (CFM) for enhancement of wines with residual sugar.

These technologies were tested for stopping alcoholic fermentation during the development of sweet wines. The comparison was carried out with the addition of SO_2 as "SO_2 mutage" and DMDC - Dimethyldicarbonate, recently permitted by EU bjut not accepted for organic wine-making.
The wines were developed to make white or rosé wines and the fermentations were stopped with a low alcoholic degree to reach a difficult situation of stability.

Experimental procedure:

- Grapes of mourvèdre (domaine INRA of Gruissan – 11430) were crushed and pressed to obtain a juice (14 % vol of potential alcohol. The fermentation was stopped when the wine reached about 12% vol). All treatments received 8 g/hl of SO_2, except for SO_2 alone treatment (5g/hl for the "mutage" + 8 g/hl SO_2 like the other treatments).

- An experimental design (in Erlenmeyer of 200 ml) was followed for each process (except DMDC) with the contamination by yeasts (S. Cerevisea K1) (3 levels: 0, 10Ç, 104 cells by milliliter) and 4 levels of SO2 (0, 4, 8,12 g/hl) and 2 repetitions of each treatment (trials car-ried out in 200 ml erlenmeyers until the end of fermentation – 2 x 3 x 3 x 4 = 72 tanks of 200ml – registering of weights of the erlenmeyers).

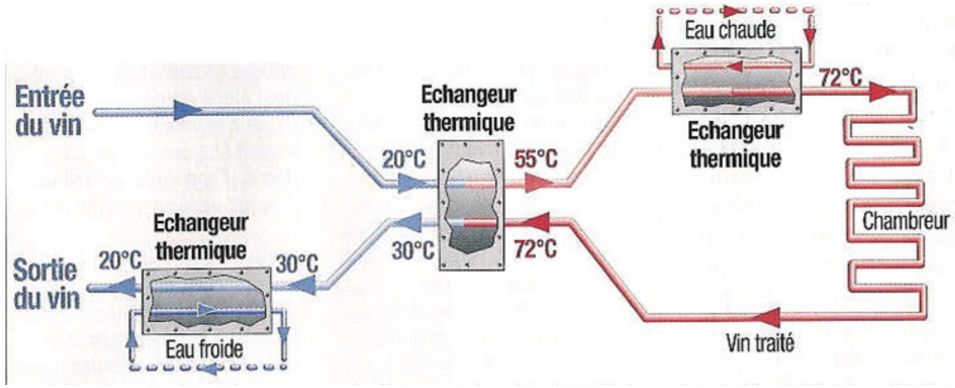


Fig. 93: Technical principle of flash-pasteurisation.

Results:
The analyses of the wines were almost identical. The combination of SO_2 is a little higher for the reference "SO_2". The benefit of the SO_2 control is only 20 mg/L. There were no significant differences in colour.

There is no significant difference (test 5%) between aromatic profiles, except for the SO_2 control where panelists found bad smells. Consequently the quality of this treatment is lower than for the other wines.
The acidity of SO_2 control is lower than the others (no differences in the analysis). The body intensity of the DMDC treatment seems to be lower than for other wines (near 5%). The other characteristics are not significant.
The level of overall pleasantness is significantly higher for CFM wine in comparison with the SO_2 treatment (bad smells) and the two other wines were in between.

After 5 months in the Erlenmeyer test there are only 2 treatments with SO_2 stabilisation. Where fermentation is still proceeding whatever the level of the yeast population 8 g/hl of SO_2 are necessary to stop the refermentation.

Fig.94: *Technical equipment for flash-pasteurisation.*

In the other cases, the re-fermentation is aleatory, and there is no link with yeasting.
The effect of mutage with CFM and FP is very strong. It's possible to reduce SO_2 without fermentation risks with these technologies.

These technologies can produce good microbiological stabilisation but the combination of the SO_2 is the same as the control. If there is a need to obtain the same concentration of free SO_2 in these different final wines, the reduction in the amount of SO_2 to be added is very low (about 20 mg/L in these experiments).

DMDC seems to represent a good alternative for "mutage" in replacement of SO_2. But thechemical origin of this product seems to not conform in an organic way.
The sensory test has shown that CFM wine is the best wine in this trial. The different tested technologies do not change sensory profiles of wines.

5.5.3. Flash-pasteurisation (FP), Cross-Flow microfiltration (CFM) for bacteria stabilisation

These technologies were tested for stopping lactic bacteria fermentation during the making of white wines. The difference between SO_2 and lysozyme additions was assessed.
These technologies were tested for red wines after lactic bacteria fermentation but before wine ageing and storage. Again the difference between SO_2 and lysozyme additions was assessed.

White wine experimental procedure:
Organic white wine was selected in a cellar (organic winery) just after the end of alcoholic fermentation. Four treatments were studied (SO_2, Lysozyme, Flash-Pasteurisation, Cross-Flow Microfiltration) with 2 levels of SO_2 concentration at bottling.

An experimental laboratory procedure was followed for each process, with controlled contamination of lactic bacteria at different inoculum levels, and with different levels of free SO_2 (0, 10, 30 mg/L).

Results:
The wine analyses were very similar with the exception of acidity. The SO_2 and FP treatments resulted in a lower concentration of tartaric acid. The precipitation of both tartaric acid and potassium ions was more efficient for these treatments.
The combination of SO_2 is a little higher for the control "SO_2" but only for the "high SO_2" treatments.
The net benefit of SO_2 usage is only about 10 mg/L.
In the sensorial testing, there is only one 5% significant difference. The treatments: FD low SO_2 and high SO_2, CFM low SO_2 and high SO_2. High level are less 'vegetal' than the other wines. The other differences are not significant.
The lysozyme treatments appear to exhibit a more aromatic intensity but there is no preference between the different wines.
There appears to be a difference in between the wines, but there is no link with the applied treatments (similarly for bitterness). For overall quality, the CFM treatments gave the lowest scores.

In the results of laboratory tests (table 15), there were no differences between the treatments with bacterial inoculation, except for the lysozyme samples where the inoculation was not adequate to induce lactic bacteria fermentation. With bacterial inoculation, it seems that FP and the CFM treatments were a little more unstable from a microbiological point of view. However, due to the length of the experiments, these results might have resulted from accidental contamination.

Table 15: Results of bacterial inoculation – White wines – IFV ORWINE 2007-2008

FML duration (days)	CFM			FP			SO2			Lysozyme		
SO2 modalities	0	10	30	0	10	30	0	10	30	0	10	30
Bacteria 0	> 90	N	N	90	N	N	N	N	N	N	N	N
Bacteria 10^2 cfu/ml	90	N	N	45	> 90	N	50	N	N	N	N	N
Bacteria 10^5 cfu/ml	40	70	N	30	60	N	40	80	N	N	N	N

Red wine experimental procedure:

Organic red wine was selected from an organic winery just after the end of malolactic fermentation. Four treatments were studied (SO_2, Lysozyme, Flash-Pasteurisation, Cross-Flow Microfiltration) with 2 levels of SO_2 concentration at bottling (0 and 2 g/hl).

Results:
Wine analyses were very similar for all treatments tested. K+ and tartaric acid concentrations are lower for SO_2 and Lysozyme treatments. The final concentrations of SO_2 in the different treatments are lower than expected. The combination with SO_2 is higher than expected for all treatments. There are no significant differences on colour or 'vegetal' assessments between the treatments. It seems that the "vegetal" parameter is higher for certain treatments but without clear linkage with the technologies used.
It is the same for the gustative parameters, except for the tendency of global quality. The best scores are obtained with CFM modalities.

Conclusions:
The technologies used in these trials are able to stabilize the tested wines. In all cases, there is a reduction of the quantity of SO_2 needed (it is possible to completely avoid SO_2 use).
For total control of bacteria the use of lysozyme is the only alternative to SO_2. In order to avoid malolactic fermentations even after inoculation or contamination with bacteria.
If the objective is to reach a certain concentration on free SO_2 after bottling, it should be noted that all tested alternatives give wines with the same concentration of total SO_2. SO_2 combinations are

near the same in all treatments. With such technological alternatives it is therefore only possible to decrease the concentration of total SO_2 about 10 to 20 mg/L.

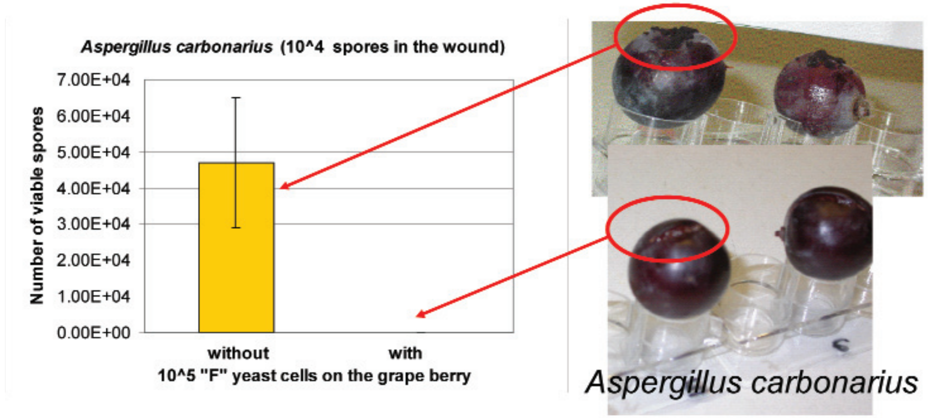
Wines without free SO_2 often exhibit oxidized olfactory profiles. The tested alternatives (chemical or physical) cannot replace the specific action of SO_2 (protection against oxygen). A strict hygienic control and an efficient bottling process are necessary to achieve a reduction of free SO_2 concentration. A new analytical approach using sensitive crystallisation can be carried out which will give a better assessment of the tested technologies.
This approach involves the reading of crystallizations in Petri boxes and is not easily adapted to standard scientific testing. The final interpretation is more literary than scientific! In these trial experiments, the expertise of Margaret he Chapelle who has worked on such analyses for 25 years should be used. Thus in conclusion these technologies did not make a lot of differences on the final tested wines. The only differences are in relation with Flash-Pasteurisation treatments on white wines. The pictures of crystallization of these treatments are very different from the others. The explanation concerns the "life of wine" (swift time of aroma).

