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Review

Starter Cultures for Sparkling Wine

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Abstract: The sparkling wine market has expanded in recent years, boosted by the increasing demand of the global market. As for other fermented beverages, technological yeasts and bacteria selected to design commercial starter cultures represent key levers to maximize product quality and safety. The increasing economic interest in the sector of sparkling wine has also implied a renewed interest in microbial resource management. In this review, after a brief introduction, we report an overview of the main characterization criteria in order to select *Saccharomyces cerevisiae* strains suitable for use as starter cultures for the production of base wines and to drive re-fermentation of base wines to obtain sparkling wines. Particular attention has been reserved to the technological characterization aspects of re-fermenting phenotypes. We also analysed the possible uses of selected non-*Saccharomyces* and malolactic strains in order to differentiate specific productions. Finally, we highlighted the main safety aspects related to microbes of enological interest and underlined some microbial-based biotechnological applications helpful to pursue product and process innovations. Overall, the sparkling wine industry may find a relevant benefit from the exploitation of the wide resources associated with vineyard/wine microbial diversity.

Keywords: sparkling wine; starter cultures; *Saccharomyces cerevisiae*; non-*Saccharomyces* autolysis; flocculation; alcoholic fermentation; re-fermentation

1. Introduction

Humans have produced alcoholic beverages for millennia and these products have been traditionally used for medicinal, nutritional, and social purposes [1,2]. During the centuries, the technical procedures for their production have continuously evolved, since the discovery of spontaneous fermentations to the industrial application of starter cultures. The microbial strains mainly used for this last purpose belong to the *Saccharomyces* species. However, non-*Saccharomyces* species, previously considered spoilage yeasts, have also been recently used as fermentation starters, in the perspective of wines designed to respond to consumer demands [3–6].

During alcoholic fermentation, yeasts produce several compounds, mainly ethanol and carbon dioxide, with the latter released directly into the atmosphere if the process is conducted in an open vessel. This is what happens during the production of most wines, normally defined "still" wines for the negligible amounts of carbon dioxide that they still contain. Contrariwise, wines containing a relevant concentration of carbon dioxide are referred as "effervescent" wines, distinguished into

semi-sparkling (1–2.5 atmospheres of pressure in the bottle) and sparkling varieties (3–6 atmospheres of pressure in the bottle) [7].

Almost all sparkling wines are the result of two fermentation steps. During the first fermentation, the must is converted into wine (usually denoted as "base" wine), whereas in the second fermentation step, the base wine is firstly added with several ingredients (e.g., sucrose, yeasts cells, nitrogen source) and then re-fermented in a cellar for, at least, 9–12 months [8].

The sparkling wine market has expanded in recent years, boosted by a high global consumer demand [9,10]. The production of sparkling wine has significantly increased, showing a rise of 40% in the last ten years, while that of non-sparkling wines only increased by 7% over the same period [11].

Sparkling wines have an important economic impact due to their high added value. Moreover, considering the consumers' attention toward quality and safety of fermented beverages, research in the sparkling wine sector is nowadays also focuses on biotechnological innovations to improve product qualities, to simplify the production process and, at the same time, to enhance the preservation of typical and unique product characteristics [12–14].

Among the steps of production of sparkling wine, an important phase for ensuring the quality of the final product is the aging. In fact, at the end of second fermentation, sparkling wines undergo an aging period during which yeast autolysis occurs, with the consequent release of several cellular compounds, such as amino acids, proteins, carbohydrates, and lipids, that improve the quality of sparkling wines [15].

Modern biotechnologies can be used not only to improve the quality of sparkling wines, but also to reduce their production time and costs [8,14,16,17]; for example, some authors suggest new methods to accelerate the above-described autolysis process. Starter cultures for sparkling wine production need to be selected in order to produce either quality base wine or to vigorously promote the second fermentation, which occurs in a harsh environment, mainly due to the elevated ethanol content, low pH (2.8–3.3), and carbon dioxide-induced pressure [18].

