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Editorial

Advances in Wine Fermentation

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Abstract: Fermentation is a well-known natural process that has been used by humanity for thousands of years, with the fundamental purpose of making alcoholic beverages such as wine, and also other non-alcoholic products. From a strictly biochemical point of view, fermentation is a process of central metabolism in which an organism converts a carbohydrate, such as starch or sugar, into an alcohol or an acid. The fermentation process turns grape juice (must) into wine. This is a complex chemical reaction whereby the yeast interacts with the sugars (glucose and fructose) in the must to create ethanol and carbon dioxide. Fermentation processes to produce wines are traditionally carried out with *Saccharomyces cerevisiae* strains, the most common and commercially available yeast, and some lactic acid bacteria. They are well-known for their fermentative behavior and technological characteristics, which allow obtaining products of uniform and standard quality. However, fermentation is influenced by other factors as well. The initial sugar content of the must and the fermentation temperature are also crucial to preserve volatile aromatics in the wine and retain fruity characters. Finally, once fermentation is completed, and most of the yeast dies, wine evolution continues until the production of the final product.

Keywords: wine; fermentation; yeasts; lactic acid bacteria; micro-oxygenation; vessels

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1. Wine Fermentation: New Approaches for a Traditional Process

The process of wine fermentation has been carried out for millennia without human intervention beyond the harvesting of grapes, mechanical processes to obtain the must, and relying on the proper development of the process to obtain a wine of acceptable quality. Technology has advanced considerably over the last century, both in terms of the knowledge of the metabolic processes developed by the yeasts and in terms of winery technology. The existence of increasingly demanding consumers, eager to discover new wines, has encouraged the adoption of new technologies and their fine-tuning in wineries. In this monograph, we will look at some of the latest innovations that we can bring to the world of wine from different aspects of its production. Consumers increasingly require wines of proven quality, both in terms of organoleptic attributes such as aroma, flavor and mouthfeel, as well as chemical composition. The use of techniques such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) has boosted the study of the metabolic profile of wines. The data generated by these analytical platforms are complex, requiring the use of statistical methodologies capable of processing and analysing the resulting information [1].

1.1. Saccharomyces Cerevisiae Is Not the Unique Microorganism in Wine Fermentation

In the past, *Saccharomyces* spp. yeasts were almost the only option for use in modern winemaking due to their unparalleled ability to metabolize all grape juice sugar into ethanol. For this reason, until some years ago, all commercial dry yeasts were *Saccharomyces* spp. For several years, non-*Saccharomyces* were forgotten at the industrial level, and some of them were even considered as spoilage microorganisms. Non-*Saccharomyces* only played a significant role in limited productions that perform spontaneous fermentations following organic polities. However, over the last decade, several researchers have proved numerous non-*Saccharomyces* to be able to improve wine quality and to solve some modern enology challenges. Some of the factors that can be improved are acidity, aromatic complexity, glycerol content, ethanol reduction, mannoproteins, anthocyanins, and polysaccharide concentrations. They can also decrease the concentrations of unwanted compounds that affect food safety, such as ochratoxin A, ethyl carbamate, and biogenic amines. Due to all those scientific advances, the main manufacturers have just started to commercialize dry non-*Saccharomyces*, such as *Torulaspora delbrueckii, Schizosaccharomyces pombe, Metschnikowia pulcherrima*, *Lachancea thermotolerans*, and *Pichia kluyveri*. Other non-*Saccharomyces* species with special enology abilities such as *Candida zemplinina, Kloeckera apiculata, Hanseniaspora vineae, Hanseniaspora uvarum, C. stellata*, *Kazachstania aerobia*, or *Schizosaccharomyces japonicus* could follow a similar progress. The aim of the chapter is to show the main abilities and advantages of these non-*Saccharomyces* in modern winemaking.

In modern traditional winemaking, *Saccharomyces cerevisiae* has been considered as the main species used in the production of quality wines. The incidences of non-selected *Saccharomyces* or non-*Saccharomyces* opportunistic yeasts during fermentations were usually related to off-flavours such as high levels of acetic acid, ethyl phenols and great levels of higher alcohols. On the other hand, at present, scientists and winemakers have started to believe in the helpful effect of some non-*Saccharomyces* in winemaking in matters such as aroma complexity [2–7].

