Post-Fermentation and -Distillation Technology

Stabilization, Aging, and Spoilage



Edited by

Matteo Bordiga

CRC Press
Taylor & Francis Group

Post-Fermentation and -Distillation Technology

Stabilization, Aging, and Spoilage

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Post-Fermentation and -Distillation Technology

Stabilization, Aging, and Spoilage

Edited by
Matteo Bordiga

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper

International Standard Book Number-13: 978-1-4987-7869-5 (Hardback)

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

To My Son

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Contents

Preface ix
Editor xi
Contributors xiii

Chapter 1 Fermentation Process 1
Tiziana Nardi and Matteo Bordiga

Chapter 2 Distillation Processes and Distillates 41
Frank Vriesekoop and Dawid Ostrowski

Chapter 3 Proteins and Bentonite in Winemaking: Chemical and Practical Aspects and Sensory Consequences 81
Federico Casassa

Chapter 4 Wine Aging and Spoilage 113
Pilar Rubio-Bretón, Teresa Garde-Cerdán, Juana Martínez, Ana Gonzalo-Diago, Eva P. Pérez-Álvarez, and Matteo Bordiga

Chapter 5 The Stability and Stabilization of Beer 159
Paul S. Hughes

Chapter 6 Beer Conditioning, Aging, and Spoilage 183
Roger Barth

Chapter 7 Vinegars: Process, Aging, and Spoilage 207
Paolo Giudici, Tommaso Bonciani, Federico Lemmetti, and Giuseppe Corradini

Chapter 8 Stabilization of Distilled Spirits 235
Paul S. Hughes

Chapter 9 Distillates Cask Aging 251

Thomas Collins

Chapter 10 Marketing Beer, Wine, Spirits, and Vinegar: Who Are the Consumers? What Do They Want? How to Talk to Them so They Hear You 263

Julie Kellershohn and Inge Russell

Index 275

Preface

This book focuses on the post-fermentation and -distillation technology applied to wine, beer, vinegar, and distillates in a broad spectrum. Stabilization, aging, and spoilage represent the three major sections of the text. The book provides a comprehensive overview of all of the post-fermentation operations related to these products, focusing on the complex issue of their stability. Wines, for example, must be stable against microbial activity as well as undesirable chemical and physical–chemical reactions occurring in the bottle. There are five goals of "finishing" a wine: clarity, stability, compositional adjustment, style development, and packaging. It is important, especially in white wines, that the wine at the point of consumption not be cloudy or contain any haze or precipitate. Currently, haze represents a visual defect associated with spoilage in the eyes of the consumer.

It is also important to prevent unwanted microbial growth from occurring in the wine after the primary fermentation is complete, as this affects the flavor and aroma profile in unpredictable ways. *Saccharomyces* autolysis will replenish nutrients in the wine, making them available for other organisms. *Saccharomyces* do not consume all possible bacterial energy sources. Many spoilage organisms are obligate aerobes, so the wine must be protected against exposure to air once the carbon dioxide blanket generated during fermentation has dissipated.

Similarly, the discussion also involves beer, vinegar, and distillates. This represents a novel approach, not limiting the book to the issues of aging, stabilization, and spoilage of wine. Covering three other main fermentation categories certainly makes this project interesting. New technologies as well as new materials are included in the discussion (e.g., square barrels, synthetic closures, tetra pak®). The book is a good mix of referenced research with practical applications, also reporting case studies of these various applications of novel technologies. Consumer packaged goods companies are forced to re-evaluate packaging formats and materials, as products and their packaging are inextricably linked to the trends taking place within the consumption habits. At the same time, as consumers grow more concerned about energy consumption and the environment as a whole, they are also increasingly demanding more eco-friendly and socially conscious products and packaging.