2. Sparkling Wine: Production Process, Legislation, and Classification

Sparkling wines can be produced according to two main procedures, the traditional, also called as "champenoise", and the Charmat methods. The traditional method, is performed by an in-bottle secondary fermentation, while in the Charmat method, secondary fermentation is carried out in hermetically-sealed tanks [11].

2.1. Production of Sparkling Wine Using the Charmat Method

The Charmat method is characterized by sealed tanks provided with agitating mechanisms, with the aim to mix the yeast uniformly into the base wine during secondary fermentation [8]. Base wines are usually corrected with 20–24 g/L of sugar and then pasteurized with the aim to accelerate sucrose hydrolysis. Briefly, the base wine integrated with sugar is added with yeast into a pressure tank made of stainless steel, built to resist the pressure. When all of the sugar is transformed into alcohol and carbon dioxide, the yeasts are removed and the wine is bottled in an isobaric, refrigerated environment. The duration of fermentation usually influences the quality of the final product and a prolonged fermentation protects the wine aroma and allows maintaining bubbles that are more durable. At the end of secondary fermentation and after clarification, sparkling wine is bottled. After that, it should be aged at least 20 days before sale, during which aging wine remains in contact with yeast lees [19]. The Charmat method is simpler and cheaper than the traditional one. However, the process cannot be used for sparkling wines with specific regional designations.

2.2. Production of Sparkling Wine Using the Traditional Method

The traditional method is referred to as "méthode champenoise", but this expression can be officially used only for sparkling wines produced in the Champagne region (EU regulation

number 3309/85). Consequently, all other sparkling wines can be identified by the expression "traditional method", "classic method", or similar terms.

Production of sparkling wine using traditional method includes two steps (Figure 1): primary fermentation and secondary fermentation. During primary fermentation, the grape juice is converted into base wine, while during secondary fermentation (also known as "*prise de mousse*") the alcoholic fermentation of the sucrose-base-wine mixture produces the sparkling wine, with its typical characteristics, flavour, and foam.