5.6. Evaluation of yeast spraying as a tool for reducing fungus diseases on grapevines (Salmon, J.M.)

Introduction

The main objective of this task was the potential reduction of microbial diseases on damaged grape berries by spraying S. cerevisiae yeasts on the grapes by creating competition amongst micro-organisms on their surfaces. Surface competitions were successfully performed to control post-harvest diseases (moulds) of fruits or vegetables by pre-harvest applications of yeasts. Natural saprophytic yeasts were generally used for this purpose. Such natural yeasts (mainly Cryptococcus and Rhodotorula spp.) are known to colonize plant surfaces or wounds for long periods under dry conditions utilizing available nutrients for rapid multiplication and to be minimally affected by pesticides. Limitation of the use of such yeasts relies on the fact that their mass production on an industrial scale is very difficult, or even impossible. However it is not known whether anyone has tried to test standard industrial S. cerevisiae strains, which are easily available in large amounts, for their ability to control fungus development. The choice of oenological S. cerevisiae strains was dictated by the fact that most of these available strains were originally isolated from grapes or wines, and there-fore seemed more adapted to the specific substrate as represented by damaged grape beries.

Fig. 95: Effect of simultaneous yeast inoculation on Aspergillus carbonarius infected damaged berries.



Effect of simultaneous yeast inoculation on A. *carbonarius* infected damaged berries

Results and Conclusion

The first trial was on the effect of *S. cerevisiae* on the development of undesirable bacteria or fungi at the surface of deliberately damaged grape berries.
The effectiveness of yeast spraying by different commercial *Saccharomyces cerevisiae* strains was evaluated on two different species of fungus diseases: *Botrytis cinerea* (invasive disease fungus) and *Aspergillus carbonarius* (undesirable fungus responsible for ochratoxin A (OTA) production), as well as on an invasive bacterial species (*Gluconobacter oxydans*).

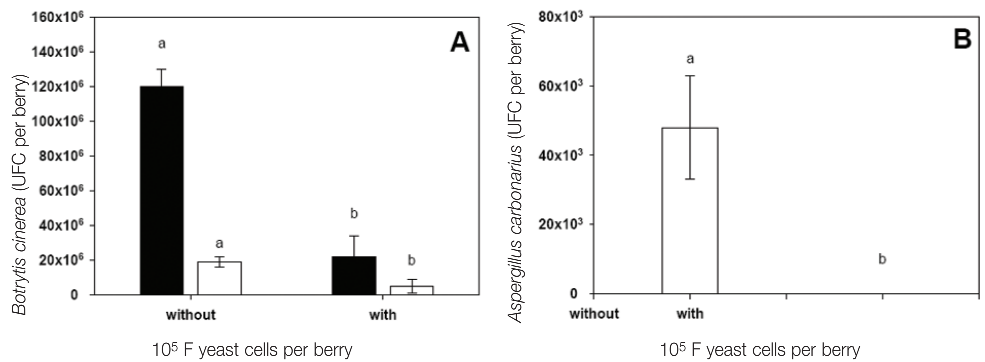


Fig. 96: Effect of the inoculation of 105 S. cerevisiae F cells on the surface of wounded grape berries previously and then inoculated with (A) 106 B. cinerea M04/51 (black boxes) and M04/63 (white boxes) spores or (B) 104 A. carbonarius spores. Assessment of Fungi was carried out after 48 h incubation at 28°C (mean and standard deviation of two replicates of three grape berries for each situation). The same letters indicate homogeneous groups at the 95% confidence level, as tested by Tukey statistical test.

A general inhibition effect was observed in vitro by a set of 17 industrial *S. cerevisiae* strains against *B. cinerea* and *A. carbonarius mycelia* growth, but not against bacterial growth (*Gluconobacter oxydans*). However, only few of them are really very efficient. Thus only the most promising S. cerevisiae strain, named F was conserved.

In a second set of experiments, it was demonstrated that the spreading of *S. cerevisiae* F strain at the surface of previously damaged grape berries contaminated with different microbial species was very efficient for reducing fungus mycelium growth after 48 h of incubation (Figure 96). This was not the case for bacterial *G. oxydans* contamination, where no effect is observed. From this first part of the work, it could be roughly concluded that *S. cerevisiae* F spraying by its mass impact could lower grape infection by fungi.

In a third set of experiments it was demonstrated that yeast spraying should be done about 2-5 days after initial infection by the fungi in order to get an optimal antagonistic effect. After this period, the potential of fungi to initiate disease remains, indicating that a competition for nutrients has taken place between protagonists. The effect of yeast spraying on A. *carbonarius* development on the grape berries was particularly significant. From all these experiments it is thought that such yeast spraying before grape harvest could represent for the viticulturist a biological alternative for limiting the occurrence of *A. carbonarius* in the vineyard.

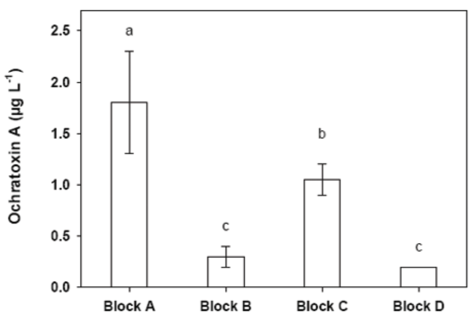


Fig. 97: Ochratoxin A levels (Ìg L-1) in the finished wines (mean and standard errors of duplicates of 2007 vintage). Each block contains 2 rows of 38 vine stocks of Mourvèdre variety. Two blocks (A and C) were con-taminated by A. carbonarius spores (103 spores per bunch), one month before harvest. Two blocks (B and D) were sprayed with commercial S. cerevisiae "F" cells (107 cells per bunch), one week before harvest. The 304 vine stocks of the 4 separate blocks were hand-picked, separate fermentations (2 x 1 HL) were performed on the grapes harvested in each block: identical starter yeast inoculation, identical alcoholic fermentation conditions, and wine ageing. The same letters indicate homogeneous groups at the 95% confidence level, as tested by Tukey statistical test.

Fig. 98: Additional infected berry by Aspergillus carbonarius; natural infected berries by Botrytis cinerea, Penicillium expansum, Trichothecium roseum and Acetic-acid bacteria's.

In subsequent field scale experiments performed during 2007 and 2008 vintages, it is shown that yeast spraying with the selected industrial *S. cerevisiae* F strain on an artificially *A. carbonarius* infected vineyard was able to reduce the spread of *A. carbonarius* inside the grape berries, even if the external black mycelia form of *A. carbonarius* is not observed at the grape berry surfaces. From the obtained results, it is possible to deduce that yeast spraying on the surface of intact grape berries partially reduces *A. carbonarius* penetration into undamaged grapes. Moreover, the reduction of the spread of *A. carbonarius* was accompanied by a significant reduction in the final level of ochratoxin A in the corresponding wines (Figure 97). The chemical and sensory properties of the final wines were also not detrimentally affected by yeast spraying.

5.7. Environmental Assessment (Capri, E.; Fragoulis G., Trevisan M.)

An Environmental Impact of Organic Viticulture Indicator (EIOVI) has been developed that can be reliably used in the management of organic vineyards. EIOVI can be used as a decision support system for farmers and other property managers by evaluating the potential ecological impact of their choices, thus optimizing management options. The tool allows the simulation of vineyard management based on six agricultural and ecological modules. EIOVI is a fuzzy expert system, which calculates the relationship between the modules on the basis of a set of 64 decision rules. The assessment tool is organized in 6 modules: a) pest and disease management b) soil management and machinery use c) fertilizer use management d) irrigation management e) soil organic carbon and f) biodiversity of flora and fauna. The modules are activated one by one. Specific functions are then selected, which apply the indicator for assessing the relevant environmental protection end-point.

The objective of an agro-ecological indicator is to render reality intelligible and the objective of an expert system is the simulation of human actions. Thus, the validation of the indicator requires the determination of its value to potential users. Before being reworked and presented to a broader public, EIOVI must be tested in several stages, appropriate to the target audience for whom it was designed: e.g. wine-makers wishing to obtain information about the ecological quality of their overall-management, ecological consultants advising wine-growing estates on their management strategy or environmental agencies evaluating the ecological impact of viticulture at a regional level. The first step of testing involved 20 simulations for six Swiss wine-growing estates. The estates are located throughout Switzerland, and vary in size between 0.12 and 20 ha. As well as typical vine varieties of Vitis vinifera, some of these estates produce modern, fungi-resistant vines, so called hybrids, resulting from the breeding of the European Vitis vinifera with Northern American or Asian varieties. This permits the demonstration of tangible differences in management between the respective blocks.