Matteo Bordiga

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Editor

Dr. Matteo Bordiga received his PhD in food science from the Università del Piemonte Orientale, Novara, Italy in 2010. He received his MS in chemistry and pharmaceutical technologies from the same university. Dr. Bordiga's main research activity concerns food chemistry, investigating the different classes of polyphenols from analytical, technological, and nutritional points of view. More recently, he moved his research interests towards wine chemistry, focusing his attention on the entire production process—from vine to glass. He has published more than 30 research papers in peer-reviewed international and national journals. Since 2013, he has been an editorial board member of the *International Journal of Food Science & Technology*. In 2014, he was appointed editor-in-chief for *Wine Studies*, a peer-reviewed international open-access journal focused on wine-related topics. He served as editor of the book, *Valorization of Wine Making By-Products* (CRC Press/Taylor & Francis Group, 2015). All his research activities have been developed through important collaborations with foreign institutions (Foods Science and Technology Department and Foods for Health Institute, University of California, Davis; Fundación Parque Científico y Tecnológico de Albacete, and Instituto Regional de Investigación Científica Aplicada, Universidad de Castilla-La Mancha, Ciudad Real, Spain).

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Contributors

Roger Barth
West Chester University
West Chester, Pennsylvania

Tommaso Bonciani
University of Modena and Reggio Emilia
Reggio Emilia, Italy

Matteo Bordiga
Università del Piemonte Orientale
Novara, Italy

Federico Casassa
California Polytechnic State University
San Luis Obispo, California

Thomas Collins
Washington State University
Richland, Washington

Giuseppe Corradini
University of Modena and Reggio Emilia
Reggio Emilia, Italy

Teresa Garde-Cerdán
Institute of Vine and Wine Sciences (CSIC-CAR-UR)
Logroño, Spain

Paolo Giudici
University of Modena and Reggio Emilia
Reggio Emilia, Italy

Ana Gonzalo-Diago
Institute of Vine and Wine Sciences (CSIC-CAR-UR)
Logroño, Spain

Paul S. Hughes
Oregon State University
Corvallis, Oregon

Julie Kellershohn
Ryerson University
Toronto, Ontario, Canada

Federico Lemmetti
University of Modena and Reggio Emilia
Reggio Emilia, Italy

Juana Martínez
Institute of Vine and Wine Sciences (CSIC-CAR-UR)
Logroño, Spain

Tiziana Nardi
Council for Agricultural Research and Economics
Research Center for Viticulture and Enology (CREA-VE)
Conegliano, Italy

Dawid Ostrowski
Ostra Distillers
Robinvale, Victoria, Australia

Eva P. Pérez-Álvarez
Institute of Vine and Wine Sciences (CSIC-CAR-UR)
Logroño, Spain

Pilar Rubio-Bretón
Institute of Vine and Wine Sciences (CSIC-CAR-UR)
Logroño, Spain

Inge Russell
Heriot-Watt University (ICBD)
Edinburgh, Scotland

Frank Vriesekoop
Harper Adams University
Newport, Shropshire, United Kingdom

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

1 Fermentation Process

Tiziana Nardi and Matteo Bordiga

CONTENTS

1.1 Wine 2
1.1.1 Alcoholic Fermentation 2
1.1.2 Malolactic Fermentation 3
1.1.2.1 Lactic Acid Bacteria in Grapes and Wine 3
1.1.2.2 The Chemistry of MLF 5
1.1.3 Sensory Impacts of Malolactic Fermentation 6
1.1.3.1 Acidity Reduction 6
1.1.3.2 Production of Flavor-Active Compounds 6
1.1.3.3 Risk of Organoleptic Defects 8
1.1.4 Current Knowledge on Malolactic Fermentation: Managing the Process 10
1.1.4.1 Physicochemical Parameters Affecting MLF Development 10
1.1.4.2 Use of Starter Cultures 10
1.1.4.3 Control of MLF to Avoid Further Microbial Spoilage 14
1.2 Beer 16
1.2.1 Microbial Aspect of Malting and Brewing 16
1.2.1.1 Barley 16
1.2.1.2 Malt 17
1.2.1.3 Wort 17
1.2.1.4 Beer 17
1.2.1.5 Beer Maturation Lagering Tanks with Single Jacket (Air Cooled) 18
1.2.1.6 Beer Maturation Lagering Tanks with Double Jacket (Liquid Cooled) 19
1.2.1.7 Brewing Yeast 19
1.2.2 Reaction Mechanisms of Aging Processes in Beer 20
1.2.2.1 Reactive Oxygen Species in Stored Beer 20
1.2.3 Aging Reactions Producing Carbonyl Compounds 22
1.2.3.1 Oxidation of Higher Alcohols 22
1.2.3.2 Strecker Degradation of Amino Acids 22
1.2.3.3 Aldol Condensation 22
1.2.3.4 Degradation of Hop Bitter Acids 23
1.2.3.5 Oxidation of Unsaturated Fatty Acids 23
1.2.3.6 Acetalization of Aldehydes 23
1.2.3.7 Maillard Reactions 24

