MICROBIAL CONTAMINATION IN WINE

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Extract from Technical Notes of Code of Best Practice for Organic Winemaking, produced under the EU FP6 STREP project ORWINE

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 *ssp.*. 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℃ and shows a maximum growth in grape juice at around 35℃. The presence of alcohol reduces the optimum to 26-28℃. *Kloeckera* is more active than *Saccharomyces cerevisiae* at temperatures of 4-10℃, 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 a 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 Lactobacillus, *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 restricted to the bottling phase after wine filtration but not acceptable for organic wine-making.

Dimethyl-Dicarbonate (DMDC)

This has recently 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. Iacts as an immediate steriliser of yeasts and, after few hours, decomposes into methanol and carbon dioxide but not acceptable for organic wine-making.

ACKNOWLEDGEMENT

The authors gratefully acknowledge from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Specific Targeted Research Project “ORWINE” SSPE-CT-2006-022769.