Results of on-farm testing

EIOVI was presented to the managers of the estates that were also provided with a questionnaire on the application of the indicator. The comments received from the managers of the estates used in the farm testing indicate the strengths of an EIOVI indicator and the improvements that could be incorporated into the system as well. Table 15 summarizes the comments of all the farm owners (managers) that took part in this survey. The estate managers agreed that EIOVI motivates managers to consider the wine-growing estate as a whole by dividing up all aspects of management for consideration. The visualization of results (Fig. 99) is very tangible and clearly shows how management could be improved. The estate managers argued that the information obtained by the use of

EIOVI was generally already known. Although at first glance this may be seen as a weakness of the indicator, however this statement from the farm managers itself reflects the main strength of the indicator. The six wine-growing estates that took part in the validation of the indicator are modern organic farms inspected and certified by various certification agencies and follow rigorous soil analysis and fertility program. This means they spent a lot of money in order to obtain all the information they need regarding the impact of their management within the boundaries of their farm. So, the fact that this indicator confirmed the information they already had implies that using this user friendly and freely available software the organic farmer can obtain valuable information that will help him with the farm management. The presented version of EIOVI can be accurate for single blocks, but doesn't reflect sufficiently the overall management of the wine-growing estate as a whole. If some improvements to the tool are implemented, EIOVI could also be used for management planning.The comments of the 6 organic vineyard owners are that the evidence from the model is realistic and reproduces the agronomic practices allowing the farmer to cover gap in knowledge

Environmental inpact of organic viticulture indicator (EIOVI)

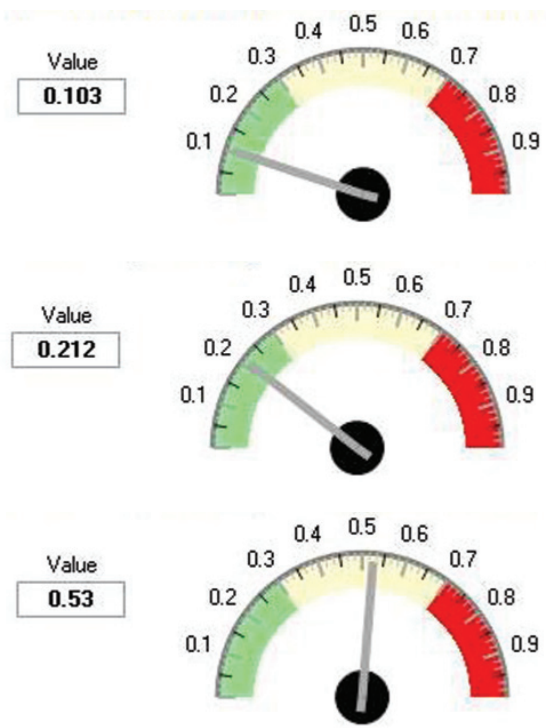


Fig. 99: EIOVI from different simulations. From a block of a fungi-resistant variety (A), a block of a "European" variety with low disease pressure (B), and a block of a "European" variety with high disease pressure (C). The differences are mainly induced by the PDMI (Pest and Disease Management Indicator) SMMUI (Soil Management and Machinery Use Indicator).

Table 16: On farm testing. Summarization of managers' responses to the questionnaire on the application of EIOVI

No	Question	Response
1	Does EIOVI give a realistic overview of the vineyard and its management?	Fairly realistic.
2	Is an improvement in the ecology to be expected, if the winegrower applies EIOVI?	Can detect the area of management that needs improvement.
3	Is the structure of EIOVI fitting to practice?	Yes
4	Did you, through the application of EIOVI, note weaknesses of your management strategies?	Yes
5	Is there missing information, which you consider necessary to evaluate the ecological impact of your management strategies?	Personalize the indicator on basis of the farm estate
6	Does EIOVI give you a base to improve your management strategies in a more ecological/sustainable direction?	EIOVI has indicated the more critical areas in the vineyard management.
7	Are the costs for gathering primary information on soil and irrigation water to high?	No
8	Did EIOVI give you information which you didn't have before?	Generally the information EIOVI gave us was already known.
9	How do you evaluate the evaluation of your management which EIOVI gives you?	Very good
10	If your production area is not continuous, how does EIOVI reflect your overall management?	Partial result. Simulation must be repeated for every different production area.

Discussion

The results of the validation of the indicator were promising. The first test of the tool revealed that the modular organisation of EIOVI already reflects well the complexity of agriculture. If some improvements to the tool are implemented, EIOVI could also be used for management planning and will be a helpful assessment tool for vine growers, consultants, environmental agencies and scientists. The tool could even be extended to other branches of agricultural production by including perennial cultures, vegetable crops, crop rotation or livestock husbandry. Stakeholders such as farmers associations and decision makers have already been contacted and new farm testing will be carried out with the new release of the software.

The EIOVI indicator is the first known tool to evaluate the environmental impact of viticulture. It takes into account the different agronomic practices used in organic viticulture (pest and disease management, fertilisation and irrigation management, soil management and machinery use) as well as estimating the effect of vineyard management on soil organic matter and the biodiversity. The fuzzy set theory adopted provides an elegant and quantitative solution to determine cut-off values for input variables and for output results. The hierarchical structure of this technique, through the use of decision rules and by combining weighted fuzzy values, allows the aggregation of indices into first level fuzzy indicators and then into a second level fuzzy indicator for the whole system. The system has a modular structure and thus provides a synthetic indicator reflecting the overall impact for the whole system as well as detailed information through its six modules.

The fuzzy expert system reflects an expert perception of the potential environmental impact of organic viticulture. Despite the fact that the theory behind the indicator is quite exhaustive, the tool is presented with an easy to use Graphical User Interface (GUI) that requires only basic input data which are not too expensive or difficult for users to obtain, be they vine growers, consultants or scientists.

References:

Fragoulis G., Trevisan M., Di Guardo A., Sorce A., Van der Meer M., Weibel F., Capri E. (2009).
A management tool to indicate the environmental impact of organic viticulture. Journal of Environmental Quality. Vol. 38, Nr.2

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6. WORKING PROTOCOLS (Zironi, R.; Comuzzo, P.; Scobioala, S.; v.d. Meer, M.; Weibel, F.; Trioli, G.)

2006 – Red Wine Protocols

Protocol 1	Protocol 2	
	Wine pH > 3,4	Wine pH < 3,4
Current farm production protocol	Sound (selected) grapes	Sound (selected) grapes
	10-20 % of the grapes (part A) are picked the day before harvest, crushed, destemmed, transfer to a fermenting tank and immediately seeded with an amount of selected dried yeasts calculated for the whole lot[22]	10-20 % of the grapes (part A) are picked the day before harvest, crushed, destemmed, transfer if a fermenting tank and immediately seeded with an amount of selected dried yeasts calculated for the whole lot 1
	Yeast nutrient supplementation during rehydration[23] (optional)	Yeast nutrient supplementation during rehydration[2] (optional)
	Lysozyme (20 g/hl) is also added to part A	
	The remaining production of grapes is harvested the day after (part B)	The remaining production of grapes is harvested the day after (part B)
	Immediate crushing and destemming (no SO^2 addition)	Immediate crushing and destemming (no SO^2 addition)
	The mash is added to the same tank containing the lot (now fermenting) picked the day before	The mash is added to the same tank containing the lot (now fermenting) picked the day before
		At 12-36 hours, co-inoculum of MLB (direct inoculum starters, 1 g/hl)
	$\frac{1}{2}$ Fermentation: yeast nutrient and O_2 supplementation[24]	$\frac{1}{2}$ Fermentation: yeast nutrient and O_2 supplementation[3]
	Skin Maceration - Draining / Pressing	Skin Maceration - Draining / Pressing
	Continue alcoholic fermentation	Continue alcoholic and malo-lactic fermentation
	MLB inoculum immediately after AF (direct inoculum starters, 1 g/hl)	
	Bacteria specific nutrient supply at inoculum[25]	
	End of MLF: 20 g/hl lysozyme and SO_2 (30 ppm) addition	End of MLF: SO^2 (30 ppm) addition
	Store avoiding oxygen contact (under N2) till bottling	Store avoiding oxygen contact (under N2) till bottling
	Raking - Fining with limited O_2 contact	Raking - Fining with limited O_2 contact
	SO_2 addition (20-30 ppm) before bottling (eventual)	SO_2 addition (20-30 ppm) before bottling (eventual)

[22] e.g. for a final volume of 40 hl and 25 g/hl of selected yeasts, 4- 6 hl are picked the day before harvest; 1 Kg of dried yeasts is immediately added, and they will acclimatise during the next 24 hours

[23] Thiamine (maximum allowed dosage) and inactive yeasts, according to supplier's dosage