The main problem about using non-*Saccharomyces* in oenology is their inefficiency to finish alcoholic fermentation properly. So, most of the time, it the combined use of *Saccharomyces cerevisiae* strains is required during alcoholic fermentations in order to ensure a proper fermentation end without any residual sugar at the industrial level. The production of remarkable metabolites by non-*Saccharomyces* in higher amounts than *S. cerevisiae* such as glycerol, pyruvic acid and mannoproteins has awakened special interest during the last few years [2,4–7]. The better performance of enzymatic activities by non-*Saccharomyces* such as the type glycosidase or β-lyase is a relatively new issue in modern oenology. The use on non-*Saccharomyces* also looks to be the only microbiological way to make wines with lower alcohol contents in warm areas.

Some studies have analyzed the use and influence of different non-*Saccharomyces* species in wine quality. Some of this yeast species are *Kloeckera apiculata, Hanseniaspora uvarum, Hanseniaspora vineae, Torulospora delbrueckii, Metschnikowia pulcherrima, Starmerella bacillaris, Zygosac-charomyces bailii, Pichia guilliermondii, Schizosaccharomyces pombe, Lachancea thermotolerans* and *Hansenula anomala* [2].

The chance to modify the flavor and elegance of fermented beverages through different fermentation methodologies is increasing the awareness in researching most imaginable blends of non-*Saccharomyces* and *Saccharomyces* [8]. Regarding this matter, most scientific trials performed fermentations with non-*Saccharomyces* strains on their own, with mixed fermentations (synchronized) and sequential inoculation, comparing them against an alcoholic fermentation performed by *S. cerevisiae* by itself. Most studies consider sequential inoculation as the finest option in winemaking.

Among non-*Saccharomyces* yeast genera, *Schizosaccharomyces* has been traditionally used to reduce acidity in wines presenting high levels of malic acid. This fact is related to its unique ability to transform L-malic acid into ethanol [9–11]. Nevertheless, novel uses of these *Schizosaccharomyces* species related to different abilities not so well-studied until the last few years have been developed to increase wine quality and food safety [12,13].

Today, efforts are focused on obtaining new strains with enzymatic activities that provide improvements in the aroma of wines [14]. Producers of glycolytic and other enzymatic activities are welcomed by the wine industry [15].

1.2. Genetic Modification of Yeasts and Vines

The great advance in wine production focuses on the immense possibilities offered by biotechnology, focused on the genetic modification of both the vines and the microorganisms that intervene in the production of wines, mainly yeasts, but also lactic acid bacteria [16]. The technological possibilities are ahead of the market, since there are currently advances that allow simultaneous alcoholic and malolactic fermentations [17]. The inoculation of lactic acid bacteria together with yeast starter cultures is a promising system to enhance the quality and safety of wine. However, there are numerous legal limitations as in the case of Europe, as well as the reluctance of some consumers who choose not to consume products manufactured in whole or in part, using genetic manipulation. In the US and Australia, it is not a problem, nor is the marketing of these wines in other regions of the world.

1.3. Micro-Oxygenation

Another advance in the winemaking process is oxygenation. There are two stages in the winemaking process where it is particularly important—fermentation and ageing. Oxygenation is intrinsically correlated with phenolic compounds, directly influencing the quality of the wine. Among polyphenols, anthocyanins and tannins contribute to the organoleptic characteristics of wines, improving both color and astringency. Since the phenols extracted from grapes change gradually due to biochemical reactions, and this promotes a decrease in astringency and a stabilization of colour, the addition of a small amount of oxygen was proposed to improve the quality of the wine. This process, called micro-oxygenation, accelerates the transformation of phenols. The final objective is to obtain products with more color and less astringency [18,19].