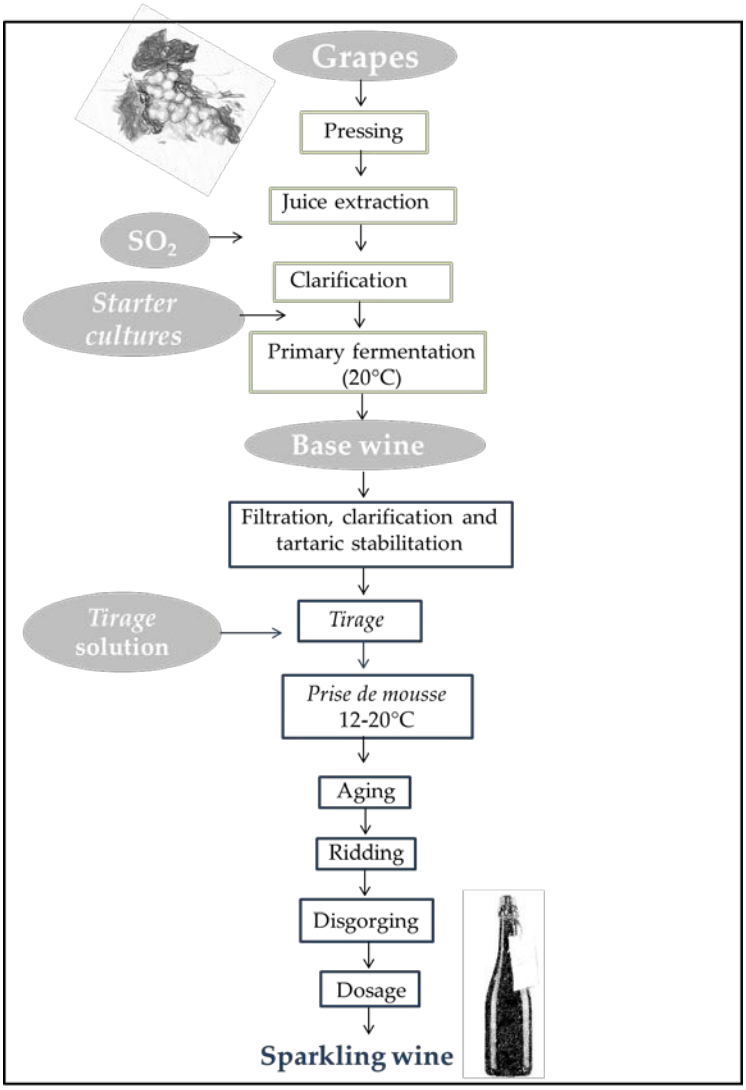

Figure 1. Flowchart for sparkling wine production using the traditional, or *champenoise*, method.

A typical base wine usually presents a moderate alcohol concentration (10%–11% *v*/*v*), low levels of sugar and acetic acid (volatile acidity), and a high concentration of other organic acids (total acidity) [8]. In particular, a significant value of the total acidity of base wine (about 12–18 g/L measured as tartaric acid), represents a critical point for sparkling wine production, because during the production process, the total acidity may decrease due to several factors, such as malic acid degradation by yeasts and lactic acid bacteria and potassium bitartrate precipitations [20].

A common practice to avoid insufficient total acidity is to slightly anticipate harvesting with respect to traditional white wine grapes. Harvesting, in fact, is a very delicate step during base wine production. Grapes are usually hand-picked and collected into small tanks to avoid berry breakage. Only sound grapes are collected and quickly transported to the cellar, in order to prevent

spontaneous fermentation. Usually, grape berries are immediately pressed, without crushing to avoid oxidations, macerations, and the development of flat aromas and browning, to obtain a good quality must. Grape juice extraction must be extremely meticulous to avoid vegetal and bitter defects, due to an excessive maceration of skins. Base wines should also have a fruity flavor and a pale color. Indeed, color extraction should be avoided, especially for white sparkling wines obtained from red grapes, also named "*blanc de noirs*" [21]. After must extraction, the next steps are (i) the addition of sulphur dioxide (apart from that added before crushing) to prevent oxidation and undesired fermentation; (ii) the clarification with pectolytic enzymes to remove solids and minimize phenol oxidations; (iii) the inoculation of starter cultures of *Saccharomyces cerevisiae*, which drive the alcoholic fermentation process in stainless steel tanks under temperature control.

At the end of primary fermentation base wines are subjected to several manipulations, such as further clarification, decantation, and filtration. Common practices also include the addition of bentonite and cold precipitation, to promote protein precipitation and potassium bitartrate precipitation, respectively. After such treatments, the base wine is ready for the secondary fermentation and selected starter cultures (*S. cerevisiae*) are added within a so-called "*tirage*" solution (containing ingredients such as saccharose 20–25 g/L, yeasts, grape must or wine, and bentonite) [22]. After the addition of the *tirage* solution, the wine is bottled and the bottles are sealed with a crown cap, that underneath it has a "*bidule*", i.e., a plastic cylinder where the lees will accumulate. The bottles are then horizontally stacked in special aging rooms at low temperature (12–15 °C). Sparkling aging usually takes place horizontally, as this position promotes an efficient contact between wine and yeast sediment, with a slow release of several compounds originating both from yeasts and wine. After the production of the desired CO_2 concentration and aging, a disgorging procedure, traditionally denoted as "dégorgement" [22], carries out the removal of lees.

The kinetics of the secondary fermentation depend on various factors, such as the yeast species/strains, temperature, and chemical composition of base wine. Usually, at 12–15 °C, the secondary fermentation takes almost 15–45 days and can be monitored by checking the internal pressure using an afrometer.

Aging duration is regulated by national legislation; as a consequence, it may vary according to country. Nevertheless, sparkling wine maturation is a slow process and it takes from a minimum of nine months for the "Cava" (Spain) to 12 months for "Talento" (Italy) or "Champagne" (France) wines [23].