1.2.3.8 Synthesis and Hydrolysis of Volatile Esters....24
1.2.3.9 Degradation of Polyphenols....24
1.2.4 Oxidative versus Nonoxidative Beer Aging....24
1.2.4.1 Antioxidants and Reducing Agents versus Pro-Oxidants....25
1.3 Vinegar....25
1.3.1 Overview....25
1.3.1.1 Wine Vinegar....25
1.3.1.2 Beer Vinegar....26
1.3.1.3 Fruit Vinegars....26
1.3.1.4 Balsamic Vinegar....26
1.3.1.5 Malt Vinegar....27
1.3.1.6 Rice Vinegar....27
1.3.1.7 Cane Vinegar Production....27
1.3.1.8 Others....28
1.3.2 Vinegar Production....28
1.3.2.1 Bacterial Cultures....28
1.3.2.2 Processing Methods....28
1.3.2.3 The Orleans Method....29
1.3.2.4 Generator Method....29
1.3.2.5 Submerged Fermentation Method....30
1.3.3 Quality Control....31
1.3.4 Maturation and "Oaking" of Vinegar....32
References....34

1.1 WINE

1.1.1 Alcoholic Fermentation

Winemaking represents the process of wine production, from the selection of grapes to the bottling of finished wine. Grapes must be healthy, without defects due to bacterial and/or fungal infections. Winemaking must be performed as soon as possible to prevent wine defects. If grapes are damaged during harvest and/or transport to the winery, issues or defects may arise due to the activity of acetic and/or lactic bacteria. It is possible to obtain about 70 L of must from 100 kg of grapes. The stem, generally separated from the grapes and discarded, represents a lignified vegetable structure containing a high concentration of polyphenols. Polyphenols and aroma compounds are present in the skin of grapes. Acids (tartaric, malic, and citric—about 0.5%–1.5% w/v), sugars (fructose and glucose—about 15%–30% w/v), and small quantities of aromatic compounds and polyphenols are present in the pulp.

Grape crushing represents the first activity of winemaking. Usually, grapes are treated with a mechanical crusher/destemmer. Once crushed, berries are then transferred to the fermentation tank. For white wine production, grape must be treated very carefully because the compounds present in the skin and stem must not pass into the must. Usually, for this typology, crushing is generally obtained with a simple grape pressing. When the must has been prepared, selected yeasts, sulfur dioxide, and nutrient substances are added. Yeasts are generally already present on the grapes

and in the winery environment but they can give detrimental results. For this reason, selected commercial strains of yeast are added to the must. Sulfur dioxide is used in winemaking (generally at 50–100 mg/L) due to its inhibitory activity against the natural microflora (bacteria and yeasts) in the juice, thus facilitating the action of the selected yeasts added to the must. Another activity related to this compound acts to inhibit oxidative enzymes responsible for wine browning. Ammonium salts and vitamins (biotin and thiamin) are often added as nutrients for the yeast. If the sugar concentration is low, sugar may be added. However, this addition is strictly subject to local regulations. For example, in Italy it is only possible to add concentrated and rectified must, not sucrose. After about 10 hours from inoculation with yeast, the primary, alcoholic fermentation (AF) starts. This fermentation generally lasts for about 8 days and, during this period, the yeast cells metabolize the sugars in the must, producing carbon dioxide gas and alcohol. The temperature during the fermentation affects the taste of the product. The proper temperature for red wines is typically from 25°C to 28°C, while for white wines it is from 20°C to 25°C. Other substances (minor products) are produced during alcoholic fermentation (e.g., glycerol, acetic acid, higher alcohols, and acetaldehyde). Wine quality is also defined by low concentrations of acetic acid, higher alcohols, and acetaldehyde.