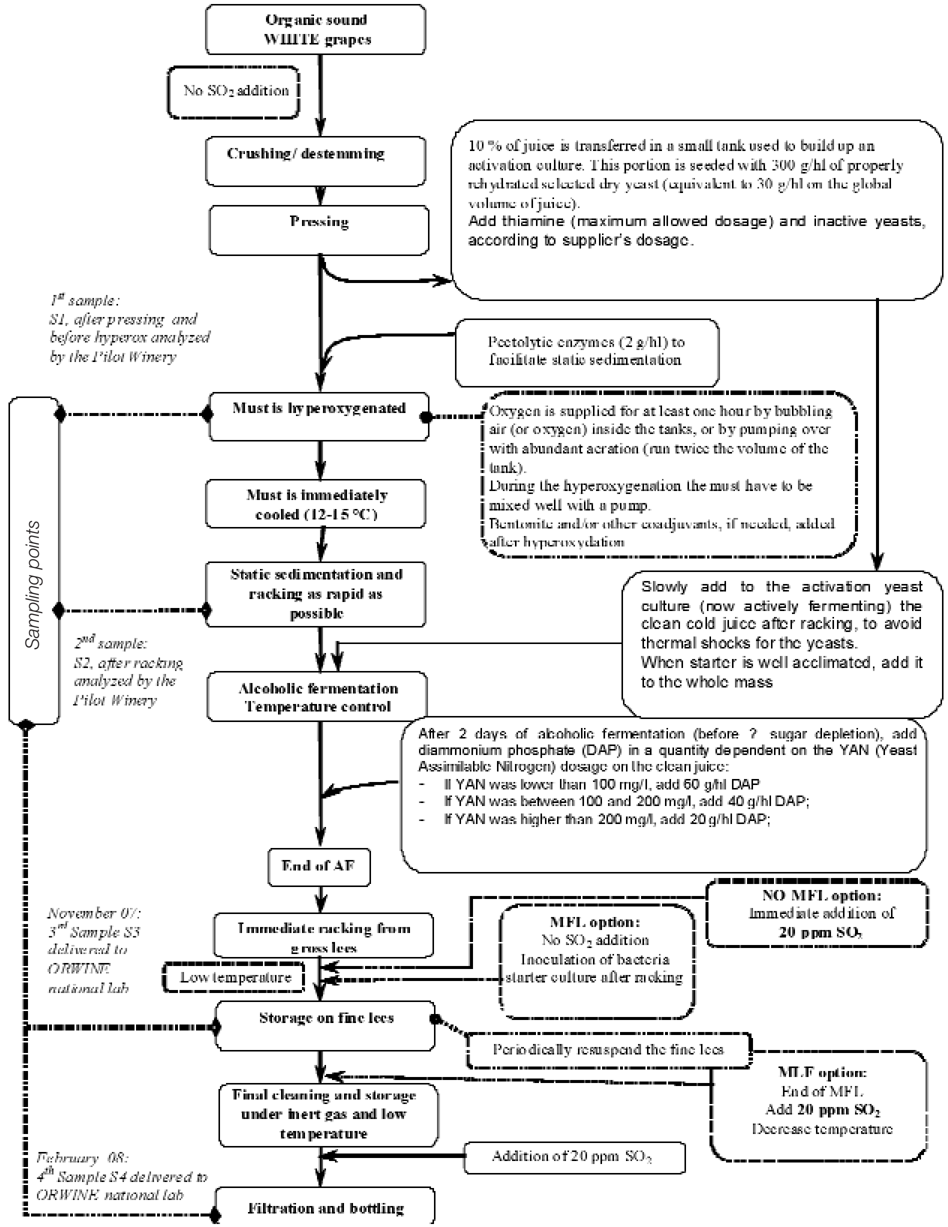
[24] Between $\frac{1}{2}$ and 2/3 of sugar depletion, add 30 g/hl or more of diammonium phosphate (DAP) and 5-10 mg/l oxygen (by specific device or through pumping over with aeration of a volume equivalent to the double of the tank)

[25] According to supplier's instructions

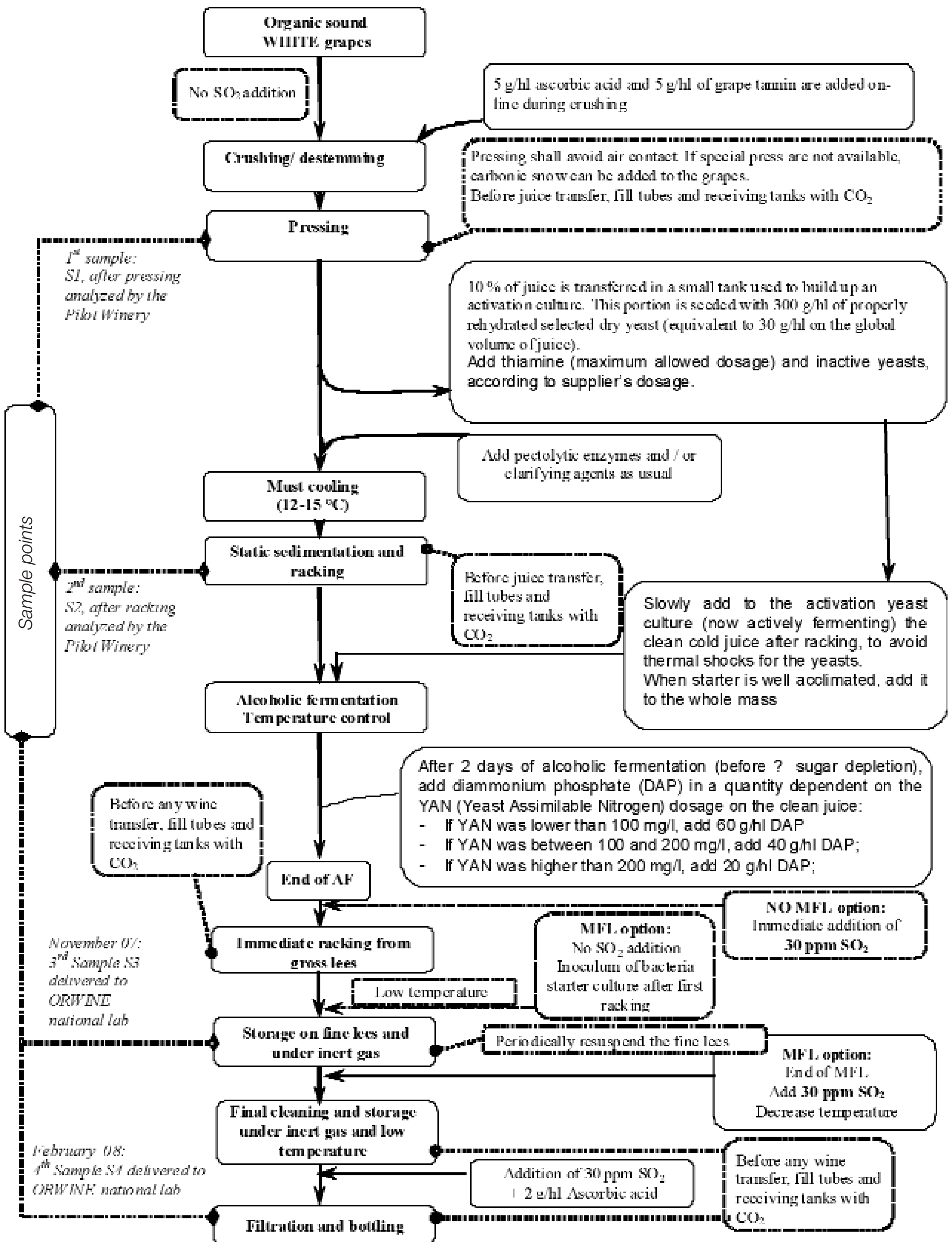
2006 – White Wine Protocols

OptionA [withoutMLF]	Option B [with MLF]	Option C [with MLF]
Sound grapes	Sound grapes	Sound grapes
100% of the grapes are picked and pressed immediately in one day. 10% of the juice (part A) is transferred to a fermenting tank and immediately seeded with an amount of yeast[1] calculated for the whole lot. 90 % (part B) goes to a separate settling tank.	100% of the grapes are picked and pressed immediately in one day. 10 % of the juice (part A) is transferred to a fermenting tank and immediately seeded with an amount of yeast[1] calculated for the whole lot. 90% (part B) goes to a separate settling tank.	100% of the grapes are picked and pressed immediately in one day. 10% of the juice (part A) is transferred to a fermenting tank and immediately seeded with an amount of yeast[1] calculated for the whole lot. 90% (part B) goes to a separate settling tank.
Yeast nutrients[2] added during rehydration of dry yeast culture for part A	Yeast nutrients[2] added during rehydration of dry yeast culture for part A	Yeast nutrients[2] added during rehydration of dry yeast culture for part A
Overnight settling (low temp.) racking after 24 h	Overnight settling (low temp.) racking after 24 h	Overnight settling (low temp.) racking after 24 h
Part B of the juice is added to the tank containing part A	Part B of the juice is added to the tank containing part A	Part B of the juice is added to the tank containing part A
1/3 fermentation: yeast nutrients[3] Option: pumping over 2 x the volume for sterol synthesis if fermentation conditions difficult	1/3 fermentation: yeast nutrients[3] Option: pumping over 2 x the volume for sterol synthesis if fermentation conditions difficult	1/3 fermentation: yeast nutrients[3] Option: pumping over 2 x the volme for sterol synthesis if fermentation conditions difficult
Performing alcoholic fermentation	Performing alcoholic fermentation	Performing alcoholic fermentation
Addition of 20 g/hl lysozyme	Co-inoculation of MLB **during** AF	Inoculation of MLB **after** AF
	Sur-lie aging (optional)	Sur-lie aging (optional)
	Addition of 20 g/hl lysozyme	Addition of 20 g/hl lysozyme
Racking, Fining (Bentonit,…?) with limited O_2 contact	Racking, Fining (Bentonit,… ?) with limited O² contact	Racking, Fining (Bentonit,…?) with limited O_2 contact
Store wine avoiding oxygen contact	Store wine avoiding oxygen contact	Store wine avoiding oxygen contact
If necessary: acidity management (methods ?), stabilisation of tartrate		
SO_2 addition 30 ppm before bottling. Optional: ascorbic acid <100ppm, well-balanced with SO2-addition	SO_2 addition 30 ppm before bottling. Optional: ascorbic acid <100ppm, well-balanced with SO_2-addition	SO_2 addition 30 ppm before bottling. Optional: ascorbic acid <100ppm, well-balanced with SO_2-addition
1 25 g/hl selected dry yeast 2 Thiamine, inactive yeast 3 Thiamine, ammonium- phosphate		