A prolonged aging is essential to improve and develop the organoleptic properties of sparkling wine since it is correlated with roundness, flavor, complexity, and foaming [14,15,24]. During aging, the characteristics of sparkling wines change due to the release of yeast cytoplasmic and cell wall compounds into the wine, by the autolysis process promoted by the activity of hydrolytic enzymes. Several authors, who suggested its positive effect on sparkling wine quality, have investigated this biological event [14,24]. Proteins released by yeast into sparkling wine show a positive correlation on "body sensation" and foam stability, while polysaccharides should improve wine stability against protein haze [14]. Contrariwise, yeasts wall should adsorb volatile compounds affecting the aroma of sparkling wine [25].

When the aging is complete, the next step is the riddling or "*remuage*", i.e., a kind shaking of the bottles to convey yeast lees into the *bidule*. This step is improved by adding a small amount of bentonite to the *tirage* solution and by yeast's aptitude to flocculate. During riddling, each bottle should be hand-rotated one eighth (of the total rotation) each day for 15 days until bottles are practically perpendicular to the floor. Riddling should promote the subsequent disgorging process, during which, lees collected at the neck of the bottle are removed, thanks to freezing and internal pressure in the bottle. Then, it is a common practice to add a dosage solution, traditionally called "*liqueur d'expédition*", to compensate liquid lost during disgorging. It consists of a mixture of variable composition with pure sparkling wine, sparkling wine containing sucrose, grape must, brandy, SO_2, or other components

typical of a determined production area. The dosage solution and its composition influence sparkling wine characteristics, and will give to each sparkling wine a distinctive structure and aroma.

3. Yeast Characterization for Wine Base Production

Wine organoleptic properties are strictly correlated with the physiological and metabolic characteristics of *S. cerevisiae* and non-*Saccharomyces* strains used as starters. Indeed, such microbial component influences the production of several compounds and transform grape compounds with positive or negative effects on fermentative or secondary aromas [26,27].

A starter culture is a microbial strain that is characterized and selected for its fermentation properties. Specific criteria have been indicated to select *S. cerevisiae* starter cultures with exquisite oenological properties. However, the first fundamental step for the selection of oenological starters is the availability of genetics and molecular diagnostic tools that allow a quick and accurate yeast identification, at either species or strain level, and their monitoring during wine fermentation [28–32].

3.1. Yeast Genotypic Characterization: Methods to Differentiate Saccharomyces Cerevisiae Strains

Several molecular techniques have been developed and successfully applied to the identification and characterization of yeasts that allow to differentiate *S. cerevisiae* at the strain level [28,33–38] (Table 1).

Table 1. *Saccharomyces cerevisiae* genotypic characterization.

Molecular Method	Reference
Random amplified polymorphic DNA (RAPD) PCR	[39]
Interdelta sequences analysis	[40]
Pulse field electrophoresis (PFGE) electrophoretic karyotypes	[38]
Mitochondrial DNA (mtDNA) restriction analysis	[33,34,36,41]
Polymorphic microsatellite loci (SSRs, simple sequence repeats)	[40,42]
Multilocus sequence typing (MLST)	[43,44]

The first technique used to reveal *Saccharomyces* strain diversity is pulsed field electrophoresis (PFGE) i.e., separation of intact chromosomes by pulsed field agarose gel electrophoresis, also called electrophoretic karyotyping [38].

Random amplified polymorphic DNA (RAPD-PCR) also has been powerful to differentiate *S. cerevisiae* strains; nevertheless, other methods are more discriminating [41].

Some authors have suggested that mitochondrial DNA (mtDNA) restriction analysis (mtDNA RFLP) could be an efficient technique to differentiate at the strain level [33,34,36,41]. In particular, this molecular technique has been used to check the dominance of *S. cerevisiae* starter cultures, thanks to the marked mtDNA polymorphism of wine *Saccharomyces* strains [45–47].

Another commonly used molecular approach relies on sequencing the interdelta element, whose amplification by PCR allows differentiating at the strain level *S. cerevisiae* strains [33,48]. Other powerful molecular tools for *S. cerevisiae* strain differentiation are the amplification of polymorphic microsatellite loci, also called simple sequence repeats (SSRs) [40,42], the multilocus sequence typing (MLST) and yeast killer virus (virus dsRNA) [43,44,49,50].