1.1.2 Malolactic Fermentation

Malolactic fermentation (MLF) represents the "second fermentation" of wine that in traditional winemaking used to take place during storage. In modern winemaking the timing of occurrence of MLF is advancing from springtime to autumn (in the Northern Hemisphere), taking place after alcoholic fermentation or during alcoholic fermentation, due both to climate change and to an increasingly careful management of this step. Nevertheless, MLF is the microbial transformation that, more than others, affects postfermentation stages such as aging, stabilization, and possible spoilage. In this context, MLF management has been recently recommended by the Organisation Internationale de la Vigne et du Vin (OIV), together with other biological methods, as a good winemaking practice to avoid wine spoilage that causes major economic losses (OIV, 2014).

1.1.2.1 Lactic Acid Bacteria in Grapes and Wine

Lactic acid bacteria (LAB) perform malolactic fermentation and constitute a ubiquitous group of bacteria that occur in a range of environments, including many foods and beverages. In the oenological environment, they can be found throughout all stages of winemaking. LAB can be isolated on many surfaces and environments including grapevine leaves, grapes, various winery equipment, and barrels. At harvest, low numbers (fewer than 100 cells/g) of LAB can be found on grapes, although acetic bacteria and yeast are found in much higher numbers (Fugelsang, 1997). Lactic acid bacteria display high morphological and physiological diversity; in fact, the term lactic acid bacteria emerged at the beginning of the twentieth century to describe a heterogeneous group of bacteria that are currently defined as spherical (cocci) or rod-shaped (bacilli), Gram-positive, catalase-negative, immobile, nonsporulating, anaerobic, aerotolerant producers of lactic acid as a primary metabolite

of sugar (Fugelsang, 1997). Concerning the specific environment of winemaking, the most studied and well-known species of LAB is *Oenococcus oeni*s because it is the predominant species conducting the biological transformation called malolactic fermentation. Nevertheless, the LAB composition of grape must at the beginning of the alcoholic fermentation is much more variable (the most common isolates are part of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Oenococcus*) and generally dominated by *Lactobacillus* species (Betteridge et al., 2015). *Pediococcus* can be found mostly after the MLF as well as in wines of higher pH. Indeed, wine does not differ from other foodstuffs in which LAB are responsible for a critical fermentation step (here, MLF), but they can also cause changes that adversely affect the organoleptic properties of the final product. Therefore, a relevant part of studies about lactic acid bacteria in foods is the study of the negative effects they have on the quality and composition of the final product. A number of recent molecular identification studies have detected new species of lactic acid bacteria in both musts (*Lactobacillus bobalius*, Mañes-Lázaro et al., 2008a; and *Lactobacillus uvarum*, Mañes-Lázaro et al., 2008b) and wines (*Lactobacillus nagelii*, Edwards et al., 2000; *Lactobacillus vini*, Rodas et al., 2006; and *Lactobacillus oeni*, Mañes-Lázaro et al., 2009). Moreover, in recent studies, genera and species not frequently detected in wines such as *Weissella*, *Fructobacillus*, and species such as *Oenococcus kitaharae* and *Lactobacillus fabifermentans* were identified during AF (González-Arenzana et al., 2016) or in grape marc after fermentation (Maragkoudakis et al., 2013). The density of LAB in the initial phases of winemaking (the must phase and first stages of AF) ranges from approximately 103 to 104 colony forming units/mL. Afterwards, only a few species are capable of surviving in grape must and fermenting wine because of the hostile conditions that they encounter: mainly low pH, a lack of nutrients, and the presence of ethanol.