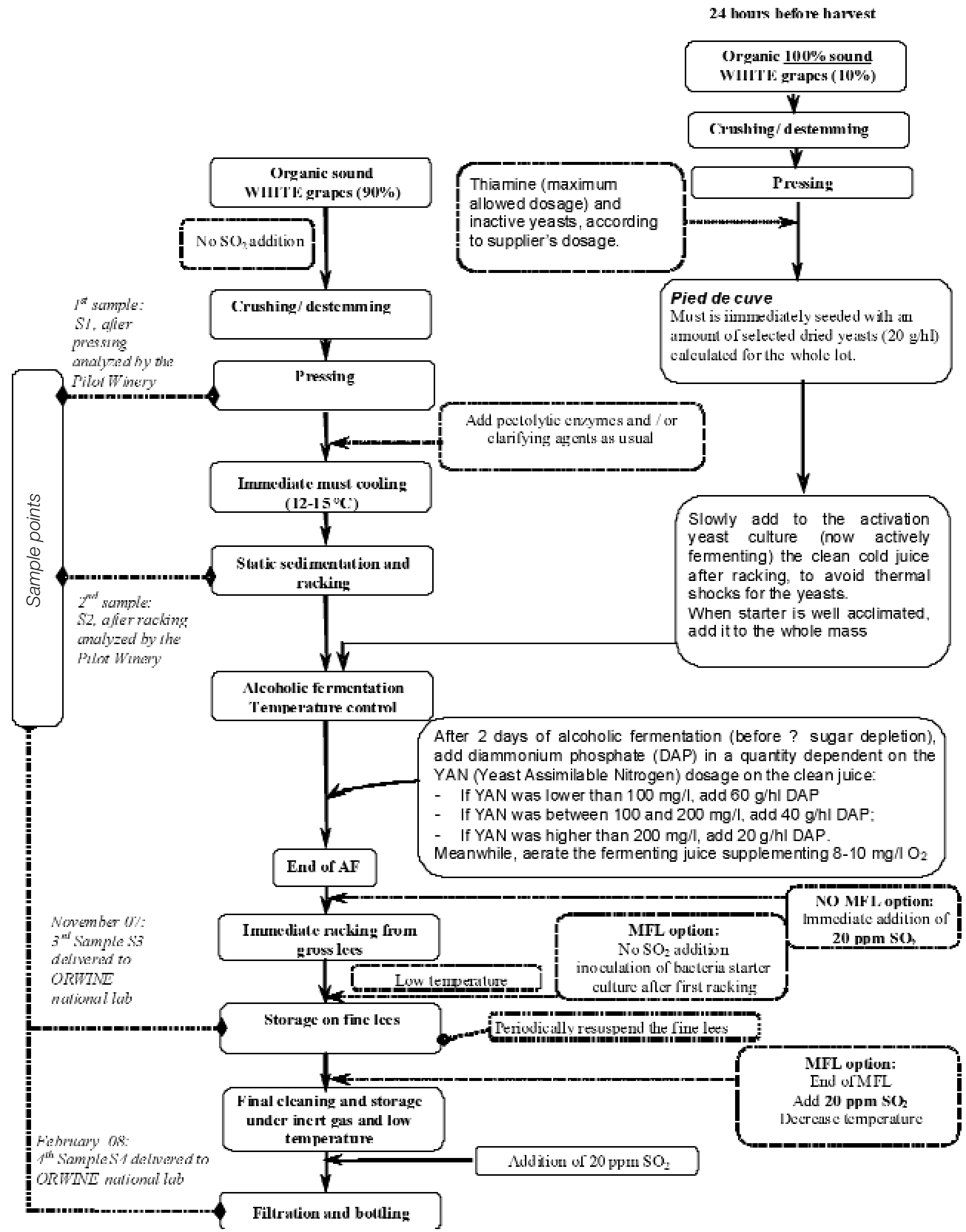
2007 Protocol A. HYPEROXYGENATION - WHITE WINES



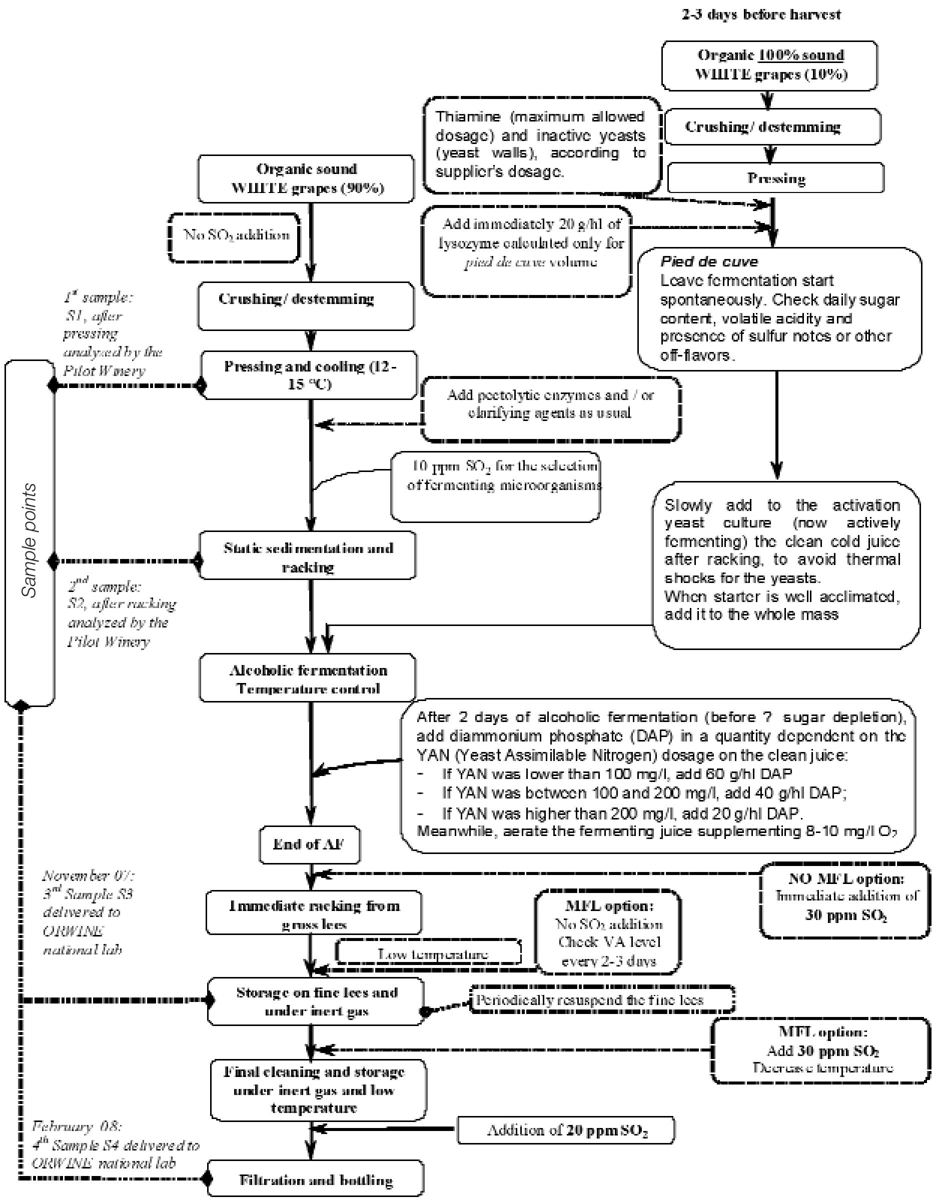
2007 Protocol B. ASCORBIC ACID + TANNINS – WHITE WINES