1.4. Low-Alcohol Wine Production

Moderate wine consumption is beneficial to health, but when consumed in excess, ethanol is cytotoxic and causes health problems. Obtaining a wine with less alcohol, reducing the risks associated with its consumption, but allowing consumers to maintain their lifestyle, is one of the most desirable advances in oenology. There are viticulture methods, both upstream and downstream of fermentation itself, in which the aim is to produce wine with a low alcohol content. There are different techniques in both viticulture and winemaking whose ultimate goal is to reduce the alcohol content in the final wine. Viticulture aims to reduce the surface area of the leaves, thus reducing the accumulation of sugar in the berries. During the vinification process, it is possible to mix grape musts with a high sugar concentration with other low-sugar musts. Enzymes can also be added, such as glucose oxidase obtained from the fungus *Aspergillus niger*, which catalyzes the conversion of glucose into gluconic acid and hydrogen peroxide, and reduces the ethanol concentration in the resulting wine by 0.7% v/v. At the technological level, the fermenter design is also used to control both aeration and fermentation temperature. At the microbiological level, we chose to use yeast strains that produce lower ethanol concentrations. Once fermentation is complete, action can be taken to physically remove the alcohol from the wine. Engineering options to precisely reduce the sugar content of the juice and the alcohol concentration in the wine include membrane-based systems (such as reverse osmosis), vacuum distillation and rotary cone distillation [20,21].

1.5. Other Types of Fermentation in Winemaking

1.5.1. Malolactic Fermentation

The development of the malolactic fermentation, bioconversion of L-malic acid to L-lactic acid, is a difficult and time-consuming process that does not always proceed favorably under the natural conditions of wine [22]. Instead of yeasts, bacteria play a key role in malolactic fermentation, that has the advantage of reducing some of the acidity and making the resulting wine taste smoother. Depending on the style of wine the winemaker intends to produce, malolactic fermentation can take place at the same time as the yeast fermentation. Moreover, some yeast strains can be developed that can convert L-malate to L-lactate during alcohol fermentation [17].

1.5.2. Bottle Fermentation

When we talk about fermentation in wine, we usually refer to the alcoholic fermentation carried out by yeasts in large vessels made of different materials, but this common term encompasses other processes that also receive the same denomination. Thus, for example, we find fermentation in the bottle [23]. It is a typical process associated with the production of sparkling wine, initiated in the Champagne region and extended to other areas of the world. After the first fermentation carried out by the yeasts, the wine is bottled and undergoes a second fermentation in which sugar and additional yeast, known as *liqueur de tirage*, are added to the wine. This second fermentation produces the characteristic carbon dioxide bubbles that identify sparkling wine [24].

1.5.3. Carbonic Maceration

This process is characterized by the induction of fermentation inside the grapes, without the addition of external yeasts. The production of Beaujolais wine, which consists of storing whole bunches of grapes in a closed container in which oxygen is replaced by carbon dioxide, is an example of this process. In this process, enzymes inside the grapes break down cellular matter to form ethanol and other chemical properties. Wines made by carbonic maceration presented higher aromatic quality due to their higher total content of esters and acetates, as well as a greater color intensity due to a higher phenolic content and higher rates of ionization and polymerization. In addition, it was observed that the antioxidant activity, the content in coumaroyl derivatives of anthocyanins and the vitisins A and B were considerably greater in wines made by carbonic maceration [25].

1.6. Vessels

The maturation of wine requires the use of barrels suitable for each type of wine [26]. Traditionally, the criteria for selecting the type of wood, the site where the trees were felled or even the type of grain were considered important in order to define the final aromas. These decisions have become more and more science-based, and now it is scientific data that are the driving force. Vicard, a French cooperative, analyzes tannins using near infrared spectrometry to increase consistency and reduce the number of barrels winemakers need to declassify. New technologies have also come into play. There are already proposals on the market to create vessels using 3-D printers that are vying to replace earthenware vessels (Qvevri.XYZ). Halfway through, hybrid barrels with oak heads and stainless steel bodies to provide the right combination of flavours and oxygenation to enhance the quality obtained using traditional barrels.

1.7. Turning Wine Waste into Fuel

Innovation in the world of wine goes one step further, and today's trends are environmentally friendly. Wineries are trying to use the waste from pressed grapes to create alternative fuels. As with the production of biofuel from other agricultural waste, the creation of biofuel from grape residues is based on the use of micro-organisms to break down the sugars into water and hydrogen. The hydrogen is then converted into energy. Eroglu et al. [27] introduced a novel two-stage hydrogen production process from Olive oil Mill Wastewaters (OMW) that included a pre-treatment by dark fermentation followed by a photofermentation process to produce biohydrogen. Anaerobic dark fermentation was first applied by using activated sludge cultures to degrade phenolic compounds, after which *Rhodobacter sphaeroides* was used anaerobically to produce biohydrogen. This and similar approaches have recently been reviewed [28,29].

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