A child-centric microbiology education framework



Wine (Ramón González, Miguel Mejías-Ortiz, Andrea M. Guindal, Pilar Morales)

Wine. According to the International Code of Oenological Practices, "Wine is the beverage resulting exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must. Its actual alcohol content shall not be less than 8.5% vol". Fermented beverages from other fruits are also called wine in many countries, but this word should be used together with the name of the fruit used in its production. Wine production and trading are highly regulated, and most producing countries and many importing countries are member states of the International Organisation of Vine and Wine (OIV), an intergovernmental organisation informing national regulations and promoting harmonisation of winemaking practices.

There are many wine-producing countries, covering both warm and cold climate regions. But three Mediterranean countries, Spain, France and Italy, account for about a quarter of the world's wine production by volume.

How do we make it?

As already said, wine is the result of alcoholic fermentation of grapes. In this process, two simple sugars, glucose and fructose, are fermented to ethanol by yeasts. There are two main schemes for wine production, one for red wines and one for whites.

In white wine production, fresh grape berries (usually white varieties) are crushed to produce *must*, from which the stems, skins and seeds are removed, and which then undergoes alcoholic fermentation. In red wine production, the grape skins and seeds are left in the *must* and, with the help of the alcohol produced by fermentation, compounds are extracted from them that provide red wines with their distinctive colour and aroma.

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Also typical in red wine production is the malolactic fermentation, a second fermentation process that most often takes place after the first, alcoholic, fermentation is completed. This process is performed by special lactic acid bacteria and mainly consists of the conversion of malic acid to lactic acid. This transformation makes the red wines more palatable and reduces wine acidity. In addition to yeast and bacterial fermentations, the transition from grape juice to bottled wine

involves many other chemical reactions and winery practices: enzymes are used to improve juice yield and extraction of desirable compounds, nutrients are commonly added to help microorganisms successfully complete fermentation, sulphiting agents are often used to prevent oxidation and microbial spoilage, solids and materials with precipitation potential are removed at different stages of the process, wooden barrels are used for aging, etc.

Yeasts and bacteria involved in wine fermentation

We should differentiate the natural microbiota involved in winemaking from microbial starters for alcoholic and malolactic fermentation. However, we must also be aware that most wine starters were originally selected from the successful individuals from spontaneous fermentations. What makes starters conceptually different from the natural microbiota is the fact that they are inoculated in high quantities.

After the grapes are crushed or pressed and the *must* is transferred to the fermentation vat, many different yeast species, both from the berries themselves but also from the cellar environment, start growing and consuming sugars. *Hanseniaspora*, *Torulaspora*, *Metschnikowia*, *Candida*, *Pichia*, or *Lachancea* are among the most frequently reported yeast genera during the first stages of alcoholic fermentation. This activity results in consumption of oxygen and, after several hours, the conditions of the fermentation vat become anaerobic.

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However, it is *Saccharomyces cerevisiae* that is primarily responsible for alcoholic fermentation. Several features of this species explain its dominance in this environment: it is well adapted to long periods of anaerobic growth; it is also highly tolerant of grape juice composition (high sugar concentrations and acidity), ethanol released during fermentation, and sulphites used to prevent wine spoilage. These are also the reasons why many *S. cerevisiae* strains are used by winemakers as starter cultures. The use of *S. cerevisiae* starters helps to circumvent some risks of spontaneous fermentations, namely sluggish or stuck fermentation and low consistency in terms of quality.

By the way, S. cerevisiae is a "superbug", involved in the fermentation of many other foods and used as model organism by thousands of scientists (have a look at the MicroStars Gallery and other fermented foods in this Gallery). Nevertheless, we currently know that other yeast species like *Torulaspora delbrueckii*, Metschnikowia pulcherrima, Pichia kluyveri, Lachancea thermotolerans, Candida zemplinina, or Hanseniaspora vineae, formerly considered neutral or spoilage yeasts, make interesting contributions to wine quality. Some winemakers have begun to use them as starter cultures, together with S. cerevisiae, in order to improve wine quality and typicity.

