

Effect of Production Phase on Bottle-Fermented Sparkling Wine Quality

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ABSTRACT: This review analyzes bottle-fermented sparkling wine research at each stage of production by evaluating existing knowledge to identify areas that require future investigation. With the growing importance of enological investigation being focused on the needs of the wine production industry, this review examines current research at each stage of bottle-fermented sparkling wine production. Production phases analyzed in this review include pressing, juice adjustments, malolactic fermentation (MLF), stabilization, clarification, *tirage*, lees aging, disgorging, and *dosage*. The aim of this review is to identify enological factors that affect bottle-fermented sparkling wine quality, predominantly aroma, flavor, and foaming quality. Future research topics identified include regional specific varieties, plant-based products from vines, grapes, and yeast that can be used in sparkling wine production, gushing at disgorging, and methods to increase the rate of yeast autolysis. An internationally accepted sensory analysis method specifically designed for sparkling wine is required.

KEYWORDS: *sparkling wine, wine production, aroma, flavor, foaming*

INTRODUCTION

Bottle-fermented wine production is increasing on a global scale, and there is a growing focus on alternative grape varieties in emerging sparkling wine regions that can produce quality sparkling wine. The more famous sparkling wines include Champagne and crémants from France, cava from Spain, prosecco from Italy, sekt from Germany, Cap Classique from South Africa, and sparkling icewine from Ontario (made with grapes picked by hand and pressed in their natural frozen state at approximately -10°C). Semi-sparkling wines such as Frizzante are found in Italy and white Vinho Verde in Portugal. Bottle-fermented sparkling wine quality, defined as flavor, aroma, and foaming height and stability, can be affected both positively and negatively at every stage of production. Factors that affect the quality of sparkling wine include grape variety, yeast strain, oxygen management, malolactic fermentation (MLF), fining, filtration, *tirage* recipe, length of lees aging, *dosage* recipe, and lees aging post-disgorging. The current literature has been critically analyzed to identify previous research regarding bottle-fermented/méthode champenoise/traditional method sparkling wine at every stage of production (Figure 1). Additionally, the authors aim to identify areas of future research that will benefit winemakers, contribute to increased wine quality, and advance scientific knowledge.

VITICULTURAL EFFECTS ON SPARKLING WINE QUALITY

This section of the review is a summary of viticultural effects of grape variety, clone, grape maturity, leaf removal (specifically

fruit exposure), and yield and their impact on sparkling wine flavor, aroma, and foaming of sparkling wine.

The selection of grape variety for sparkling wine takes into consideration climate, foaming properties of the juice, the base wine composition (sugar, acidity, and pH levels), aging ability, and the wine style required. Cava wine is predominantly produced from white varieties, Macabeo/Viura, Xarel.lo, Parellada, and Verdejo with a small proportion of Chardonnay and Malvasia Riojana/Subirat Parent. Red varieties in cava wine production include Monastrell, Pinot noir, Garnacha, and Trepat.^{1,2} The traditional Champagne grapes are Chardonnay, Pinot noir, and Pinot meunier, but a revival in interest of varieties such as Petit meslier, Arbane, Pinot blanc, and Pinot gris in Champagne is taking place.³ An increase in the use of alternative grape varieties for sparkling wine is underway. Research has recently focused on varieties such as Albariño, Verdejo, Godello, Garnacha, and Prieto Picudo in Spain,⁴ Baga and Fernão-Pires in Portugal,⁵ Italian Riesling/Welschriesling, Manzoni Bianco, and Moscato Embrapa in Brazil,^{6,7} Mieli in China,⁸ and Emir and Dimrit in Turkey.^{9,10} This increase in monovarietal sparkling wines is resulting in cultivar-specific, regionally focused research at various stages of the sparkling wine process.

Chardonnay clones in California, used for sparkling wine, were compared to Champagne clones in a 4 year study.¹¹

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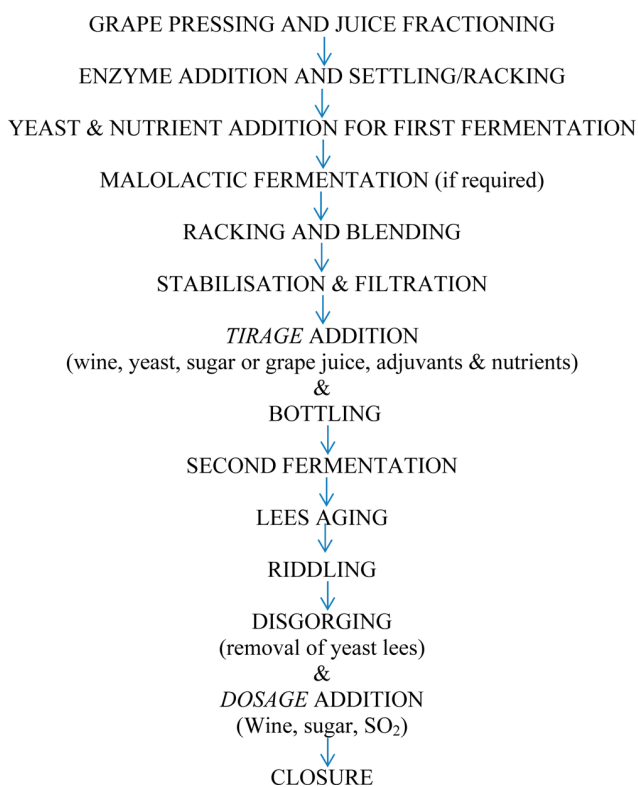


Figure 1. Simplified flowchart of bottle-fermented wine production stages.

American clones had higher acidity at harvest, which was attributed to climatic differences of the two regions. The significant difference between reproductive factors was greatest between the American clones.¹¹ Pinot noir clone performance in California was found to be significantly different in a 3 year study, with some clones suffering from excessive vegetative growth and others from high yields.¹² Making a viable comparison of the performance of clones for sparkling wines in different countries is extremely difficult because studies rarely have more than a few clones in common.¹¹ Vine spacing, row orientation, training system, canopy management, and soil and climate differences in each country or region or even from one vineyard to another are all factors that influence clone and rootstock performance.^{11,12}

A study in Portugal investigated the impact of soil type and grape maturity on sparkling wine aroma quality. More herbaceous notes were apparent in sparkling wines made from grapes picked one week before commercial harvest. Unfortunately, sugar levels, acidity, and pH values of the grapes were not presented in the study.¹³ Esteruelas et al.¹⁴ recently reported that sparkling wines produced from early harvested Macabeo/Viura, Xarel.lo, Parelada, and Chardonnay vines had higher foaming properties compared to those harvested later. However, the first grape harvest was determined by acidity levels and the second harvest by sugar levels, so alcohol differences also contributed to differences in foaming properties.

Viticultural Effects on Phenolic Compounds. Several studies have investigated the effect of viticultural practices on phenolic compounds in sparkling wines due to their importance on rosé, red, and white sparkling wine color and their impact on foaming and flavor.^{15,16} Prewinning leaf removal resulted in

higher levels of hydroxycinnamates in Chardonnay base wines and increased anthocyanin levels in Pinot noir base wines in Tasmania, Australia.¹⁵ Hydroxycinnamates are the major class of phenolic compounds in Champagne and cava wines. They affect the texture and mouthfeel of sparkling wines, thereby affecting quality.^{1,14,16–19} With regard to yield effects, Parelada sparkling wines from high-yielding vineyards in Spain had higher levels of phenolic compounds and fusel alcohols compared to low-yielding ones.¹⁷ Sensory panelists preferred wines from the low-yielding vineyard, although this may have been due to the spontaneous malolactic fermentation that took place in the high-yielding wines, making direct comparison to low-yielding wines difficult.

Phenolic compounds in grape skins and seeds can be a result of environmental factors, vintage, training system, clonal selection, light, temperature, canopy management, and water availability.^{5,14–16} Tastes and textures elicited by the presence of phenolic compounds in still white table wines are more prominent at lower alcohol levels and at moderate pH levels, indicating that the presence of phenolics is more important in the context of lighter bodied wines.²⁰ This could have implications for the quality of sparkling wines with low alcohol levels.

Nineteen phenolic compounds were identified in both Chardonnay and Pinot noir monovarietal Champagne wines from 2000 and 2001 vintages, although the quantity of each varied between the grape varieties.¹⁶ In agreement with a previous study, the variation between phenolic concentrations in the wines was due to variety and vintage, but press pressure (which affects phenolic extraction) was not considered.^{14,15}

Although common in many regions, mechanical harvesting is banned in Champagne. This is due to detrimental anthocyanin extraction from red grapes destined for white sparkling wine. Limited space between the grapevine rows in the Champagne region prohibits mechanical access, so this is unlikely to change in the future. Excessive anthocyanin extraction from mechanical harvesting can result in juice requiring charcoal or polyvinylpyrrolidone (PVPP) treatment and can cause a loss of aromas and foamability in the final wine.⁴ Additionally, an increase of pathogenesis-related (PR) proteins from mechanically harvested white grapes compared to hand-picked fruit has been reported.^{21–23} Results were attributed to their extraction from skins as opposed to physiological wounding response by the berry. PR proteins are largely responsible for protein haze, have low isoelectric points (pI) and molecular weights (MW), and are resistant to low pH as well as enzymatic and non-enzymatic proteolysis.²³ Phenolic compounds negatively affect foaming properties and have been implicated in “gushing” during disgorging (see Gushing).^{1,24}

Recently, a comprehensive review regarding viticulture for sparkling wine production was published that highlights viticultural research including grape varieties, clones, rootstocks, soil impact on flavor, precipitation in cool/cold climates, and training systems as part of climate change adaptations.²⁵

■ PRESSING

Traditional Champagne wine production uses gentle whole bunch pressing due to the use of red grapes for white wine production to prevent excessive anthocyanin extraction. A study in 1980 compared musts pressed by screw press, cylindrical Willmes press, and Willmes press with pressing aids and reported pressing effects on aroma constituents.²⁶ It is known that high pressures at pressing increase proteins and polyphenol

oxidase (PPO) and that oxidation of phenolic compounds is the main source of browning in wines due to PPO activity.²⁷ An increase in browning of cava wine (measured at 420 nm) due to the oxidation of phenolics has been previously reported.¹⁸ A comparison of three presses, two traditional basket presses (with and without an outflow central star, respectively), and a pneumatic press, revealed that oxidation level of musts obtained from traditional Champagne presses were high as early as the press exit.²⁷ Crushed and destemmed juice from Spanish Albariño, Verdejo, Godello, Garnacha, and Prieto Picudo grapes was found to have high levels of phenolic compounds, which were later removed from base wines with PVPP.⁴