3.2. Yeast Technological and Qualitative Characterization for Starter Culture Production

Yeasts, mainly *S. cerevisiae* strains, have a fundamental role during winemaking and alcoholic fermentation. Grape sugars, in particular hexoses, must be rapidly and completely converted into ethanol and CO_2, with the associated production, by the yeasts, of several metabolites important to confer wine typical organoleptic properties (but also the possible release of off-flavors) [51–54]. Nevertheless, it is important to underline that a complete transformation of sugar occurs on dry wine but not on sweet wine.

Must/wine system represents a hostile environment due to several factors, such as high sugar concentration (average 200 g/L), growing ethanol and glycerol amount, low pH (3–3.5), the presence of sulphites, and progressive consumption of nutrients (such as nitrogen sources, vitamins, and lipids) [55].

Usually, starter cultures or autochthonous strains should be selected on the basis of typical oenological traits due to the peculiar characteristics of grape juice, base wine, and to desirable qualities of wines. Indeed, an efficient procedure to characterize *S. cerevisiae* starter strains selected from natural fermenting needs biotechnological tools/criteria to optimize global wine quality [51–55]. Several authors proposed technological and qualitative criteria to select yeast strains with desirable features. Among these, tolerance to alcohol, resistance to sulphur dioxide, several enzymatic activities, osmotic properties, killer factor, and low production of H_2S are determinants [26]. Table 2 reports the most important technological and qualitative criteria to select yeast starter cultures.

Table 2. Yeast technological and qualitative characteristics for starter cultures production.

Technological and Qualitative Characteristics	Reference
Resistance to low pH, sugars, ethanol, and sulphur dioxide contents	[26,46,51,55–57]
Low volatile acidity production	[52,58–60]
Low production of sulphur compounds (H_2S, SO_2)	[26,46,47,51–53,61]
Fermentation vigour	[51]
Desired enzymatic activities (e.g., β-glucosidase, β-xylosidase, protease, polygalacturonase, pectinase, glucanase, xylanase, and decarboxylase activities)	[46,47,57,62,63]
Desired fermentation-associated metabolites (glycerol, succinic acid, acetic acid, acetaldehyde, n-propanol, iso-butanol, isoamyl alcohol, and β-phenylethanol)	[46,47,57,62–64]
Implantation aptitude	[65–67]

Other relevant features include the strain-specific formation of fermentation-associated metabolites (such as glycerol, succinic acid, acetaldehyde, n-propanol, isobutanol, isoamyl alcohol, and β-phenylethanol) and the presence of specific extracellular enzymatic activities (β-glucosidase, β-xylosidase, protease, polygalacturonase, pectinase, glucanase, xylanase, and decarboxylase) [46,47,57,62–64]. Obviously, implantation aptitude of starter cultures also is a criterion to be checked during starter technological selection programs. Indeed, several studies suggested that starter cultures dominance is not always guaranteed, as a function of the diversity associated with the naturally present microbial consortia, and that during winemaking indigenous yeasts can survive and grow, affecting starter dominance [65–67].

4. Yeast Technological Characterization for Secondary Fermentation of Sparkling Wine Production

The starter cultures used for the secondary fermentation in the traditional method need to possess several additional technological properties to those of the yeast used in the primary fermentation (Figure 2).

Chemical composition of the base wine and the sparkling wine production process represent a hostile environment for yeast growth and fermentation efficiency [23]. Base wine usually is characterized by consistent ethanol concentration (about 10%–12% *v*/*v*), low pH (2.8–3.5), high total acidity (5–7 g/L H_2SO_4), and total SO_2 contents (50–80 mg/L). In addition to these critical factors, we have to consider low temperatures occurring during the secondary fermentation (10–15 °C) and the high amount of CO_2/high pressure (usually 6 atm) associated with this process [68]. Hence, yeast starter cultures for secondary fermentation have to be selected in order to survive the above-described stresses, and, in particular, to high ethanol concentration and low pH value [69]. Ethanol in base wines affects yeast growth, viability, and ability to carry on the secondary fermentation [70]. Analysis for ethanol tolerance involves the yeast exposure to several increasing concentrations of ethanol and the monitoring of its growth [71]. It is generally recognized that yeasts should be adapted