The lactic acid bacterium *Oenococcus oeni* is to malolactic fermentation what the yeast *S. cerevisiae* is to alcoholic fermentation. It is the responsible agent of spontaneous malolactic fermentation, and many *O. oeni* strains are now used as starter cultures. An alternative species used as malolactic starter is *Lactiplantibacillus plantarum*. The use of starters for malolactic fermentation helps avoiding spontaneous fermentation by other bacteria (e.g., the genera *Pediococcus, Leuconostoc, Lactobacillus*) that might spoil the wine, by producing off-flavours or biogenic amines.

Main wine styles around the world

The combination of vine varieties, climatic factors, agronomic and winemaking practices, and spontaneous or starter microorganisms involved in wine production gives rise to hundreds of different wine styles around the world. Ageing time in wood barrels (and the type of barrel) and in bottle is key to shaping the aroma and flavour of wines. Its impact is so important that it is regulated in many wine producing regions. Wood ageing is much more common in red wines than in white wines. In fact, the majority of white wines are marketed as young wines (i.e., "unaged").

Sweet wines

Most wines are dry, which means that the yeasts have left almost no unfermented sugar. However, sweet wines are also popular depending on the type of consumer and the consumption circumstances. Sweet wines may be the result of a stuck fermentation because the sugar content of the grape *must* is very high at harvest and *S. cerevisiae* is unable to thrive in the resulting combination of high sugar and high alcohol levels. This is the case for Ice wines and Noble-rot wines. Sweet wines can also be produced by winemakers stopping the fermentation before all the sugar is converted to alcohol, by cooling or adding sulphites (or a combination of both) and removing the yeast cells. This results in sweet wines with lower alcohol content than above. Or they can be fortified wines produced when fermentation is arrested by addition of ethanol from wine distillation. Fortified wines typically undergo biological or oxidative aging, as in Sherry or Port wines.

Botrytis cinerea is a phytopathogenic mould responsible for the spoilage of many different fruits, including grapes. Grape growers make much effort to reduce the incidence of *B. cinerea* on their plots, as it can destroy the quality of their wines. However, in some regions, pre-harvest weather conditions may favour a type of *B. cinerea* infection that causes partial dehydration of the berries. As a result, the juice of noble rot grapes is very rich in sugars and is used to produce various types of sweet wines, the best known of which are produced in France and Hungary.

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Sparkling wines

The most popular sparkling wines are Champagne and Cava wines (from France and Spain, respectively), which are produced by the "traditional method". In this method, wines undergo a second fermentation in the same bottles that the consumer receives. First, a basic "still" wine is produced. In a second step called "tirage", it is bottled with added sucrose and a S. *cerevisiae* starter (specially adapted for this application). It is then maintained at 14-16°C for fermentation and aging. Finally, the yeast cells are concentrated in the bottle neck and removed with minimal liquid loss in a procedure called "disgorgement". Lost liquid is replaced by wine from other bottles and/or a mixture of sugar and liquor depending on the kind of sparkling wine desired. The final stopper is then fitted to maintain the carbon dioxide and internal pressure until consumption. There is a less laborious version, the Charmat method, which carries out the second fermentation and ageing in large pressure vessels.

Biological aging

This is a process carried out by yeasts after the alcoholic fermentation is completed. Instead of consuming sugars, in this case yeast cells use ethanol as their food. Alcohol metabolism requires oxygen, which is only available at the surface of the liquid in the nearly full barrels used for ageing. Specific *S. cerevisiae* yeast strains adapted to this process form a biofilm called a "velum" which floats on the surface and grants them access to the air. The velum protects the underlying wine from excessive oxidation, while yeast cells release compounds, like acetaldehyde, that provide these wines their characteristic properties (e.g. Manzanilla and Fino Sherry wines).

Beneficial properties:

Wine has been linked to human cultures for several thousands of years. Archaeological and historical references tell us about the hedonistic, ceremonial, religious and nutritional use of wine. The alcohol content also made wine an antiseptic that helped to make water drinkable and even as a skin disinfectant. Currently, there is a clear medical consensus on the risks of excess alcohol in relation to various diseases, as well as violent and risk-taking behaviour. On the other hand, as part of the Mediterranean diet (recognised as health-promoting by the WHO), and with the proposal of the "French paradox", many scientific studies have linked moderate wine consumption to the prevention of some cardiovascular diseases as well as other diseases and conditions. Those benefits have been linked in part to moderate ethanol intake, but mostly to the polyphenol content of wines. However, taking into account the risk-benefit ratio, no definitive recommendations can be made on a specific intake dose that would benefit most diseases.