A study of a central membrane press compared to a side membrane press on red grapes for still table wine found that the central membrane press produced a higher juice yield as well as lower phenolic levels in juice fractions.²⁸ Few studies have compared flavor, aroma, and foaming properties of sparkling wines processed using different presses, yet this technology has vastly improved in the past decade. Additionally, comparisons of press performances have been discussed but not with specific regard to sparkling wine quality.²⁹ The addition of pressing aids, such as rice hulls, to the press with the grapes has been found to increase free run juice from 5 to 15%.^{29,30} However, their addition may influence aroma development in sparkling wine, and their use may be dependent on the type of press being used as well as the press cycle program.²⁹

Recent grape processing innovations have been developed that include automatic grape box unloading in Champagne in addition to the separation of press fractions partitioned into distinct juice trays using gravity flow with automatic SO₂ addition.³¹ Further technological methods to preserve potential grape aromas and quality of the wine have been developed and are currently used in Champagne wine production. These include vibrating hoppers that deliver grapes directly to the press and inert gas (nitrogen) to protect grapes from oxidation in press.³¹

■ PRESS FRACTIONS

The main objective of grape pressing, as previously mentioned, is to use minimal rotation to avoid oxidation and minimal mechanical pressure on the grapes to minimize total insoluble solids that negatively affect wine quality. Studies have found a decrease in protein concentration and an increase in browning of the juice in the crumbling cycle of a Willmes press.^{32–35} This oxidation could affect the foaming ability of the final wine if these fractions are included in the *cuvée* (first press fraction) or *taille* (subsequent press fractions referred to as first *taille* and second *taille* in Champagne). Further studies identified *Vitis vinifera* proteins including a class of IV chitinase, thaumatin-like proteins, putative thaumatin-like protein, and several uncategorized proteins in Pinot noir press fractions of *Botrytis*-contaminated grapes.^{34–37} These proteins decreased in the return/retroussé cycle, which could have a negative impact on foaming. Most proteins have a pI ranging from 2.5 to 4.5 and MWs of 12–65 kDa.^{36–38} Some grape-derived proteins may not survive two fermentations. However, grape pathogenesis (PR)-related proteins, found in high concentrations in grapes, are thought to be the main contributors to protein haze in still white table wines.^{38–40} Although if numerous proteins were isolated and characterized according to their MW, pI, hydrophobicity, glycans, amino acid composition, and origin, there would still be little information concerning foaming

properties of specific proteins or those responsible for foaming height and stability.^{38–42} Turbidity levels decreased during the pressing but increased at the return/retroussé stage.³⁵ In the same study, post-pressing and after 24 h of flocculation, the early fractions had settled and decreased dramatically, from 300 to 20 nephelometric turbidity units (NTU), whereas the return/retroussé fractions were only marginally reduced. Foamability decreased during pressing, probably due to protein reduction or proteins binding to phenolic compounds.³⁵ Acidity decreased during sparkling grape pressing and pH increased. Additionally, phenolic extraction increased during the length of the pressing with increased pressure of each press cycle.³⁵ Press-fractioning impact on base wine and sparkling wine chemical composition will depend on grape variety, type of press, the pressure exerted during each cycle, and the length of each cycle.

■ CHAPTALIZATION, ACIDIFICATION, AND DEACIDIFICATION

Chaptalization. Chaptalization (sugar addition to increase final alcohol level) using cane or beet sugar is common in some cool-climate sparkling wine production regions, that is, Champagne and England. It is often suggested that 17 g/L additional sugar is required for white wines to reach 1% volume increase in alcohol content.⁴⁴ However, for low-temperature fermentations such as sparkling base wines, 16 g/L sugar likely contributes 1% volume of alcohol to the wine.³⁹ Guidelines regarding the level of chaptalization and its timing are controlled by regulatory bodies in each wine region. Sugar addition is not permitted in cava production, but rectified grape must (RGM) is allowed, but only in difficult years when climate conditions have affected grape ripeness.^{14,44} Brazilian legislation allows the addition of sugar to grape must during the initial fermentation to increase ethanol content by up to 3 °GL [Gay-Lussac (GL) is the volumetric percentage of alcohol in a beverage].⁴² However, in California, legislation permits sugar addition only for the second fermentation.⁴² One study found that juice chaptalization had a significant influence on the perceived sweetness, body, and balance between acidity and flavor in still table wines.⁴⁵ The study investigated fruit ripeness and juice chaptalization on the sensory properties and typicality of Sauvignon blanc wines in Marlborough, New Zealand. Since Goresline and Champlin,⁴⁶ there have been few studies regarding the effect of sugar type (i.e., cane, beet, dextrose) added prefermentally on sparkling wine quality. Chemical analysis of sparkling wines pre- and post-sugar addition, at first and/or secondary fermentation, investigating its effect on aroma, flavor, and wine quality has not been completed. However, it is likely that any required sugar addition prior to first fermentation will not affect aroma but increase alcohol and enhance the balance of acidity and sweetness.

Acidification. Acidification of juice is carried out by the addition of tartaric acid or, in some countries, cation exchange and is legal in some warm wine regions (i.e., California, Australia). Tartaric acid addition decreases the potassium ions and increases equivalently the concentration of hydrogen ions.⁴⁷ The pH reduction from acid addition is not directly related to the amount of acid added due to variations in the buffering capacity of different wines.⁴⁴ However, it is possible to make calculations for additions based on the level of pH reduction required.⁴⁴ Tartaric acid has also been found to positively affect foaming.^{43,47–49} Lowering the pH without lowering titratable acidity requires the addition of calcium

sulfate (CaSO_4).⁴⁷ However, this is more suited to juice, and wines, to be aged prior to bottling and is not authorized by OIV regulations (except in Jerez, Spain, for historical reasons). The effect of acidification with tartaric acid on long-term sparkling wine quality and flavor remains unclear due to a lack of studies. However, any long-term effect on sparkling wine is likely to be minimal due to high tartaric acid being beneficial for sparkling wine foaming and stability.

Deacidification. Uncommon in cool and cold climates except in exceptionally poor growing seasons, the removal of tartaric acid or excess L-malic acid before or after fermentation may be necessary to balance wine acidity and stability.⁴ Blending wines with high-acid wines or musts is not always possible, and amelioration (water addition) is detrimental.⁵⁰ Double-salt precipitation treatment (Acidex or DICALCIC) uses a 1% calcium tartrate and malic acid salt mixture as a seeding agent and is used to treat a small quantity of the wine, which is blended back into the untreated sample.⁵⁰ Boulton⁵¹ advocated that treatment of juice is preferable to treatment of wine and stated that a juice with a pH of >3.8 and a titratable acidity of >10 g/L requires addition of tartaric acid after the double-salt method. This encourages potassium bitartrate crystal (KHTa) precipitation during and after fermentation, to lower the extent of exchange and pH.⁴⁶ Further details of deacidification can be found in a review by Volshenck et al.⁵⁰

Malolactic Fermentation (MLF). MLF carried out post-first fermentation or simultaneously with alcoholic fermentation [cofermentation of yeast with lactic acid bacteria (LAB)] is common among winemakers in Champagne but results in a change in acid that may contribute to a reduction in aging.⁵² Nonetheless, in difficult years in some wine regions when juice has high acidity, or for stylistic reasons, wineries might consider MLF. MLF not only reduces the acidity but improves the biological stability of the wine and modifies sparkling wine texture.⁵³ *Oenococcus oeni* is the main LAB that conducts MLF by the decarboxylation of L-malic to L-lactic acid.⁵⁴ *Lactobacillus* and *Pediococcus* LAB can conduct MLF but can be associated with wine spoilage and off-flavors.⁵⁴ Malic acid has been found to positively affect foaming height but negatively affect foam stability,^{1,24,55,56} whereas lactic acid has been found to be beneficial for foaming stability but negatively affects foaming height.^{24,56} Furthermore, malic acid and citric acid have both been found to inhibit calcium tartrate precipitation in model wine.⁵⁷ Several extensive reviews regarding MLF chemistry have been published but are not the subject of this review.^{58,59} They detail bacterial strain performance, inoculation timing, and factors that affect MLF, those being ethanol, sulfur dioxide (SO_2), medium-chain fatty acid MLF inhibition, pH, temperature, nutritional requirements, and phenolic compounds as well as effects on wine aroma and lysozyme.

To prevent MLF, hen egg lysozyme (peptidoglycan N-acetylmuramylhydrolase, EC 3.2.1.17) can be used to reduce SO_2 levels in must or wine and prevents MLF due to the sensitivity of *O. oeni* to it. It is not permitted for use in all wine regions (i.e., Canada), and its high cost can be prohibitory.^{60,61} Two genetically modified yeasts for MLF have been released onto the market but have limited availability in only a few countries due to public perception and lack of acceptance.^{62,63}

Anecdotally, manufacturers of LAB are investigating the use of region-specific strains for MLF by identifying strains in vineyards/wineries, isolating suitable candidates and testing them on wines prior to their manufacture. MLF technological innovations include a study that used a two-stage nanofiltration

technique (demalication) and compared its efficiency to inoculated MLF in six grape varieties, including Chardonnay.⁶⁴ Further research is required, though, because volatile aroma compounds, flavor analysis, and sensory analysis were not carried out in the study. Provided that aroma and flavor are not negatively affected, and beneficial compounds such as proteins for foaming are not removed in this process, it could have application in sparkling wine production.