In an interesting recent work, molecular advanced techniques—next-generation sequencing (NGS)—have been used to obtain a more complete picture of how microbial communities change during grape fermentation and how different fermentation techniques might affect the microbial community composition, including bacteria with undesirable effects on wine quality (Piao et al., 2015). A better understanding of the microbial dynamics and their effect on the final product will likely be of great importance to help winemakers produce wine styles of consistent and high quality in the future. Both implantation effectiveness and bacterial metabolism can differ widely depending on species and strains involved in MLF. In general, lacking heme-linked cytochromes and catalase, all LAB obtain energy from carbohydrates by fermentative metabolism (Kandler, 1983). Overall, the LAB group can utilize a wide range of carbohydrates, including the hexoses (glucose, fructose, mannose, and galactose), as well as other pentoses, polyols, and oligosaccharides. This capability is dependent on the species and strains involved, as well as the pH of the medium. Moreover, since malic acid cannot be used by wine LAB as a sole carbohydrate source (see next paragraph), the availability and utilization of fermentable carbohydrates in wine by LAB is essential to enable the onset of bacterial growth and the occurrence of MLF. Furthermore, recent studies have clearly demonstrated that grape-derived phenolic glycosides also significantly stimulate the growth of *O. oeni* (Schopp et al., 2013).

1.1.2.2 The Chemistry of MLF

The term "malolactic fermentation" describes the enzymatic conversion of L-malic acid to L-lactic acid and CO_2 by LAB (Wibowo et al., 1985); thus it does not technically refer to a fermentation process, but rather to a decarboxylation reaction (Figure 1.1). Malic acid is a major acid in wines; the conversion of a dicarboxylic acid (L-malic acid) into a monocarboxylic acid (L-lactic acid) increases pH and modifies the sensory profile of wine. In 1901, Seifert could already describe for the first time that lactic acid bacteria from wine transformed L-malic acid into L-lactic acid and carbon dioxide via a direct reaction, meaning that the intermediate pyruvic acid was not formed during this conversion (Munoz et al., 2011). This reaction is catalyzed by the enzyme malate decarboxylase—often referred to as the malolactic enzyme—that requires cofactors NAD+ and Mn++ (Lonvaud-Funel and De Saad, 1982; Spettoli et al., 1984). The malolactic enzyme, which was purified for the first time in *Lactobacillus plantarum*, has been found in all species of lactic acid bacteria isolated in wine (Munoz et al., 2011).

Malic and citric acids do not serve as the sole energy sources for the growth of LAB (Liu et al., 1995). Consequently, malolactic bacteria require sugars as a carbon source. However, under conditions of limiting sugar availability or of low pH, which inhibit sugar metabolism, energy (ATP) generated from MLF is beneficial to cell growth (Henick-Kling, 1993). Another, minor (<1%) activity of the malolactic enzyme has also been suggested to stimulate the metabolic activity and initial growth rates of wine LAB (Morenzoni, 2006). All wine lactic acid bacteria are able to perform the malolactic reaction. However, *Oenococcus oeni* is the major actor that carries out this fermentation since it is the best, well-adapted wine–associated species (especially when pH is beyond 3.5) and, for a long time, it has been used almost exclusively for the induction of malolactic fermentation in wines (Bartowsky et al., 2015; Wibowo et al., 1985). Among LAB, *Oenococcus* was separated from the genus *Leuconostoc* by 16S rDNA sequence analysis in 1995 (Dicks et al., 1995). For a long time, this genus only included one species: *O. oeni*. In 2006, *Oenococcus kitahareae* (Endo and Okada, 2006) was described, isolated from a composting distilled shochu residue and, more lately, a third species was ascribed to the genus *Oenococcus alcoholitolerans*, isolated from cachaça and ethanol fermentation processes (Badotti et al., 2014). In recent years, a number of scientific studies have focused on *Lactobacillus plantarum* as a potential starter for MLF, also leading to industrial interest in this genus (Berbegal et al., 2016; Iorizzo et al., 2016; Spano and Capozzi, 2011; du Toit et al., 2011). These studies open a perspective on the potential of *Lactobacillus* as the next generation of MLF starter cultures, discussing their occurrence during winemaking,