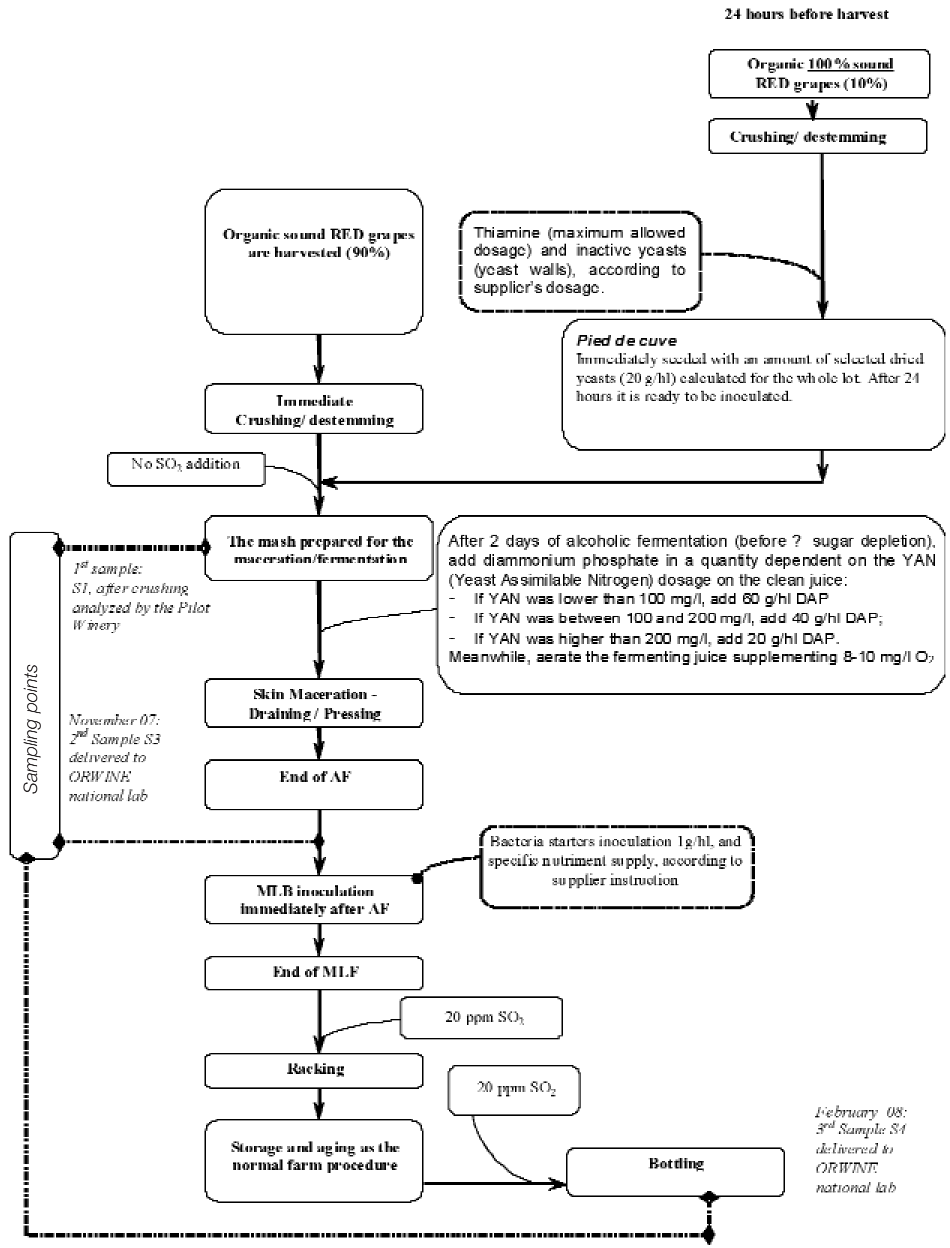
2007 Protocol C. PIED DE CUVE WITH SELECTED YEASTS - WHITE WINES



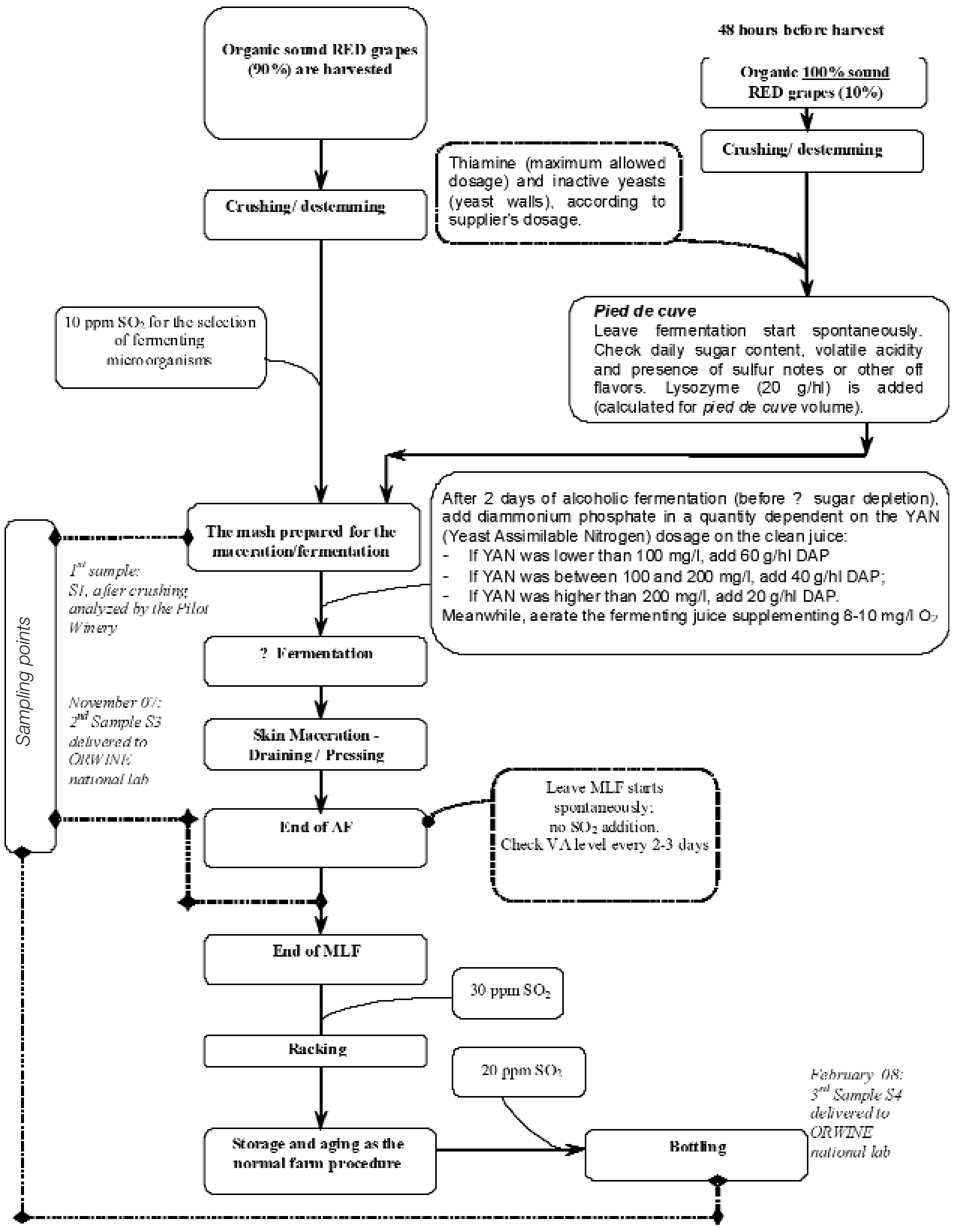
2007 Protocol D. MANAGEMENT OF SPONTANEOUS FERMENTATION - WHITE WINES