An immobilized lactic acid bacterium continues to be investigated for MLF due to its ability to perform MLF in a low-pH environment.^{65,66} Materials used to suspend lactic acid bacteria have included grape skins, corn cobs, and delignified cellulosic material (DCM) from sawdust, but they are not yet available commercially.^{65,66} Sequential alcoholic and MLF using immobilized cells have been found to increase the speed of wine production, but fermentations were influenced by the pH of the medium, with higher pH levels being less successful.⁶⁷ This method may be more suitable for large-scale wineries, but further research is required regarding the flavor and aroma profile of the finished sparkling wines. The selection criterion for LAB has been highlighted and includes stress resistance, technological performance, and safety.⁶⁸ With recent progress being made on the stress adaptations, survival rate of *O. oeni*, and proteome map of *O. oeni*, future research is focused on decreasing the time and improving the success rate of MLF in high-acid wines to improve production efficiency.⁶⁹

Recently, *Schizosaccharomyces pombe* yeast with malic dehydrogenase activity that metabolizes malic acid with the production of ethanol has been trialed.^{47,63,70} Traditionally described as wine spoilage yeast, *S. pombe* has malic dehydrogenase activity, produces pyruvic acid, breaks down ethyl carbamate precursors, and has a cell wall structure that ensures the autolytic release of mannoproteins and polysaccharides during lees aging, yet only a limited number of commercial strains are available.^{63,70} Its main drawback is the strong acetic acid production from unselected strains currently used in wine research.⁷⁰ However, it has potential use in sparkling wine production including the partial reduction of malic acid in wine. Instead of using it for a complete MLF, it could be used to start MLF, which could be followed by inoculation with *O. oeni* when acidity is reduced. Additionally, sequential inoculation with selected *Saccharomyces cerevisiae* strains at fermentation and the selection of low acetic acid forming strains for sparkling wine are further options. Further information regarding MLF and LAB can be found in published reviews.^{50,59,71} The effect of these various deacidification methods on long-term sparkling wine flavor, aroma, and quality remain unclear due to a lack of comparative published research.⁴⁷

■ ALCOHOLIC FERMENTATION OF SPARKLING BASE WINES

Alcoholic fermentation wine kinetics has been extensively investigated.^{62,71} The key metabolic process during winemaking is alcoholic fermentation, the conversion of sugars into ethanol and carbon dioxide by yeast: $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2$.⁷² Base wine flavor composition, aroma, and foaming potential are influenced by many factors that include grape variety, clone, rootstock, yield, the health of the grapes (i.e., disease severity), sugar levels, acid levels, nutrient levels, choice of yeast, and press fractions. The alcohol level of sparkling wine has been found to have a negative effect on foam. Chardonnay and Paredada wines made from less ripe grapes in Spain had

higher foamability, due to lower sugar levels at harvest, compared to the grapes picked later.¹⁴ Fermentability of botrytized base wine is reduced because of compositional effects including grape metabolites and protein composition of the *Botrytis*-affected berries.⁷³ *Botrytis* has been implicated in the destabilization of sparkling wine foam in several studies as well as organoleptic properties, thereby affecting final wine flavor and quality.^{73–76} *Botrytis*-affected grapes also require higher SO₂ additions during pressing, which itself is known to negatively affect foaming.^{1,49,56,77} The addition of a mixture of potassium caseinate, bentonite, and microcrystalline cellulose mixture to Parellada and Macabeo/Viura juice prefermentation produced wines with lower polyphenol content (less browning capacity), high foam stability time, less foamability, and foam collar persistence than the ones treated with bentonite.⁷⁸ It was reported that volatile aroma profiles of the wines treated with the caseinate mixture were different in comparison to the bentonite-treated wines, even though the study did not report any effect on sparkling wine quality.

Enzymatic preparations are used in winemaking to increase color extraction and filterability and improve aroma release due to β -glycosidase activity.⁷⁶ Pectolytic enzymes (added to juice during or after pressing) can increase juice yield and are used to settle solids in juice by breaking down pectins to smaller compounds. Grape protopectin and pectin levels are affected by climatic conditions and affect extraction of phenolic compounds. Insoluble protopectin is high in unripe grapes and is converted to pectin by hydrolyzation during ripening. Pectins are linear polymers of galacturonic acid, often possessing multiple esterified methyl groups, and complexed to various degrees with rhamnogalacturonan and chains consisting of arabinans and arabinogalactan.²⁹ However, pectolytic enzyme addition to Macabeo/Viura, Xarel.lo, and Parellada juice had a negative impact on foaming, possibly due to the settling and removal of solids that included polysaccharides and/or proteins.⁷⁹

Few comparative published studies exist regarding the effect of oak barrels, steel tanks, and cement eggs and their impact on the final sparkling wine flavor and aroma, undoubtedly due to the number of vessels required and the length of time needed from base wine to finished wine for comprehensive chemical and sensory wine analysis. House style (reductive/oxidative) will dictate percent of oak used during production, which ranges from 10 to 100% depending on style. However, a study of the phenolic profiles of Chardonnay and Picapoll varieties of still wines fermented in stainless steel and American oak barrels has been published.⁸⁰ Unsurprisingly, total phenolics were found to be higher in wines fermented in oak, which may have detrimental effects on the finished wine foam qualities.^{55,79} Several aromatic volatile compounds were reported in the oak-fermented wines [coniferaldehyde, sinapinaldehyde, syngaldehyde, 4-ethylguaiacol, 4-vinylphenol, eugenol, β -methyl- γ -octalactone, furan compound, and a coumarin (scroproletin)] but not in the stainless steel wines, but their impact on sparkling wine aroma and flavor by sensory analysis was not examined.

The effects of six commercial yeast strains were investigated using Macabeo/Viura, Xarel.lo, and Parellada base wines.⁸¹ Yeasts produced wines with different aroma concentrations of esters, glycerol, phenylethanol, terpenes, isoamyl acetate, medium-chain fatty acids, and alcohols. The study illustrates chemical compositional changes attributed to yeast strain. The dynamics of *S. cerevisiae* in controlled and spontaneous fermentations, of two base wines from wineries in Franciacorta,

Italy, were investigated.⁸² The highest level of biodiversity of yeast isolates was found in the spontaneous fermentation, but the study did not investigate the sensory impact on the final wines. Although it is known that *pie de cuvée* is carried out in some sparkling wine regions (grapes are picked earlier and used to inoculate the first fermentation), there remains a lack of published studies concerning this inoculation method on yeast profile and its effect on sparkling wine foaming, flavor, or aging ability. The potential benefits of non-*Saccharomyces* yeasts (i.e., *Torulaspora delbrueckii*, *Pichia kluyveri*, *Lachancea thermotolerans*, *Candida/Metschnikowia pulcherrima*) on still table wine are well-known.⁸³ Nevertheless, their effect on bottle-fermented sparkling wines (if used for first fermentation) remains unclear, particularly in regard to their effect on glycerol (important for mouthfeel) that affects wine viscosity, foaming, and volatile aroma compounds.

Low nitrogen concentration in must and excessive ammonium addition have both been found to affect fermentation length, volatile acidity, and glycerol content and wine aroma profile.⁸⁴ A study in Australia with Chardonnay investigated nutrient concentration effects on fermentation kinetics and composition.⁸⁵ The project surveyed Chardonnay juice composition from cool and warm climates destined for still and sparkling wines, so there was a wide range of pH and acidity levels. They found that low potassium concentrations at low pH negatively affected fermentation performance of some yeasts, thereby affecting the wine quality due to acetic acid production. However, the study did not include the effect of yeast strain or type of nutrient addition on aroma compounds. Grape variety has an influence on nitrogen concentrations, which influences the fermentation kinetics and aromatic profile of the wines.⁸⁶ For instance, cava wines manufactured from Monastrell grapes had lower concentrations of fatty acids and ethyl esters as well as different nitrogen concentrations compared to wines produced from Trepat and Macabeo/Xarel.lo/Parellada blend.⁸⁶

Alternatives to SO₂ addition to wine to prevent oxidation and microbial instability are a major focus of current enology research, and studies have used ascorbic acid, glutathione, yeast lees, and yeast autolysate.⁸⁷ The yeast derivative glutathione (GSH) (a tripeptide composed of glutamic acid, cysteine, and glycine) was found to be the one additive that performed most like SO₂ in a base wine in Prosecco DOCG when added to Pinot noir and Chardonnay must and base wine.⁸⁸ The study found that addition to must had greater oxidation protection than base wines, but addition to base wines resulted in retained free SO₂ levels. Phenolic levels were found to be lower in the sparkling wine that had GSH addition to must. The authors suggest further studies to assess the influence of GSH on MLF and to elucidate the effect of GSH on the levels of free SO₂. These preliminary results indicate that GSH has a promising role in future sparkling wine production, particularly due to the possible reduction of SO₂ use. However, studies into its effect on sparkling wine flavor, aroma, and lees aging are also needed. Additionally, the consequences of oxygenation of must and base wines on the synthesis and preservation of varietal aromas in the finished sparkling wine remain unclear, despite a comprehensive review of wine aroma aging in still wines by Ugliano.⁸⁹

With regard to sparkling base wine aroma, different yeasts produce wines with different base wine aromas, particularly ethyl esters of fatty acids, depending upon yeast strain, fermentation temperature, oxygen management, and sugar

contents.⁹⁰ They are predominantly produced during alcoholic fermentation from reactions between alcohols and acetyl-CoA catalyzed by alcohol acetyltransferase.¹³ The most important ethyl esters found in Chardonnay base wines were ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl lactate, and diethyl succinate.⁹⁰ Higher molecular weight alcohols found in Brazilian Chardonnay base wines included the aliphatic alcohols propanol, hexanol, dodecanol, 2,3-butanediol, and 2-phenylethanol.⁹⁰

Grape variety has an effect on the aroma of base and sparkling wines. As an example, Ferñao-Pires grapes produced sparkling wines with higher aroma potential than Baga grapes in Portugal.¹³ Although there are differences in the aroma compounds between base and sparkling wines, the main volatile compounds that differed between Brazilian Chardonnay base wines and the finished sparkling wines were C₁₃-norisoprenoids (TDN, vitisparine, and β -damascenone), esters (laurate, 2-hydroxybutanoate, decanoate, 2-hydroxypropanoate, pentanoate ethyl esters), alcohols (4-butoxybutanol, 1-propanol, methanol), aldehydes (3-phenyl-2-propenal, nonanal, undecanal), acids (acetic, 2-ethylhexanoic, butanoic), ketones (acetoin, diacetyl), and phenols (4-vinylguaiacol, 4-ethylphenol).⁹¹ With regard to the color of wines after the first fermentation, Girbau et al.⁹² suggested that base wine color intensity of white wines produced from black grapes should be similar to that of wines made from white grapes, between 140 and 160 absorbance units (AU).