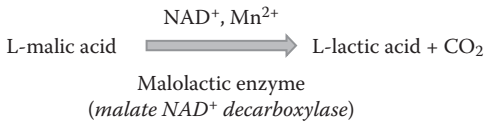

FIGURE 1.1 Reaction of decarboxylation, describing the enzymatic conversion of L-malic acid to L-lactic acid and CO_2 by lactic acid bacteria (LAB).

major metabolic activities, factors influencing their growth, and their performance in conducting MLF that will probably be further developed in the near future.

1.1.3 Sensory Impacts of Malolactic Fermentation

1.1.3.1 Acidity Reduction

The decarboxylation of malic acid to lactic acid is a biological deacidification reaction, well recognized as one of the main metabolic capabilities of LAB in wine, and its conduct is of major commercial importance to the winemaking process. Depending on the style of a wine, this enzymatic conversion can be beneficial or detrimental. It is a fact that the sensory impact of deacidification is particularly detectable and appreciated in wines with high acidity; thus the development of strategies to favor a microbial deacidification of low pH wines is particularly critical. Wines produced from grapes cultivated in cool climate areas contain a naturally high level of malic acid (up to 8 g/L) and are considered to benefit from an acidity reduction (Lasik, 2013). Moreover, in such wines the induction of MLF is often difficult to achieve due to harsh conditions (mainly, low pH), so it is important to prevent sluggish or stuck fermentation. Thus, many recent investigations focused on wines with high acidity (cool-climate whites, most of the sparkling base wines, and other varieties), improving knowledge in this field and proposing and optimizing techniques as the use of bacterial starters with early inoculation as efficient methods for achieving MLF (Guzzon et al., 2016; Knoll et al., 2012; Ruiz et al., 2012). On the other hand, wines produced from grapes grown in warm to hot regions have lower total acidity, and a further reduction in acidity from MLF can have a negative impact on their quality, causing a flat taste. Nevertheless, in wines characterized by high pH, biological deacidification is often desired because it results in improved microbial stability. In these cases, the impact of MLF is rather indirect, although the lack of acidity caused by this transformation is not strongly perceivable or even detrimental to freshness. It is usually accepted in order to remove nutrients (malic acid as a possible carbon substrate, and other compounds consumed by LAB) that would leave place for late, uncontrolled, and possibly incomplete malolactic fermentations (eventually ascribed to *Pediococcus* spp.) or for other microbial transformations including development of *Brettanomyces* in wines during aging (Morenzoni, 2006). Furthermore, a very recent frontier research approach introduced the perspective of using *Lactobacillus* strains with ability to induce biological acidification in low acidity grape musts to obtain more acidic wines, together with the achievement of malolactic fermentation. This recent study shows a selection of *Lactobacillus* strains that can grow in must, carry out MLF, and at the same time acidify grape must by synthesizing lactic acid from sugars. Indeed, homofermentative or facultative heterofermentative bacteria such as *Lactobacilli* are good candidates to be used as acidifying starters since they can synthesize only lactic acid from grape must sugars by lactic fermentation and have no danger of acetic acid synthesis (Lucio et al., 2016).

1.1.3.2 Production of Flavor-Active Compounds

In addition to the deacidification reaction that characterizes MLF, it is becoming increasingly recognized that a diverse range of other metabolic activities are

associated with the growth and development of LAB in wine, which can have a significant influence on wine quality. The complex aroma and flavor compounds found in wine largely originate from the grape, from yeast metabolism during alcoholic fermentation, and from oak when used. Bacterial metabolism during malolactic fermentation might contribute to wine flavor by the formation of additional compounds and the modification of grape-, yeast-, and oak-derived compounds (Swiegers et al., 2005). Indeed, MLF affects the final aroma and taste balance by modifying fruit-derived aromas and producing aroma-active compounds. Research has shown that LAB have the potential to impact the aroma profile of wine by