prior to inoculation for secondary fermentation, by exposing them to increasing trends of ethanol concentrations [72]. This step (known also as "*prise de mousse*") is essential for an efficient and successful secondary fermentation because it allows the yeast acclimatization to low pH and high amounts of ethanol [73]. Cell acclimatization can improve cell viability, biomass accumulation, and time required for complete secondary fermentation. In addition, improving tolerance to stress by starter yeast may reduce the production of off flavors [21,74,75]. The low pH value is one of the main negative factors that can affect secondary fermentation of a typical base wine. In fact, base wines contain generally high amounts of organic acids (such as tartaric, malic, succinic, and acetic acids) in the undissociated form (at the common pH), that are susceptible to acidify the yeast cytosol, thus leading to sluggish or stuck fermentation [68]. In particular, the parameter to be considered during the selection procedure is the yeast resistance to high concentrations of acetic acid. In fact, acetic acid combined with ethanol can affect yeast fermentative behavior by decreasing cell pH, fermentation rate, and enolase activity [76].

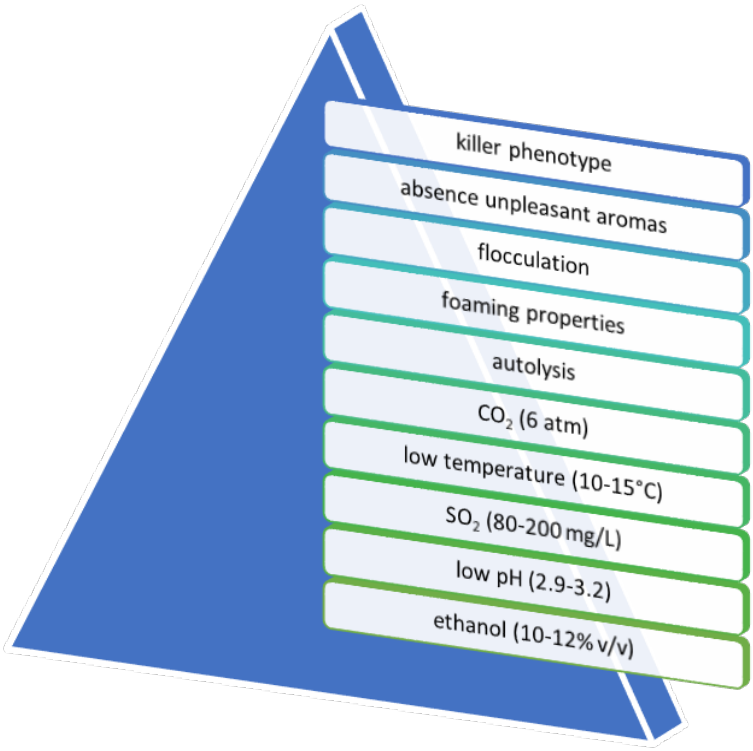

Figure 2. Yeast technological characterization for sparkling wine production.

Another biological parameter that should be considered during the selection of starter cultures for the secondary fermentation is their ability to undergo autolysis. A strain-dependent phenotype that, as a consequence of cell disruption, implies the release in the fermenting wine of several yeast-associated compounds able to influence organoleptic and foaming properties of sparkling wine [77,78]. Several reports suggested the positive effect of yeast strains with high autolytic capacity on sparkling wine quality and foaming properties [24,79,80]. This characteristic is desired at the end of the re-fermentation in sparkling wines, generally two or four months after the end of secondary fermentation. During autolysis, several compounds, such as peptides, amino acids, polysaccharides, higher alcohols, and aldehydes, are released, [69,81,82] and may improve sparkling wine flavor, because some of them (e.g., amino acids) are often precursors of aroma compounds [78]. A further technological characteristic of interest in sparkling wine production is the flocculation capacity of the candidate strain. High flocculation ability is an important criterion among sparkling starter culture selection, because yeast high flocculation aptitude facilitates the removal of sediment at

disgorging [23]. At the same time, flocculation prevent yeast sediments from remaining attached to the bottle, thus avoiding turbidity of the final product [7].