■ STABILIZATION, CLARIFICATION, FINING, AND FILTRATION

Blending of base wines has not been an area of scientific research because the blends are specific to the winery style, so in-house trials are usually conducted. Post-blended wines are stabilized to test for tartrate and protein stability, and several studies have investigated methods to assist in wine stability prior to fining (if required) and filtering.

Stabilization and Clarification. In grape juice and wine, tartaric acid (H₂T) and its salt, potassium hydrogen tartrate (KHT), are naturally present.⁹³ Tartrate instability leads to crystal formation in wine bottles, and the most common method to avoid crystals is cold stabilization (chilling wines to -4° for 10 days to 3 weeks using jacketed blankets or seeding crystals).⁹⁵ Other methods include electro dialysis, ion exchange, yeast mannoproteins, metatartaric acid, and carboxymethylcellulose (CMC).^{93,95} Stabilization and clarification along with the first 6 months on lees reduced polyphenol concentrations in sparkling wines, but the reduction was reversed by 9 months of lees aging, probably because some phenolic compounds were released back into the wine.⁹⁶ Cross-flow microfiltration is becoming more widely used for clarification and microbiological stabilization in wine production, although its effect on sparkling wine quality is unclear, particularly the effect on foaming ability and stability.^{97,98} Clarification of Champagne base wines using wheat, alfalfa, and lupine proteins reported improved clarification compared to bentonite treatment. Pea protein is now commercially available and is currently being used by some English winemakers and so could be considered.⁹⁸

CMC was authorized in 2008 by the International Organisation of Vine and Wine (OIV) at a concentration limit of 100 mg/L tartrate stabilization. It is made of polymers of β -D-glucose units on which the primary or secondary alcohol groups are esterified by sodium acetate groups ($-\text{CH}_2-$

COONa).⁹⁹ However, it is not yet available or permitted in all wine regions.^{93,100} The use of CMC can decrease winery energy costs due to a reduction in cooling while also preventing the loss of acidity. Yet CMC has been found to remove phenolics and proteins if CMC is added in high quantities, which may affect foaming. The stabilizing effect of CMC on tartrate salt precipitation results from its capacity to reduce the transfer of bitartrate molecules from the wine supersaturated with salt to the growing crystals and to decrease the speed with which crystals grow.^{94,99} The effect of CMCs with different degrees of substitution and molecular weights on tartaric stability, tartaric acid, mineral content, phenolic compounds, and chromatic and sensory characteristics of white still wines was recently investigated.¹⁰¹ All CMCs significantly reduced conductivity, and a higher addition of CMCs resulted in higher tartaric stability. In wines with high tartaric instability associated with tartaric acid and potassium concentrations, CMC is beneficial. However, a deeper understanding is necessary with regard to their structure. This will allow winemakers to choose the appropriate CMC for a given tartaric acid instability issue.¹⁰¹

Flotation is a solid-liquid separation process used when the density of the particles is lower than the liquid containing them and is commonly used in wastewater treatment.⁹⁹ It has grown in popularity for wine production in recent years for must clarification due to the subsequent reduction/elimination of fining. Flocculating agents are used to bind particles including mineral adjuvants and proteins isolated from plants.⁹⁹ It has been suggested that natural plant-based vegetable compounds can replace animal products, for example, gelatin, and could reduce phenolic content and browning in white juices destined for base wines prior to fermentation.^{98,99,102} Chitine derivatives are new biopolymers recently described as good flotation adjuvants.¹⁰³

Fining. Grape variety, vintage, grape maturity, pH, and processing techniques affect must and wine proteins.⁹⁹ Trials on still wines to remove proteins using carrageenan, wheat proteins, lupine proteins, pea proteins, pectin, laccase, aspartic acid protease, and zirconium have taken place.^{99,104-108} Aspartic acid protease BcAP8, from the fungal pathogen *Botrytis cinerea*, has recently been found to remove heat-precipitated proteins from Semillon still wines but not adequately in Sauvignon blanc still wines.¹⁰⁹ Nonetheless, Marchal et al.¹¹⁰ showed that *Botrytis* protease negatively affects wine foaming by removing proteins, although a direct causal relationship between *Botrytis* proteases and wine protein content has not yet been found.¹⁰⁹ It is unlikely that sparkling winemakers would use *Botrytis*-associated enzymes due to possible effects on foamability and flavor.

The foamability of still base wines (Pinot noir and Chardonnay) decreases when the dose of bentonite increases. Generally speaking, base wine for the production of sparkling wine should have no proteic haze risk, so bentonite fining is not required. Overfining with bentonite causes large bubbles and poor bubble stability and will affect wine aroma.⁹⁹ Bentonite treatment has a deleterious effect on wine foaming properties as the foamability of wines obtained from musts treated with bentonite at the concentration of 50 g/hL decreased by 40-60% and, correlatively, the protein content of the same wines diminished by 20-60%.⁷⁷ Bentonite, although commonly used by winemakers in the production of sparkling wines, should be avoided for sparkling base wines. This is because of its concentration of heavy metals potentially released over time and its negative effect on foaming, and it

should not be used for fining if bentonite is to be used as a riddling agent. The most commonly used protein stability test is the one referred to by Pocock and Rankine.^{105,106} Wine is subjected to 80 °C for 6 h, cooled, and then visually checked for haze with a strong beam of light.^{105,106} Unfortunately, there is currently no recommended protein level in base wines at bottling, probably due to the many variables involved including grape variety and production techniques. Unlike bentonite treatment, fining with gelatin–tannins or gelatin–silica gel significantly increases the foaming properties of the fined wines when compared to the nontreated wines.¹¹¹ The current interest in natural, plant-based winemaking products means further studies in this area are likely in the future.

Many sparkling base wines produced with Pinot noir and Pinot meunier grapes are pink in color. This is especially true for the wines produced with (1) grape berries having a high maturity level (less common in cool regions where the potential alcohol is between 8 and 10.5% v/v) or (2) the grape juice obtained in the second part of the pressing cycle. In the Champagne area, Pinot noir and Pinot meunier grape berries represent approximately two-thirds of all grapes, and many winemakers use vegetable charcoal for base wine color removal. Treatments with charcoal always diminish the Pinot noir base wine foamability.^{77,114,115} It has been shown that 40 g/hL charcoal, used to remove color from Pinot noir base wines, was required for sufficient color removal.¹¹⁴ The amount of charcoal needed to treat base wines will depend on the type of charcoal used, the wine chemical composition, and timing of addition. Charcoal contains a large quantity of air, and oxidation can occur from charcoal use due to the reduced protection that can occur when the protective phenolic compounds have been removed.¹⁰¹ Wine oxidation can be reduced and prevented if the charcoal is rapidly and meticulously removed.¹⁰¹ As with all fining agents, winery trials using different types of charcoal to determine the final type and quantity of charcoal addition are recommended.

A logarithmic relationship was found between the pink color and wine protein content. Foamability decreased by 54% with charcoal addition, and protein content decreased by 20%, suggesting that proteins in Pinot noir base wines are not solely responsible for foam quality. Nicolini et al.¹¹⁵ found that a smaller amount of active carbon (20 mg/L) added during fermentation to still white wines produced from Chardonnay and Pinot gris did not negatively affect wine color or aroma. Differences in the grape color, charcoal product, analysis method, grape variety, vintage, and ripeness parameters (pH, TA, sugar level, phenolic composition) could all contribute to the difference in results. Some considerations that should be given to base wine charcoal additions include the timing of addition (must or base wine), the grape variety, and whether during alcoholic fermentation or to the blend before bottling. Additionally, it was not established whether the color from some red grapes would diminish during bottle fermentation and/or lees aging because phenolics are absorbed by yeast cells, with tannins being absorbed before anthocyanins.¹¹² This could mean charcoal addition to base wine is unnecessary for some varieties, especially as carbon used to remove color has been shown to be detrimental to wine foaming ability.¹¹⁴

Studies into the impact of specific grape (endogenous and exogenous) phenolics on foaming, phenolic characterization of different grape varieties, and their foaming ability, along with products capable of removing anthocyanins without removing beneficial proteins, could be areas of future study.¹¹⁴ Occa-

sionally carbon can also be used to reduce base wine off-flavors (mushroomy/moldy/earthy odors), but the carbon used for color removal and that used for off-flavor treatments have different characteristics. Not all forms of charcoal can absorb aroma compounds and polyphenols.

New fining agents include yeast protein extracts for clarification and stabilization including phenolic removal but are not yet available for trials on sparkling base wines.¹¹⁶ PVPP is a high molecular weight fining agent made of cross-linked polypyrrolidone and attracts phenolic compounds by adsorption.⁹⁹ It is used to decrease bitterness in wine and oxidative browning in white wines. Potassium caseinate, either alone or in combination with other fining agents (gelatin or bentonite), was studied using Parelada juice for cava wine production. Juice filtration was found to be the most effective form of clarification, but potassium caseinate had less influence on aroma compounds and removed more phenolic compounds than other fining agents.¹¹³ More recently, chitine derivatives have been described as interacting with some polyphenols and transition metals such as iron and copper so they could act as a preservative in regard to the wine oxidation process.^{117,118}

Filtration. After cold stabilization and fining (if required), the turbidity of the wine is generally too high for bottling or secondary in-bottle fermentation so it is necessary to filter it (except for some rare wines vinified/produced and/or matured in barrels). Filtration is important for the brilliance of white wines, but results show that it modifies the turbidity of the sparkling wine. Additionally, foam formation depends on the type of filtration the wine has been subjected to.⁴⁸ Different foaming behavior has been reported in sparkling wine after different types of filtration.¹¹⁹ Two base wines were laboratory filtered (0.2, 0.45, 0.65, and 3 μm and unfiltered), and it was found that foaming substantially decreased with filtering, no doubt due to removal of compounds required for foaming. The 0.2 μm filtered wine had very poor foam, and 0.45–3 μm range results were less clear, but the 0.45 μm filtered wine had the least foam collapse and best collar stability. It has been demonstrated that the smaller the filter pore size, the lower the foaming properties.⁴⁸ These differences are due to a different rate of film rupture. Foam expansion (E) is defined as the ratio of foam volume on liquid volume under standard conditions. During foam formation, the films are still thick and are drained mainly by gravity. Assuming that the nucleation is constant, the difference of slopes (during foam formation) depends on filtration.⁴⁸ These different coalescence rates occur because during foam formation, bubbles trap substances such as proteins to stabilize their interfaces.^{120,121} If these components are lacking, the films are not stable and the surface tension is high, so coalescence takes place more easily.^{48,122}