i. The production of volatile secondary metabolites
ii. The modification of grape- and yeast-derived metabolites including ethyl esters, acetate esters, primary terpene alcohols, glycoside-related aroma compounds, acids and alcohols
iii. The removal of existing flavor compounds by metabolism and adsorption to the cell wall (Bartowsky and Henschke, 1995; Bartowsky and Pretorius, 2009; Bartowsky et al., 2015; Lonvaud-Funel, 1999; Nielsen and Richelieu, 1999; de Revel et al., 1999; Ugliano et al., 2003)

In the same studies, flavor attributes imparted by MLF are usually described as buttery, lactic, nutty, yeasty, oaky, sweaty, and earthy; MLF may also impact fruity and vegetative aromas, as well as the mouthfeel of wine. Many of these alterations are strain dependent; however, the vinification technique can also affect the final wine aroma profile and these flavor impacts of individual bacterial strains are of great interest for winemakers (Lerm et al., 2010).

From a molecular point of view, over the last 15 years, research studies have deepened the characterization of *O. oeni* diverse array of secondary metabolic activities during MLF, which can modify the sensory properties of wine (Cozzolino, 2016). These secondary activities include the metabolism of organic acids, carbohydrates, polysaccharides and amino acids, and numerous enzymes such as glycosidases, esterases, and proteases, which generate volatile compounds well above their odor detection threshold. Recent studies using array-based comparative genome hybridization and genome sequencing of *O. oeni* strains have revealed the large genomic diversity within this species, confirming that phenotypic variation between *O. oeni* strains is central for producing different wine styles (Bartowsky and Borneman, 2011; Liu et al., 2016).

From an applicative point of view, results based on wide surveys usually show that lactic acid bacteria modify the fruity notes of wines but without a specific trend (Antalick et al., 2012), since the impact is strongly dependent on the strain carrying out the fermentation and on its inoculation timing. For example, in recent studies comparing aromatic compounds released from natural precursors by selected *Oenococcus oeni* strains during malolactic fermentation, some bacteria resulted in good candidates to be used when floral wines are desired, while others were the best preserving the fruity aroma of wines (Pérez-Martín et al., 2015; Sumby et al., 2013). Other works comparing the effects of inoculating grape must with malolactic bacteria at various stages of alcoholic fermentation (usually, the beginning of

alcoholic fermentation [co-inoculation with yeast], midalcoholic fermentation, and/or postalcoholic fermentation) on the wine chemical composition showed important differences. This often suggested that co-inoculation is a worthwhile alternative for winemaking if compared with traditional postalcoholic fermentation LAB inoculation or with spontaneous MLF (Abrahamse and Bartowsky, 2012; Cañas et al., 2015; Guzzon et al., 2016; Knoll et al., 2012). In general, more and more findings illustrate that MLF is an effective and novel way of modulating the volatile and aroma compound profile of wine.

1.1.3.3 Risk of Organoleptic Defects

Many secondary metabolites produced by bacteria are volatile and potentially affect negatively wine sensory qualities. Lactic acid bacteria can spoil wine during winemaking if MLF is not controlled, takes place too early, or gets stuck or, afterwards, during maturation and aging (Lonvaud-Funel, 1999). In the first case, deviations of metabolism by the fermenting bacteria (usually *O. oeni* or *L. plantarum*) during malolactic fermentation can affect wine quality. In the second case, growth of *Lactobacillus*, *Pediococcus*, and even some *Oenococcus* species in wine, happening after malolactic fermentation, can lead to numerous spoilage scenarios, as they can form undesirable aromas and flavor compounds. Overall, the chemicals involved in LAB-caused wine depreciation include compounds ranging from acetaldehyde to acetic acid, diacetyl, tetrahydropyridines, acrolein, beta-glucans, and biogenic amines, as summarized by Bartowsky (2009). Concerning organic acids, not only malic acid but also citric acid in the wine is metabolized by *O. oeni* and by numerous genera of the lactic acid bacteria, resulting in the production of acetic acid and diacetyl. One of the intermediary compounds in the metabolism of citric acid, diacetyl, is considered one of the most important flavors produced during MLF. When present at a concentration above the sensory threshold, diacetyl gives the wine an aroma that can be characterized as buttery or nutty. It has been demonstrated that threshold values in different wines vary from 0.2 mg/L in Chardonnay wine to 2.8 mg/L in Cabernet Sauvignon. The compound can add pleasant aromas and complexity to wine at concentrations below 4 mg/L, but above this, it becomes unpleasant with overt buttery notes (Bartowsky, 2009; Nielsen and Richelieu, 1999; Swiegers et al., 2005). In general, wines that have undergone MLF have higher concentrations of diacetyl (Liu, 2002).