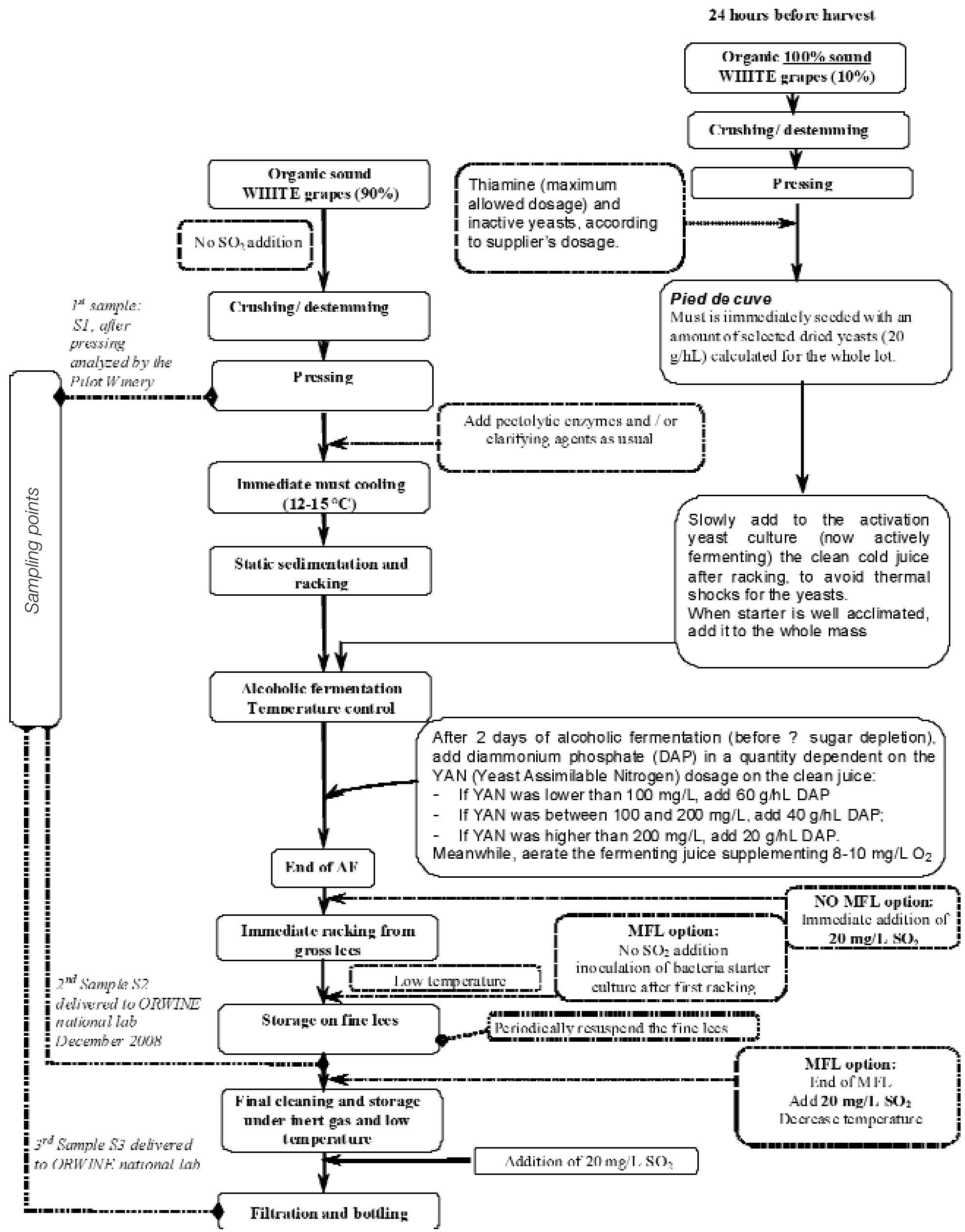
2007 Protocol E. FERMENTATIONS MANAGEMENT PROTOCOLS - RED WINES



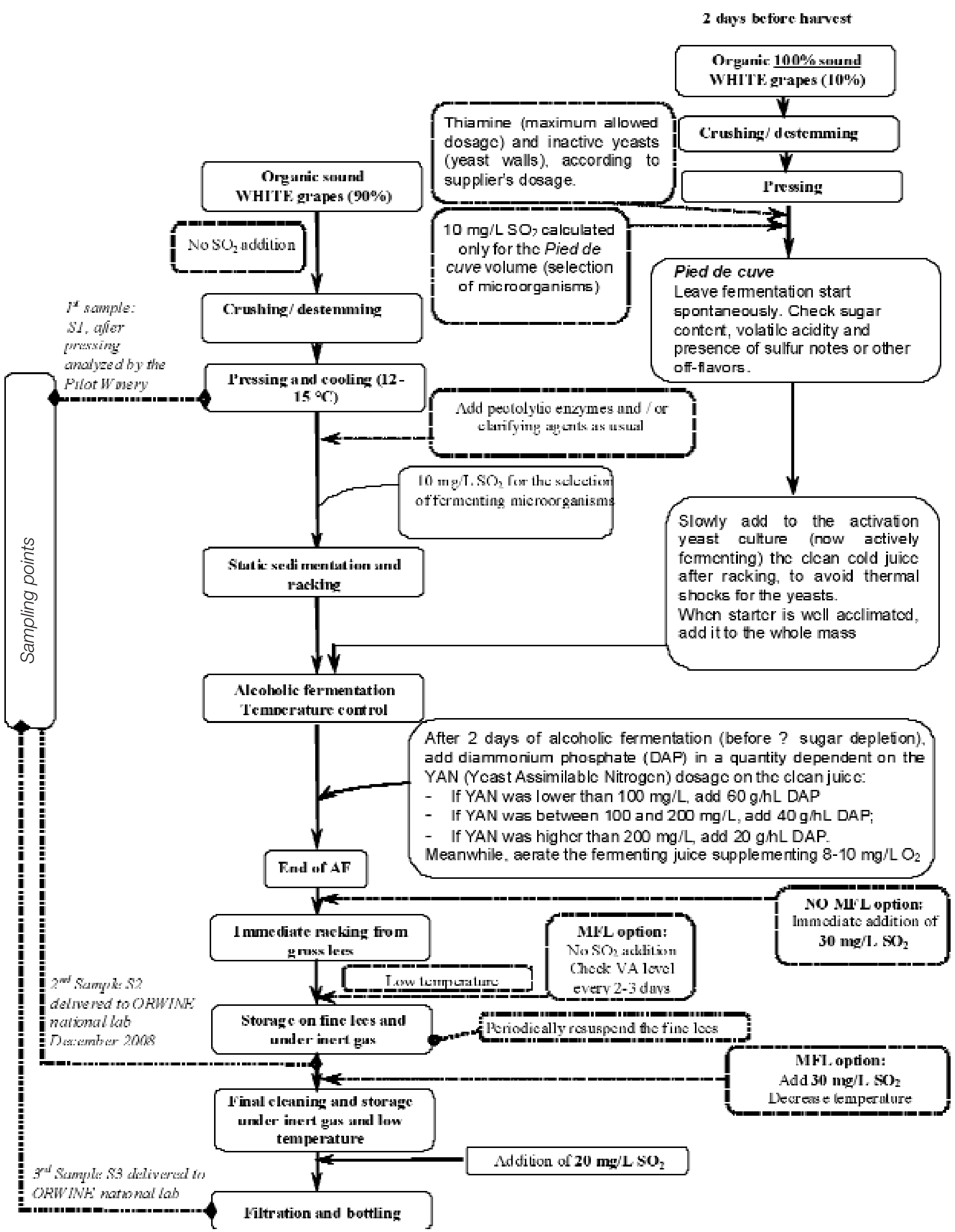
2007 Protocol F. SPONTANEOUS FERMENTATIONS PROTOCOLS - RED WINES



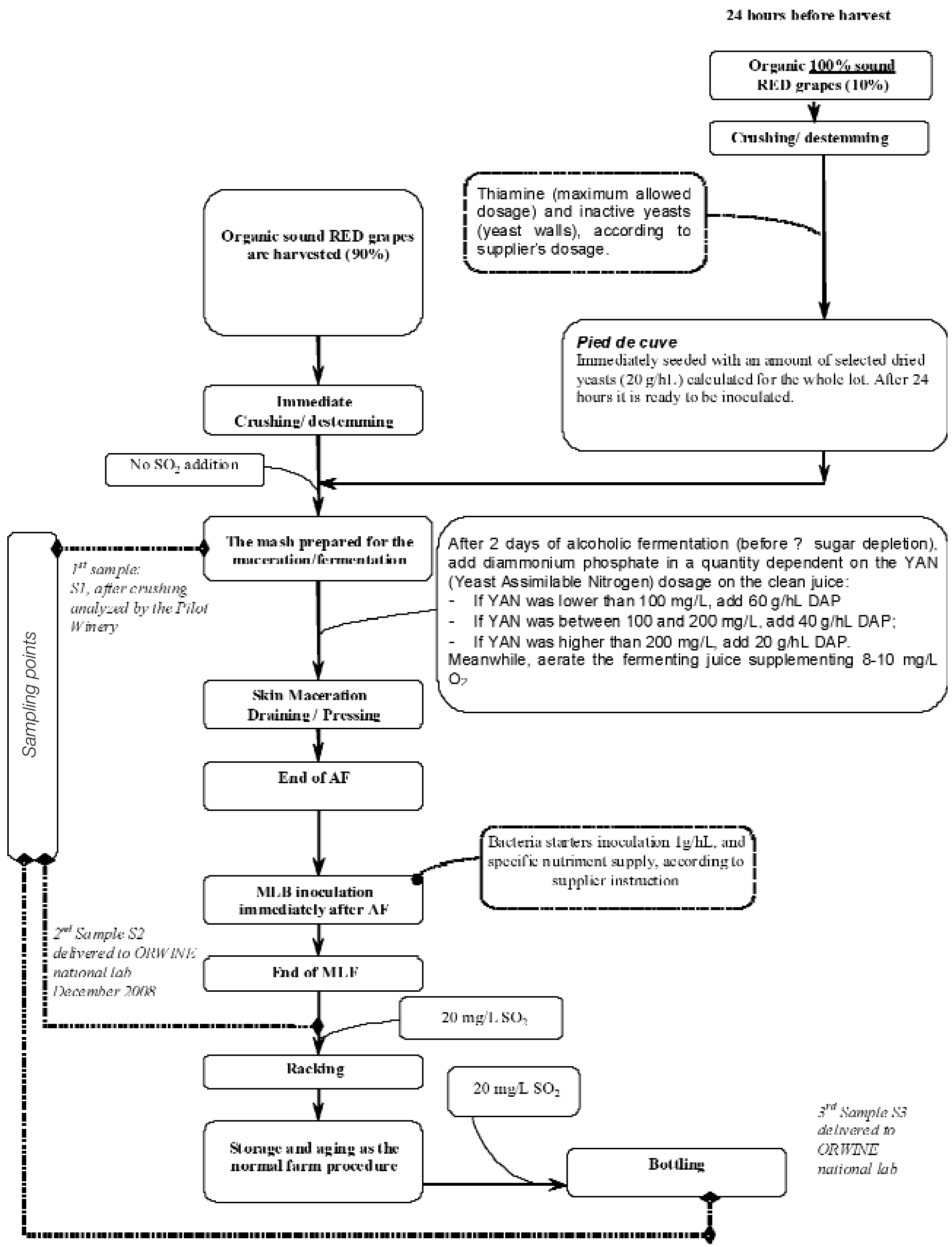
2008 Protocol A. PIED DE CUVE WITH SELECTED YEASTS - WHITE WINES