A comparison of diatomaceous earth (DE) filtration to cross-flow filtration in the food and drink industry found that DE filtering requires a low skill level and low maintenance and is cost-effective, but it is a risk to workers due to dust exposure from fine diatomaceous earth.¹²³ Apart from storage space for the DE filter and earth, consideration must be made for the spent cake disposal. Cross-flow filtration has a cost implication. It has been suggested that wine flavor and aroma can be lost in wines filtered by a cross-flow system.¹²³ However, a recent study in California using red and white still wines found minimal sensory differences between cross-filtered and unfiltered white wines.¹²⁴ Yet the red unfiltered wine changed after 2 months of post-filtration. A study of three membranes for cross-flow membrane filtration found that 0.1 μm was the

optimum cut-off point for white Riesling wines.¹²⁵ Filtering base wines to such a low level will most likely negatively affect foaming due to removal of foam-active compounds such as proteins. A review of cross-flow microfiltration in enology includes wine composition, membrane fouling, and membrane characteristics.¹²⁶ However, the effect of cross-flow filtration on sparkling wine foam, flavor, and volatile aromas remains unclear. Lenticular filtration is a relatively recent innovation rapidly replacing DE filtration in Champagne. It has no moving parts and fewer health hazards for workers than DE due to lack of dust inhalation risk and fewer disposal issues. These filters have large internal surfaces capable of retaining considerable volumes of turbid liquid (up to 3 L/m²).¹²⁷ A comparative study using the same sparkling base wine filtered using different filtration methods would increase our knowledge and understanding of how the filtration systems affect sparkling wine volatile aroma compounds, foaming, and flavor.

■ BOTTLING AND *TIRAGE* (BOTTLING FOR BOTTLE FERMENTATION)

After fermentation, the sugar level at bottling affects the bottle pressure, especially with the more permeable crown caps available to producers.¹²⁸ An amount of 22–24 g/L sugar is used in the *tirage*, although in commercial wineries it may be 22–23 g/L to avoid excess pressure that can be high if the CO₂ loss is low from a crown cap. Depending on the crown cap permeability, the loss or transfer of CO₂ from inside the bottle to the outside can be from 0.12 to 0.68 mL/day. This CO₂ permeability is correlated with the O₂ intake.^{129,130} Crown caps have been designed with seals with a range of permeability related to the desired O₂ intake depending on the quality objectives requested by the winemakers; that is, if the *cuvée* is to have long lees aging, the winemaker will choose a “closed to oxygen” crown cap to avoid oxidation during aging of the wine in the cellar. With the current crown caps on the market, the scale of O₂ intake ranges from 500 ppb to >3000 ppb (accumulated oxygen over 2 years).

All sparkling wines, but particularly rosé wines, are photosensitive and suffer from degradation over time on shelves, resulting in color, flavor, and aroma changes. Several studies have investigated the role of bottle color in wine quality.^{131–133} Low-density polyethylene films (LDPE) have been found to protect wines for 60.4% longer than bottles without photoprotection, increasing the wine's shelf life from 6 to 12 months.¹³¹ During storage Chardonnay still wine has been shown to be affected by temperature and light. The order of protection reported is Flint < Arctic Blue < French Green and Antique Green with low-wavelength visible, ultraviolet light being primarily responsible for the color changes.¹³² Bottle weight was found to have only a minor impact on rosé sparkling wine color development.¹³³ These results have implications for producers of bottle-fermented sparkling wine when choosing colored bottles for wines, especially rosé sparkling wines, when color, flavor, and aroma retention is required for quality wine.

Tirage. *Tirage* ingredients are a closely guarded secret in many wineries, but the *tirage* recipe from two studies were *S. cerevisiae* var. *bayanus* yeast (0.30 g/L) with sucrose (23 g/L) and bentonite (0.10 g/L) as a riddling aid.^{96,134} This level of bentonite addition (at 0.10 g/L) is not reflective of all commercial wineries because if a winery uses bentonite as a riddling aid, it is more likely to be between 0.01 and 0.04 g/L.^{34,35} Nutrient additions such as diammonium phosphate

(DAP) and/or thiamin can be made along with tannin to prevent reductive notes and provide structure to sparkling wine while increasing antioxidant ability of the wine.¹³⁵ Other studies have not included *tirage* or *dosage* ingredients in their experimental design, making comparisons of chemical analysis results difficult.^{136,137} The CIVC has demonstrated that the initial oxygen level at the *tirage* stage is not a limiting factor for yeast growth and fermentation kinetics. However, the initial CO₂ level in the mixing tank can be problematic for the normal development of the cells during *prise de mousse* (secondary fermentation in bottle).^{139,140} A study of the influence of the method of starter culture preparation and level of inoculation with viable yeast assessed the kinetics of yeast growth and sugar utilization in *tirage* found that starter cultures prepared aerobically (in juice or wine) started fermentations that finished at similar times.¹⁴¹ At similar levels of inoculation, the anaerobically prepared culture resulted in fermentations being completed 8–10 h earlier, due to the short period of exponential growth and earlier onset of linear growth, where 65–90% of the total sugar is utilized. Consideration must be given to alcohol level prior to sugar addition to ensure the required bottle pressure at the end of secondary fermentation is reached.¹³⁵ Researchers used a stable carbon isotope method to detect sugar additions in sparkling wines from Chile, Australia, Brazil, the United States, and Europe (France, Germany, Portugal, and Spain).⁴³ They identified sugar beet as being used for secondary fermentation in European and Chilean wines as opposed to sugar cane in Brazilian and Argentinian sparkling wines. There is unlikely to be any effect on wine flavor or volatile aroma compounds from these two forms of sugar (see Chaptalization).

Winemakers generally use active dried yeast for bottle fermentation, and many suitable strains are available on the market. The direct yeast preparation for *tirage* avoids the risk of contamination compared to a multiplication procedure from one initial batch of yeast. Yeast flocculation ability is an important consideration to facilitate sediment removal at disgorging. This explains why some companies have developed “agglomerated yeast” to avoid riddling agents and to facilitate the riddling step.^{142,143} However, the use of this particular yeast is decreasing due to some negative organoleptic effects on sparkling wine generated by some agglomerated yeast strains.¹⁴⁴

Some researchers blend bottles of sparkling wines to allow for bottle variation prior to volatile aroma analysis, whereas others use two to three separate bottles for replication without opening and blending them.^{137,145,146} Standardizing representative bottle numbers (i.e., three to five) to reduce bottle variation factors in addition to reporting *tirage* and *dosage* ingredients would improve direct comparisons of wines, research results, their treatments, and other factors associated with quality sparkling wines.

■ RIDDLING AIDS/ADJUVANTS

Bentonite. Bentonite not only is used as a fining agent for still wines (see Fining) but is also the most frequently used riddling agent for bottle fermentation. This clay efficiently removes proteins from wine due to the cation exchange capacity of the bentonite (a negatively charged adsorbent that at wine pH binds the positively charged grape proteins).¹⁰⁸ Calcium bentonite produces more compact lees than sodium bentonite and so is the preferred riddling agent for wineries that choose to use it in their Champagne sparkling wines.⁹⁹ A

study carried out by Jeandet et al.¹⁴⁶ investigated suspended particles (*voltigeurs*) thought to be bentonite in Champagne wines post-riddling. The particles were larger than bentonite, not protein related, but due to the concentration of aluminum and silicon concentration were found to be of a similar atomic composition to bentonite used in the *tirage liqueur*; however, it remains unclear why these particles did not settle during *remuage*. The use of bentonite as a riddling aid was investigated in cava monovarietal sparkling wines.¹⁴⁷ It decreased foaming height and stability in Chardonnay, Macabeo/Viura, and Xarel.lo. This was because bentonite removed favorable foaming proteins in the wine, although no significant differences were reported in Parellada and Pinot noir wines. The protein fraction, F2 and F3, which comprised proteins with molecular weights of 60 kDa (F2) and between 20–30 kDa (F3), were most affected by bentonite treatment, eliminating >80% of total soluble proteins.¹⁴⁷ These results are in agreement with another study that reported an almost complete removal of low molecular weight proteins from Chardonnay wines, illustrating that bentonite effects are different depending upon grape variety and the blend of grape varieties of the bottled sparkling wine.^{148,149} A study found that just 3 g/hL of sodium bentonite added to cava sparkling wine (blend of Macabeo/Viura, Parellada, and Xarel.lo) did not greatly affect the volatile aroma compounds.¹⁴⁴ Nevertheless, sensory analysis showed that panelists preferred the wines produced without bentonite, in agreement with Martinez-Rodriguez and Polo.¹⁵⁰ It was found that even an addition of 3 g/hL bentonite reduced the foaming capacity of cava sparkling wines, which affected the visual and sensorial properties of the wine although the grape varieties in the wines were not mentioned.¹⁵⁰ This suggests bentonite affected mouthfeel, flavor, and aroma, possibly due to the change in nitrogen content, ethyl octanoate, and ethyl decanoate concentrations and other smaller volatile aroma compounds.

Bentonite and Alginate. Some other riddling agents can contain a percentage of potassium alginate. This polysaccharide has the ability to form gel in acidic conditions or very stable gels with calcium cation.¹⁵⁰ This enables a rapid formation of film-type sediments allowing quicker riddling. This particular riddling agent is often dedicated to mechanical riddling, and a pure bentonite riddling agent is used for manual riddling.