Moreover, yeast autolysate compounds could positively influence foaming properties [1] since several macromolecules released by yeast (e.g., mannoproteins) are involved in both foam formation and stabilization [80,83].

Linked to this lytic phenotype, another yeast property to be assessed during starter selection is the presence of the killer phenotype. A killer yeast is a yeast strain which is able to secrete one or more toxic proteins, which are lethal to sensitive yeast strains. This biological phenomenon should be analyzed, checking both killer and sensible phenotypes, because yeast autolysis can be improved with a mixed inoculum of "killer" and sensitive yeast strains for the secondary fermentation [84].

5. Sparkling Wine Production: Role of Non-*Saccharomyces* and Lactic Acid Bacteria

In light of the existing innovative trends in the field of microbial resources in enology, a specific insight is needed for non-*Saccharomyces* yeasts and malolactic lactic acid bacteria (LAB) that might be involved in sparkling wine production [3,85].

Even though non-*Saccharomyces* yeasts are usually involved in winemaking to increase wine's organoleptic properties, little is known on non-*Saccharomyces* application for sparkling wine production. Gonzalez-Royo et al. [9] investigated the chemical and organoleptic properties of base wines accomplished by sequential inoculation of two different non-*Saccharomyces* strains (belonging to *Metschnikowia pulcherrima* and *Torulaspora delbrueckii* species) and *S. cerevisiae* during the alcoholic fermentation of base wines of the AOC Cava. Sequential inoculation of *T. delbrueckii* and *S. cerevisiae* on base wines led to higher glycerol content, lower volatile acidity, and higher foaming properties than their corresponding control wines, suggesting their potential application to innovate specific sparkling wine production [9].

Sequential or co-inoculation of non-*Saccharomyces* and *S. cerevisiae* could be a powerful tool to make base wines with different organoleptic properties, in particular following recent trends on winemaking that suggest the important properties of non-*Saccharomyces* in order to solve specific technological challenges and/or to differentiate the production in terms of sensorial quality [3–6,53].

Among non-*Saccharomyces* yeasts, *Schizosaccharomyces pombe*, usually recognized as a spoilage yeast, presents a malic dehydrogenase activity [86,87] that might have a role in the induction of yeast autolysis during sparkling wine production, enhancing mannoprotein and polysaccharide release during sparkling wine aging [86,87]. Although non-*Saccharomyces* biodiversity could represent a considerable resource of innovation for sparkling wines production, further investigations are needed to understand the possible role of non-*Saccharomyces* starters in the base wine re-fermentation and sparkling wine aging.

The malolactic fermentation (MLF), i.e., the decarboxylation of L-malic acid into L-lactic acid, if desired, takes place post-first fermentation or simultaneously with alcoholic fermentation (co-fermentation of malolactic LAB with yeasts). MLF attenuates acidity, enhances wine biological stability, and modifies sparkling wine sensorial qualities [88]. Several LAB are involved in MLF, the most important is *Oenococcus oeni*, while other LAB species can produce off-flavors and, for this reason, are considered spoilage LAB [89]. The induction of MLF during sparkling wine aging is a common practice in the Champagne region and it allows the production of wine denoted by a higher pH value that also has a reduction in the time requested for their maturation [90]. However, malic and lactic acids were shown to have controversial effects on sparkling wine foaming properties. In fact, malic acid can improve foaming height, while lactic acid produces an opposite action [91–93].