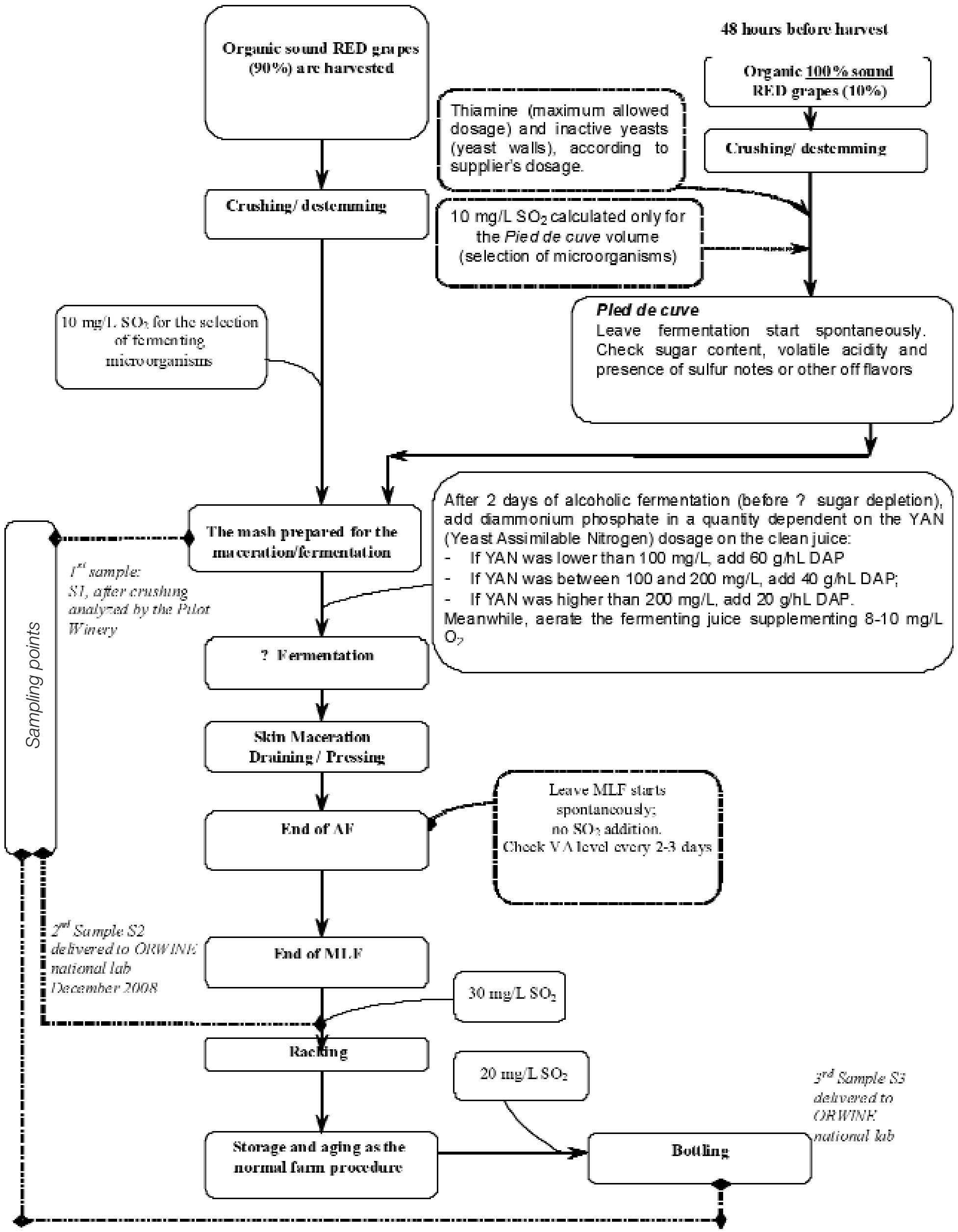
2008 Protocol B. MANAGEMENT OF SPONTANEOUS FERMENTATION - WHITE WINES



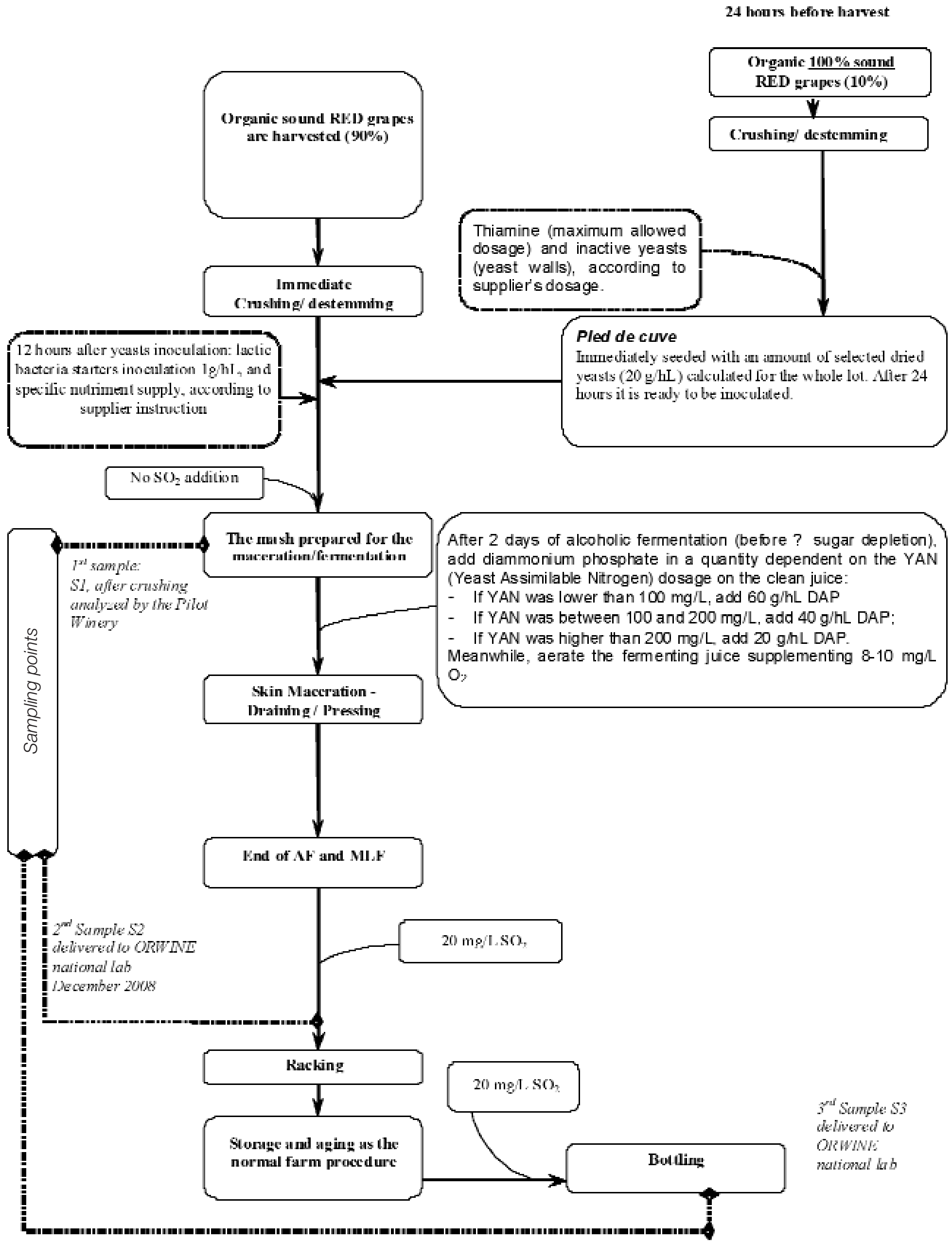
2008 Protocol C. MANAGED FERMENTATIONS PROTOCOL - RED WINES



2008 Protocol D. SPONTANEOUS FERMENTATIONS PROTOCOL - RED WINES



2008 Protocol E. YEASTS - LACTIC BACTERIA CO-INOCULATION – RED WINES



7. FACT SHEETS ONLY ON THE CD
(Jonis, M.; Pladeau, V.)

Code of good organic viticulture and wine-making:
Translated in German, French, Italian and Spanish, final output of the **ORWINE-project** – Policy-oriented Research (SSP)- Project Nr. 022769 for the European Commission.

ECOVIN - German Organic Winegrowers Association.

SIXTH FRAMEWORK PROGRAMME

Note