Immobilized Yeast. Methods for encapsulated yeast in alginate matrix dedicated to the bottle fermentation were patented in the mid-1970s.^{151–154} Valade¹⁵⁵ conducted a study into riddling methods and compared (1) classical yeast and hand riddling, (2) classical yeast and mechanical riddling, (3) mechanical riddling and agglomerated yeast, (4) technique using included yeasts, and (5) the “Millispark” cartridge (developed by Micropore in 1993). The author found no difference in the wines, and no negative effect was observed on the sparkling wine flavor quality. More recently, a study compared the use of biocapsules (co-immobilization of filamentous fungi *Penicillium chrysogenum* and *S. cerevisiae* yeast), calcium alginate beads (immobilized *S. cerevisiae* yeast trapped in calcium alginate beads), and free yeast cells with bentonite.¹⁵⁶ Calcium alginate beads completed riddling in 15 s, biocapsules in 2 min, but free yeast with bentonite took several days. Yeast cells with bentonite produced wines with reduced foaming in agreement with other studies.¹⁴⁶ Calcium alginate wines had slightly higher foamability values than those made with biocapsules. This was attributed to the matrix support of the biocapsules formed by fungus hyphae that may

have absorbed proteins required for foaming. Several studies have investigated the use of different support casings for immobilized yeasts in the beer and bioethanol industries, and these include clay and poly(potassium acrylate), but their effectiveness and impact on sparkling wine have not been investigated.^{157,158}

■ SECONDARY FERMENTATION

Yeast chosen for secondary fermentation can sometimes be the same as the first fermentation (depending on wine style and the preference of the winemaker). Invariably, yeast is chosen for its ability to ferment wines high in acid, with low pH and possessing a high ethanol tolerance. *S. cerevisiae* strains able to carry out secondary fermentation in bottle were isolated from spontaneously fermenting Macabeo/Viura, Xarel.lo, and Parellada musts in El Penedes, Spain.¹⁵⁹ The use of non-*Saccharomyces* yeast is becoming more common in winemaking, but less so for secondary fermentation in bottle. There is a lack of published research regarding their use in sparkling winemaking, possibly due to an expected increase in glycerol, which could affect viscosity (that can affect foaming), mouthfeel, and wine flavor.¹⁶⁰ The effect of conventional versus non-conventional selected wild yeast for secondary fermentation was investigated with isolated yeasts from Sicilian Malvasia delle Lipari wine.¹⁶¹ The yeasts produced sparkling wine with different aroma profiles easily distinguished in a triangle test. Further studies using wild yeast are likely to be a focus of future research, particularly with alternative varieties.

The kinetics of a second fermentation has been monitored using Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) microspectroscopy to observe the degradation of lipids, proteins, and polysaccharides in Chardonnay base wine.¹⁶² These authors suggested the technique could be used to select yeast strains according to their autolytic capacity, but wineries are unlikely to have this technology. The transcriptome profiling of secondary fermentation included the expression of genes involved in respiratory metabolism, oxidative stress response, autophagy, and peroxisomal function consistent with ethanol being the main environmental factor influencing transcriptional responses to winemaking conditions.¹⁶³ The majority of genes down-regulated during secondary fermentation are related to cell growth, linked to the biosynthesis of nucleic acids and proteins, and gene expression.¹⁶³ The study found that low pH or CO₂ pressure was not a relevant constraint for the adaptation of wine yeast cells as expected. However, phenolic compounds suppress yeast metabolism during the secondary fermentation in the bottle, which can cause gushing problems for sparkling red, rosé, and white wines.¹⁶⁴ The lower the temperature of a solution, that is, after secondary fermentation, the higher the gas solubility. After secondary fermentation, a standard 75 cL Champagne bottle has close to 9 g of dissolved CO₂ molecules.⁷²

■ AGING ON YEAST LEES

The lees present in still wine during aging are composed of tartaric acid salts, organic residues, and cells of various species of yeasts and bacteria. In contrast, sparkling wine lees are mainly composed of cells from a single species of yeast, along with the technological co-adjuvants, which help to flocculate and eliminate the yeast lees at the end of aging. The aging of sparkling wine in the bottle on yeast lees is generally longer than still wine aging, and yeast autolysis occurs under pressure

commonly at 6 standard atmospheres (atm). Yeast autolysis is considered a lytic event. It is an irreversible process catalyzed by yeast intracellular enzymes. Autolysis generally takes place at the end of the stationary phase of growth and is usually associated with cell death. The scientific basis of yeast autolysis has been the subject of numerous reviews and is not the subject of this paper.^{165–168}

Natural autolysis takes time. This is especially true in wines, in which the autolytic conditions from pH 3 to 4, aging temperature of 15°C, and presence of ethanol (12% v/v) are not the ideal conditions of 45°C at pH 5.¹⁶⁸ In sparkling wines, yeast autolysis does not begin until 2–4 months after the completion of secondary fermentation.^{165,169} Yeast autolysis can be promoted by using a mixture of “killer” and sensitive yeast for the secondary fermentation. In these conditions, the sensitive yeast cells rapidly die in the presence of the killer strains.¹⁶⁹

Hydrolytic enzymes play a major role in autolysis. Proteases are the most extensively studied of all the enzymes involved in autolysis. In sparkling wines, proteolytic activity decreases during active bottle fermentation and in the following months, but after 9 months of fermentation and aging, it gradually increases.¹⁷⁰ Leroy et al.¹⁷¹ reported that proteolytic activity during Champagne aging may also depend on the yeast strain used.

The yeast cell wall is degraded during autolysis, but few studies have investigated the enzymes involved in this process during wine production. In still wines aged on lees, it has been shown that glucanases are involved in yeast cell wall degradation.^{172,173} Cell wall degradation during autolysis results in the release of both amino acids and macromolecules, suggesting that both proteolytic and polysaccharides are involved in the degradation of enzymes.¹⁷⁶ However, in sparkling wine conditions, these activities have not been studied. A recent study investigated the evolution of polysaccharides and their molecular weights during sparkling winemaking.⁹⁶ The highest amount of mannoproteins and polysaccharides with high concentrations of arabinose and galactose with a shift to low molecular weights was reported after 6 months of lees aging, which could positively effect foaming.

Recently, it was reported that the addition of enzyme preparation rich in β -glucanase increases aging characteristic of sparkling wine and would also enhance the antioxidant properties of sparkling wine.^{174,175} Indeed, addition of exogenous β -glucanases promotes release of yeast components in sparkling wine during aging.¹⁷⁶ The optimal temperature for proteolysis in the Champenoise method has been reported to be between 10 and 12°C.¹¹⁴ Another factor that influences yeast autolysis is the yeast strain. One study suggested that yeast strains with a high autolytic capacity would produce better quality sparkling wine than yeasts with a low autolytic capacity.¹⁷⁸ They also suggested that autolytic capacity and foaming analysis should be used for selecting yeasts for sparkling wine production. The importance of the autolytic capacity of yeast strains for sparkling wine quality was confirmed by Nunez et al.¹⁷⁹ A mutant strain with accelerated autolysis was used to conduct the secondary fermentation. The resulting wine had better foaming properties than that produced with the control strain. The aging period was also reduced from 9 to 6 months with this mutant, potentially decreasing production costs.

The use of high-pressure homogenization (HPH) to accelerate yeast autolysis in sparkling wines in Franciacorta, Italy, was investigated. Results showed that HPH-treated yeast strains (except L951) significantly modified the ester profile with lower medium- and long-chain fatty acid concentrations compared to wines without HPH treatment.¹³⁸ HPH reduced aging time for most yeast strains without enzyme addition. Its role in reducing medium- and long-chain fatty acids could be utilized in the future to reduce high levels of medium-chain fatty acids to prevent stuck MLF.⁵⁹ Although the volatile aroma profile of sparkling wine is dependent upon the kinetics of volatile retention and release by yeast lees during aging, further research is needed to study the effects of HPH on sensory attributes of sparkling wine and foaming ability.⁷⁶

■ YEAST AUTOLYSIS COMPOUNDS AND THEIR IMPACT ON SPARKLING WINE QUALITY

The autolysis of yeast during aging results in the release of various compounds that modify the physical and organoleptic properties of sparkling wine.

Changes in the Nitrogen Compounds Present at Different Stages in the Traditional Method. Nitrogen release is thought to reflect the autolytic activity of the yeast-proteolytic activity in particular. Yeast autolysis does not begin until 3–4 months after the end of the secondary fermentation. The total amino acid concentration increases before the increase in free amino acid concentration. Thus, peptides are released into the medium and then broken down into amino acids. The change in the various nitrogen fractions during bottle-fermented sparkling wine aging has been studied.¹⁸⁰ Between 3 and 9 months after addition of the *tirage* solution, no change in free amino acid concentration had been observed, regardless of the grape variety used. Free amino acid concentration increased after 9 months, indicating the start of autolysis, and these results have been confirmed.¹⁷⁹ Peptide content fluctuates, peaking after 12–15 months of aging on the lees and decreasing thereafter. This behavior may reflect an initial release of peptides that are subsequently broken down. The amount of peptides released by yeast autolysis during sparkling wine aging is variable and depends on grape variety and aging time.¹⁸¹ The nature of the peptides released also changes with aging; the length of the peptides released decreased with increased aging time.^{182,183}

The amino acid composition of the peptides present in sparkling wines has been investigated.^{180–184} The peptides mostly originate from the breakdown of yeast proteins rather than grape juice proteins. An increase in protein and polypeptide levels during the first 3 months, followed by a decrease attributed to protease activity, has been reported.¹⁸² Protein and peptide contents then increased again after 6 months. Amino acid enrichment of the medium may improve the aromatic potential of sparkling wine, as amino acids are the precursors of aroma compounds. Aroma compounds may be generated by the deamination or decarboxylation of amino acid.¹⁶⁵ The levels of one lactone compound, 3-hydroxy-4, 5-dimethyl-2(*SH*)-furanone, also known as sotolon (green nut, curry odor), gradually increase during the aging of sparkling wine. Pham et al.¹⁸⁵ showed that sotolon is generated from threonine, which is transformed into α -ketobutyric acid that reacts with acetaldehyde. Vitispirane, a norisoprene-derived compound imparting eucalyptus aromas, is synthesized from methionine and has been detected in aged cava wine.¹⁸⁶

The characteristics of foam are an extremely important property of sparkling wine that is usually measured for foam height, foam area, and foam collar.¹⁷⁹ Moreno-Arribas et al.¹⁸⁷ suggested that the hydrophobicity of peptides might account for the foaming properties of sparkling wine. A few years later it was found that foam characteristics were positively correlated with the concentrations of most free amino acids and proteins, confirming the results of Malvy et al.^{183,188} However, no relationship was found between foam characteristics and the concentration of wine peptides.