6. Safety Aspects Correlated to Base and Sparkling Wine

Base and sparkling wine safety can be affected by several compounds derived from grapes (e.g., pesticides, phytosanitary products, or trace metal compounds) or from microbial metabolism (e.g., biogenic amines (BA) and the mycotoxin ochratoxin A) [94]. Concerning the compounds of

microbial origin, BA are low molecular weight compounds formed in foods by fermentative processes and during aging and storage as a consequence of microbial amino acid decarboxylation [55,95,96]. Sensitive consumers can be intoxicated by BA, which produce several physiological and toxic effects on human health, such as rash, edema, headaches, hypotension, vomiting, palpitations, diarrhea, and heart problems. BA are usually recovered from all fermented foods. Nevertheless, in alcoholic drinks, especially in wine, ethanol and acetaldehyde can enhance the negative effects of biogenic amines by affecting the efficiency of their detoxification by the human body [97–99]. In wine, putrescine represents the major biogenic amine, followed by histamine, tyramine, and cadaverine [100–102], and several authors suggested that BA are produced by LAB metabolism during MLF [98,103–105]. Nevertheless, recent studies also demonstrated that some yeast strains are able to produce BA in wines [53,106,107]. From this point of view, the selection of suitable yeast might represent a fundamental phase to 'build' wine safety.

Another toxic microbial by-product is ocratoxin A (OTA), one of the most common naturally occurring mycotoxins in wine, with several toxic effects [108]. OTA is present in grape musts as a consequence of fungal growth on grapes, particularly attributed to *Aspergillus* and *Penicillium* metabolism [109]. Several OTA-elimination methods have been proposed, from physical to biotechnological mechanisms. The latter represent the best methods to remove OTA from wine without affecting wine organoleptic properties or using toxic chemical compound [110]. Among biologically-based approaches, several studies suggested that *Saccharomyces* and non-*Saccharomyces* strains can remove OTA from wines [111–117].

Therefore, it is very important to monitor the presence of indigenous yeast or LAB during sparkling wine production to reduce the risks to consumer health due to the presence of this toxic microbial compound [118].

7. Biotechnological Applications

Several authors studied biotechnological applications to improve sparkling wine quality, with particular attention to autolysis, flocculation, and sparkling wine flavor [12,14,72,119]. The yeast autolysis, for example, is a slow process that can occur in a few months, or several years, thanks to different environmental conditions, such as temperature, pH, ethanol concentration, nutrient availability, redox potential, and yeast strain [14]. Usually, a slow sparkling wine aging corresponds to an increase of wine organoleptic properties; nevertheless, a long sparkling wine aging can affect entrepreneurial costs. However, several methods, such as adding yeast autolysates to sparkling wine, increasing the aging temperature to accelerate yeast autolysis or microbial genetic improvement, are reported [8,78,84,120]. In particular, the use of yeast autolysates or the increase of aging temperature can affect sparkling wine organoleptic properties [121], while a mixture of killer and killer-sensitive yeast seems to be more promising [32]. In fact, co-inoculation of killer and sensitive *S. cerevisiae* strains allows for increased autolysis, shortening sparkling wine aging time without affecting wine flavor [14,17,32,84]. Genetic improvement can help to design yeast strains with an increased autolytic and flocculation capacity [23]. This method allows improving autolysis and to shorten aging, without affecting wine flavor and needing to introduce modifications in the production process. Among genetic-based methods, the main techniques used are random mutagenesis (for example UV mutagenesis) and genetic engineering [24]. In addition, studies reported in the literature proposed several methods for the development of new *S. cerevisiae* strains with improved flocculation aptitudes, such as clonal selection, recombinant DNA, and hybridization [57,122–124].

With this concern, it is interesting to underline the possibility to hybridize *S. cerevisiae* and *S. uvarum* (*S. bayanus* var. *uvarum*) that led to hybrids with improved technological characteristics (higher fermentative rate, tolerance to low and high temperature, better flocculation capacity, excellent aromatic properties) compared to those of the parental strains [124–128].

8. Conclusions

Both traditional and Charmat methods for sparkling wine productions can benefit from using appropriate starter cultures that could allow the increase of both production efficiency and product quality. Important advances have already been performed by investigating the benefit associated with microbe biodiversity in the vineyard/wine environments. Furthermore, the sector of sparkling wine production will benefit from the development of novel procedures for a renewed exploitation of the enormous opportunities associated with natural microbial biodiversity.

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