Polymers. Glucanases and proteases release polysaccharides from the yeast cell wall during autolysis in sparkling wines. These macromolecules contain mainly glucose (74%) and mannose (26%). Polysaccharide concentrations in wines vary. An increase in polysaccharide content from 366 mg/L in base wine to 602 mg/L after 9 months of aging has been reported.¹⁸⁹ The effect of colloids (macromolecules) on foam quality has also been investigated.¹⁹⁰ Neutral polysaccharides are important for foam quality due to their highly significant, positive effect on foam stability.¹⁸⁷ A recent study investigated the impact of polysaccharides from Spanish varieties (Verdejo, Macabeo/Viura, Malvasía, Albarín, Godello, Prieto Picudo, Tempranillo, and Garnacha) on foaming properties.¹⁸⁴ Polysaccharides (mannoproteins, polysaccharides rich in arabinose and galactose, homogalacturons, glucans, and rhamnogalacturons type II) did not correlate with foamability [maximum height reached by foam after CO₂ injection (HM) or foam stability height during CO₂ injection (HS)].¹⁸⁴ The model to explain HS in the study was only predicted by polysaccharides rich in arabinose and galactose. However, there were positive correlations between all of the wine polysaccharides and foam stability time [defined as the time that elapsed before bubble collapse and the appearance of liquid after the interruption by CO₂ (TS)], except for rhamnogalacturonans type II. The optimum aging time for obtaining a high-quality, stable foam appears to be 18 months. Foam quality decreases after 18 months, which coincides with an increase in the level of monomeric compounds, such as fructose, that probably arise from the hydrolysis of grape components by yeast enzymes released during autolysis.⁵⁵

Lipids are important components of sparkling wines because they are a major source of flavor compounds and affect foam stability.¹⁹¹ Changes in the lipid content of sparkling wine have been the focus of several studies. Lipid content increases during the secondary fermentation, and qualitative changes occur during aging in the bottle in contact with the lees.^{192,193} The concentration of polar lipids decreases, whereas the concentration of neutral lipids increases (monoglycerides, diglycerides, triglycerides). Conflicting results have been published concerning the influence of lipids on foam. Octanoic and decanoic fatty acids have been found to reduce foam stability, but it was also reported that the addition of a lipid mixture did not affect the foam.^{77,194} It has been noted that linolenic and palmitoleic acid levels were the best indicators of foam stability, and the effect of fatty acids on the foaming properties of wine was investigated.^{49,195} The C8, C10, and C12 acids had a negative effect on foam quality, whereas ethyl esters of hexanoic, octanoic, and decanoic acids had a positive effect.

RNase is active during autolysis in Champagne, but data on the extent of nucleic acid degradation should be interpreted with caution because organic acids, phenolic compounds, peptides, and other compounds in wine can interfere with the measurement of nucleotides.¹⁷¹ Monophosphate nucleotides

(5'-UMP, 5'-GMP, and 5'-IMP) have unequivocally been identified in Champagne wine aged on lees.^{196,197} Nucleotide monophosphate concentration ranged from 50 to 500 µg/L. Recently it was demonstrated that aging on lees leads to an increase in uridine concentration, whereas purines appear as fermentative catabolites, which are derived from nucleosides or ATP degradation.¹⁹⁸ The nucleotides, especially monophosphate nucleotides, are used as flavorings in the food industry, but further studies are required to evaluate their impact on wine flavor.¹⁹⁹

Volatile Compounds Released during Autolysis. Heavy acyl chain esters have been identified in model and sparkling wines.¹⁷⁷ Terpenic alcohols and higher alcohols are also released during autolysis. Geraniol and α -terpineol, citronellol, and farnesol have all been identified. These compounds are perceived by tasters at low concentrations, from 100 to 300 µg/L. Molnar et al.¹⁷⁷ suggested that farnesol could greatly contribute to the aromatic quality of sparkling wine, and Loyaux et al.²⁰⁰ suggested that nerolidol makes a similar contribution to Champagne wines. Approximately 10 aldehydes have been identified in sparkling wines.¹⁶⁸ Methyl-3-butanal is the most abundant, accounting for 40% of all aldehydes present, and may be formed through a mechanism involving isoamyl alcohol oxidation. Most of the aldehydes identified are present at levels close to, or greater than, the detection threshold of the human nose for aqueous solutions, but their odor active values in ethanolic solution with CO₂ (sparkling wine) is unknown.

Volatile aroma compounds released during autolysis were characterized in 221 cava wines (classified into four groups according to their aging time), and diethyl succinate was identified as a possible age marker throughout the aging period.²⁰¹ Acetates appeared to decrease during aging, whereas diethyl succinate, vitispirane, and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) levels increased over time. Hexanol and 2-phenylethanol were also released during autolysis. Compounds such as vitispirane, TDN, and diethyl succinate may be good age markers and can discriminate between young and aged sparkling wines. Similar results have been obtained, and the researchers reported that some high molecular weight acetates and ethyl and isoamyl esters are typical aroma compounds in young cava wines, whereas vitispirane, diethyl succinate, TDN, hexanol, and ethyl acetate are typical aroma compounds in cava wines aged over a prolonged period.²⁰² The release of these aromas by yeast enzymes acting on glycoside precursors has been identified as a possible mechanism for their formation, with C13-norisoprenoids and vitispirane being derived from glycoside-bound carotenoids and megastigma, respectively.²⁰²

The concentration of esters has been found to decrease with aging in the presence of lees, especially 5-hydroxymethyl-2-furfural (5-HMF), synthesized from the dehydration of sugar (mainly fructose) and formed from the intermediate stage of the Maillard reaction.^{203–205} This stage begins with the Amadori/Heyns products leading to sugar fragmentation products, that is, 5-HMF, in aged bottled-fermented sparkling wines.^{204–206} 5-HMF has been described as contributing caramel, butter, musty, sharp, animal, and soap aromas to wines.^{204,208,209} However, its odor activity threshold in sparkling wine and contribution to aged sparkling wine aroma, flavor, and quality remain to be determined. Fedrizzi et al.²¹⁰ found that different lengths of time on lees did not affect sulfur compounds in Chardonnay or Chardonnay/Pinot noir sparkling wines, but ethyl pyroglutamate was found to be

much higher in lees aged wines than still table wines aged on lees, which correlated with aging. Still table wines can age on lees in stainless steel tanks or oak barrels, depending on wine style and variety, but bottled-fermented sparkling wines age on lees that are in the bottle from secondary fermentation.

Benzenemethanethiol, 2-furanmethanethiol, and ethyl 3-mercaptopropionate were found in concentrations higher than their perception thresholds in aged wines, suggesting an important role in the aroma of aged Champagne wines.²¹¹ In the study 3-mercaptopropan-1-ol (3MP) in aged Champagne wines was 4–10 times its perception threshold, which can contribute to the fruity aroma of Champagne wine. Differences in the phenolic concentration of various sparkling wine grape varieties have been reported.²¹¹ However, lees aging did not significantly affect phenolic composition; rather, viticultural effects, vintage variation, and grape variety are responsible for phenolic differences in wines.

■ RIDDLING/REMUAGE

Prior to riddling, “Le Coup de Poigné” or “Poignettage” was used in Champagne but is less common today.²⁰⁷ The process involves taking the bottle and shaking it to stir up yeast lees and then placing it on its side so that lees are dispersed over 80 cm².²⁰⁷ This practice may break apart tartaric crystals if cold stabilization was not carried out on the base wine and could possibly assist with loosening yeast “streaks” to consolidate lees sediment.

Lees aging in bottle can be from 9 months to several years, depending on the regulations of the wine region and the wine style, and is followed by *remuage* (riddling) to move sediment to the neck of the bottle.¹⁷⁶ The gradual and controlled turning of the slanted and inverted bottles brings the yeast and adjuvants (bentonite or bentonite/alginate) together.¹⁴⁶ Riddling time has been reduced to 3–4 days with free yeasts and to 2 days with agglomerated yeasts, using automated riddling machines with 504 bottles in each cage.¹⁴⁶ The method of riddling does not affect O₂ intake. A recent promising innovation in sparkling wine technology has been developed.²¹² Researchers attached to yeast cells magnetic iron oxide maghemite ($\gamma\text{-Fe}_2\text{O}_3$) that did not penetrate the yeast cell wall. These magnetized yeasts increased secondary fermentation time, and simplified and accelerated yeast removal from the bottle.²¹² The iron oxide nanoparticles are considered nontoxic and are approved by the U.S. Food and Drug Administration (FDA) for in vivo medical applications and did not exceed the permissible iron limit in European wine regulations.²¹² However, the effect on sparkling wine quality as well as yeast multiplication, foaming, oxidative potential, and long-term lees aging is currently unclear. This new magnetized yeast removal method could have a significant impact on production costs by removing the need for riddling, gyro-palette equipment, and freezing bottleneck in glycol, decreasing riddling time and production costs (especially equipment and energy costs) of bottle-fermented wine production.

■ DISGORGING/DÉGORGEMENT

Disgorging is the removal of the yeast sediment and adjuvants from the bottle. It is currently performed by inserting the neck of the bottle into a glycol or calcium chloride solution, which freezes (−25°C) the yeast sediment in the *bidule* (a small plastic cap that fits inside the crown cap to catch yeast sediment).⁷⁶ Bottles are then picked up and quickly placed

neck up; the crown cap is removed, and pressure ejects the *bidule* along with the iced sediment.⁷⁶ Marks and Morris investigated the use of ascorbic acid and SO₂ additions at disgorging on *V. vinifera* Chardonnay and Riesling sparkling wines and four sparkling wines made from hybrid cultivars (Cayuga white, Vidal, Chancellor, and Chardonnell) in Arkansas (USA). The aim was to assess the efficiency of ascorbic acid, SO₂, and a combination of the two on the oxidation of sparkling wine post-disgorging after 11 months of lees aging. SO₂ levels declined in all cultivars after the storage period, but there was no added benefit of including ascorbic acid in the *dosage*. Higher acetaldehyde levels were reported in wines after storage with SO₂ only added. Wines made from Cayuga white and Chancellor grape varieties had reduced browning and reduced color intensity when ascorbic acid was added compared to Chardonnay, Riesling, Vidal, and Chardonnell. The results indicated that ascorbic acid could be used in sparkling wine production to prevent oxidation, but only for certain cultivars.²¹³ Some studies suggest that closure is mainly responsible for the SO₂ decline and post-bottling oxidation.^{214–216,218–220} In addition to the closure effect, the presence of the yeast (*sur lattes* bottles) in the traditional method can explain the rapid decrease of SO₂ over time. Some CO₂ is also lost at disgorging, but rapid closing with traditional cork, technical cork, or other closures prevents excessive CO₂ loss. After corking the bottle, dissolved and gaseous CO₂ quickly recovers equilibrium.⁷²

“Gushing”. Gushing during disgorging has been attributed to a number of factors, specifically tannin from red grape varieties, bottle shaking, ambient temperature during disgorging, insufficient bottle cleaning, cork dust, ultraviolet (UV) lighting in the disgorging area (especially natural sunlight from open doors or through windows on bottles waiting to be disgorged), incomplete riddling, lack of tartaric stabilization, and protein instability.^{217,221,222} However, there is a lack of published studies fully investigating the causes in sparkling wine.^{221,222} The effect of gushing causes financial loss due to reduction of wine and a decrease of bottling-line speed and efficiency, but its impact on foam, volatile compounds, and flavor in the finished product has not been researched (gushing can generate various levels of O₂ intake before corking.²²⁰ Recent studies focused on beer gushing found that some proteins (hydrophobins and LTP) can be responsible even at very low levels (ppb scale) for beer gushing.^{223–225} These particular compounds, the hydrophobic biopolymers of medium MW, are excreted from some fungi (*Fusarium* and other barley contaminants) and remain stable during beer production process. No evidence exists of work being conducted into the link between hydrophobic biopolymers and gushing in sparkling wine. It is likely that some grape contaminants could exhibit the same behavior as that previously described in beer. This would provide further information about “unexplainable gushing” in sparkling wines that lack tartaric crystals, turbidity, MLF in bottle, or riddling issues as suggested by Liger Belair et al.^{226,227} The beer foaming gene, *CFG1*, has been isolated from *Saccharomyces pastorianus*, and similar fermentation foaming genes in *S. cerevisiae* are *Awalp* and *Fpg1p*. These are cell wall mannoproteins, although their contribution, if any, to gushing and effervescence in sparkling wine remains unknown.^{228,229}

“Jetting”. Oxygen is a major contributor to wine quality; therefore, managing oxygen ingress is of utmost importance during bottle-fermented sparkling wine production.²³⁰ Jetting has been developed to reduce flavor and aroma bottle variability

from the oxygen in the neck of the bottle at disgorging.²³¹ The beer industry has used this technology for several decades to avoid air intake (beer is very sensitive to oxidation). Immediately after disgorging when the headspace is 25 mL, there is potentially >7 mg of O₂ per bottle.²³² When the wine is removed, there is potentially >10 mg of O₂ per bottle. Jetting is the insertion of 100 μ L of wine into the bottleneck to induce foaming and is being used in some Champagne wine production at bottling.²³¹ The foam acts as a piston, reducing oxygen ingress in the neck space because the cork is then inserted when the foam is high. Results of a blind test sensory analysis in Champagne found that 31 of 41 panelists preferred the jetted samples, describing them as *fresh* and *fruity*.^{230,231}

Liqueur d'Expedition/Dosage. Prior to corking, the *liqueur d'expedition* (*dosage* solution) is inserted into each bottle, and it can give each wine its own unique flavor, for example, ice wine *dosage* in Ontario. The recipe for the *dosage* varies among wineries but can consist of sugar (cane or beet), the oldest sparkling wine in the cellar, oaked/unoaked still Chardonnay wine, wines aged in stainless steel, oak, or/and concrete vessels, with SO₂, citric acid, and very occasionally brandy (now illegal in Champagne), tannins, and/or copper sulfate.^{19,76} To date there are no published studies regarding sugar types/sources in *dosage* on sparkling wine quality. *Zero-dosage* is becoming more popular and contains no added sugar. This type of "no-*dosage*" is reserved for special *premium* wines in Champagne. However, it has also recently been used in a scientific study of alternative varieties for sparkling wine. In the study *liqueur d'expedition* was not added to any of the research wines. Each bottle was filled with the same wine to produce Brut Nature wines for the laboratory and sensory analysis.⁴ A recent study that used a range of wine styles in the *dosage* solutions found no difference in ethyl ester concentrations in brut sparkling wines (8 g/L residual sugar) by 15 weeks post-disgorging.²³² Interestingly, the *zero-dosage* wines (same wine inserted without sugar) showed an increase in ethyl isovalerate (fruity/minty), which the sugar added wines lacked. The wines with the same wine inserted but with sugar addition (8 g/L) showed slight increase in ethyl butyrate (artificial fruit/candy/strawberry) and ethyl isobutyrate (apple/tropical fruit/citrus). However, the effect on volatile wine compounds when aging under cork post-disgorging or the effect on sensory properties of the wines was not studied. Higher sugar additions may have more of an impact on volatile aroma compounds in sparkling wine than the 8 g/L residual sugar in the trial wines. The contribution of fusel alcohol pathways (anabolic and catabolic) that arise primarily from hexoses in wine fermentation has recently been studied.²³³ The rate of sugar hydrolysis has been found to differ between grape varieties. It has been suggested that the natural hydrolysis of a 10 g/L sucrose addition to a still wine (pH 3–3.4) devoid of invertase activity will take 2.5–5.5 months.²³⁴ The length of time between disgorging and release of sparkling wine for sale is a decision by the wine producer, but the flavor of sparkling wine during the post-disgorging time is known to differ from 1 to 3 months and continuously in bottle thereafter. However, further flavor analysis of sparkling wine aging post-disgorging will clarify the aroma and flavor chemistry occurring in the bottle. It has been demonstrated that disgorging leads to a significant increase in the concentrations of thiols, specifically 2-furanmethanethiol (toasty aroma), benzenemethanethiol (smoky aroma), and ethyl 3-mercaptopropionate (foxy aroma), in two Champagne wines across a range of vintages.²³⁵ Oxygen ingress at disgorging can negatively affect sparkling wine

quality, and comprehensive reviews about oxidation mechanisms in wines have already been published.^{219,236,237}

■ SENSORY ANALYSIS OF SPARKLING WINES

There is no internationally accepted or recognized sensory analysis method that has been specifically designed for sparkling wine and no published criteria to evaluate effervescence and foam of sparkling wines.²³⁸ Hedonic scales following panelist training to obtain consensus-based descriptors have been used by visual assessment of foam formation and effervescence as well as questionnaires during tastings, but the method utilized depends on the aims and objectives of the research.^{136,238–240} Although serving temperature, bottle opening method, pouring method, room temperature, and lighting are controlled, studies lack attention to glass type, sample order effect on foam and flavor, and the time between opening bottles and then pouring and imbibing.^{90,240,242} Sparkling wine descriptors have been generated in studies and used in sparkling wine sensory analysis that includes olfactory intensity, equilibrium, fruity, full body, vegetal, acidity, persistence, floral, varietal, bitterness, freshness, and astringency.^{96,243} Foam attributes have included initial foam, foam collar, bubble size, effervescence, and foam and require panelists to be trained in the use of these descriptors.^{96,244} A fixed-choice profiling (FCP) method was used whereby each descriptor definition was built from the consensus of the panelists.²⁴⁵ Initially 64 descriptors were generated, but this was reduced to 19. Wines were poured 5 min before panelists entered the room, so foaming height and stability were not included in the sensory analysis, which are essential to sparkling wine quality. A modified-descriptive analysis method for sparkling wine sensory analysis using calibrated glasses (cm/mm) to measure foam height would help with standardizing a sparkling wine sensory method. Criteria suggestions for consideration include (1) a specific time between opening pouring and tasting the wines or (2) immediate assessment of each wine by panelists as soon as their sample is poured, (3) set room and bottle temperature, (4) storage guidelines before tasting, (5) standardized bubble and foam classifications including persistence, stability, and collar, (6) agreed visual, aroma, and palate descriptors for sparkling wine styles, and (7) number of times to taste the same wine while minimizing bottle variation by allowing panelists to taste at least three bottles for replication purposes.

The odor activity value (OAV) of an aroma compound is an indication of the importance of a specific compound to the aroma of a wine, calculated as the ratio between the concentration of an individual compound and the perception threshold described in the literature.⁷ Until perception thresholds of volatile aroma compounds are determined in a range of sparkling wines (monovarietal and blends) for each compound, it is difficult to state with any certainty their contribution to sparkling wine aroma profile. For instance, the two 3MH enantiomers, *R* and *S*, have different aromas: *R* is fruitier with a grapefruit aroma, and *S* produces aromas reminiscent of passion fruit, which are likely to contribute differently to sparkling wine aroma profiles.²¹¹ However, although OAVs can provide important information, they do not take into account the interactions between volatile compounds and other wine compounds that can enhance or suppress aromas.²⁴¹ It is also likely that CO₂ affects odor detection when compared to the same aroma compound in an aqueous or ethanolic solution.

This review of sparkling wine production has discussed current knowledge and highlighted areas for future research that include grape varieties, *tirage* and *dosage* effects on sparkling aroma and flavor chemical composition, the need for a specific sensory analysis method for sparkling wine, markers associated with sparkling wine quality (especially fermentation byproducts from secondary fermentation), and reducing the time and efficiency of MLF.⁶² Future studies are likely to include chemical and sensory analysis related to post-disgorging aging effects on wine flavor and aroma, research related to red and rosé sparkling wines, length of lees aging for zero-*dosage* wines, low-SO₂ wines, SO₂ alternatives for sparkling wines, the effect of using different yeasts for first and secondary fermentations, acceleration of yeast autolysis, reduction of time for MLF, and enzymes involved in yeast autolysis. The mechanism for induction of yeast autolysis remains elusive as are the kinetics of glucanase activity linked to wine quality by its ability to release mannoproteins and the effect on the release of nucleotides, nucleosides, and lipids. If the chemical profile of Chardonnay including acids, genes, and proteins can be identified, then similar varieties with similar traits, and therefore aging characteristics, could be sought in sparkling wine regions. Research regarding phenolic compounds in sparkling wine is likely to focus on different grape varieties to provide information for phenolic management techniques to winemakers, especially for color stability of rosé and red sparkling wines. Research is likely to focus on “alternative” or “resurrected” varieties for sparkling wines and is likely to include the impact of non-*Saccharomyces* yeast on sparkling wine aroma, flavor, and foaming. Studies that investigate natural plant-based products from vines/grapes/yeast for use in the production process are already underway and likely to continue. The authors are aware of an international sensory analysis method currently being developed with a team of international sensory scientists, specifically for sparkling wine assessment. Although not covered in this review, organic and biodynamic effects on sparkling wine quality, plus the economical, financial, and ecological sustainability of sparkling wine production practises, are likely to be a focus of future research.

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