A number of factors, including some that the winemaker can control, particularly during malolactic fermentation, affect the final level of diacetyl in wine. The bacterial strain used, oxygen exposure, fermentation temperature and duration of malolactic fermentation, wine type, and sulfur dioxide impact diacetyl production (Bartowsky, 2009). It should be pointed out that diacetyl is formed chemically from the oxidative decarboxylation of α-acetolactate, an unstable intermediary compound produced during citrate metabolism. Its formation and degradation by bacteria are directly related to growth and to the metabolism of sugar and malic acid together with citric acid, since it is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid (Swiegers et al., 2005). Moreover, previous studies observed the utilization of diacetyl by *Oenococci*, which is not surprising, since many LAB contain diacetyl reductase that converts the flavorful diacetyl to the

much less flavorful acetoin and 2,3-butanediol; therefore some strains can decrease the amount of this molecule (Liu, 2002).

Certain strains of lactic acid bacteria (particularly *Lactobacillus* strains) are also capable of degrading tartaric acid, although this capacity is much less common than that of malic and citric acid metabolism. Tartaric acid is only degraded in certain conditions after the metabolism of other organic acids. The catabolism of this acid always alters wine by causing a slight reduction in fixed acidity and an increase in volatile acidity (Munoz et al., 2011). Acetaldehyde, a highly volatile compound with an apple-like and nutty aroma, is one of the most important sensory carbonyl compounds formed during vinification, constituting more than 90% of the total aldehyde content in wine, and originates mainly from yeast metabolism (Swiegers et al., 2005). At low levels, acetaldehyde gives a pleasant fruity aroma, but results in an undesirable aroma described as green, grassy, or apple-like when present in excess. The aroma can be masked by the addition of SO_2, but binding of SO_2 to acetaldehyde reduces its effectiveness as an antimicrobial compound and its antioxidant effect. The interaction of acetaldehyde with phenolics improves red wine color by forming stable polymeric pigments resistant to SO_2 bleaching, but it may also induce phenolic haze and eventual deposition of condensed pigments (Bauer and Dicks, 2004; Swiegers et al., 2005). Some strains of *Oenococcus* and *Lactobacillus* spp. can metabolize free and SO_2-bound acetaldehyde to acetic acid and ethanol (Osborne et al., 2000). Free SO_2 released from the degradation of SO_2-bound acetaldehyde by SO_2-sensitive strains of *O. oeni* may cause inhibition, resulting in stuck or sluggish MLF. The chemical and sensory impact of the ethanol and acetic acid formed by the metabolism of acetaldehyde by lactic acid bacteria is believed to be limited, but the reduction in the acetaldehyde pool in wine is believed to influence final wine (Osborne et al., 2006). By using efficient acetaldehyde-degrading strains to conduct MLF, the addition of SO_2 to reduce acetaldehyde aroma can be minimized (Bauer and Dicks, 2004).

Mousy wines result from the metabolism of ornithine and lysine, leading to the formation of extremely potent and unpleasant nitrogen-heterocylic compounds: tetrahydropyridines. These molecules are perceived on the back palate as a persistent aftertaste reminiscent of caged mice because of interactions with the mouth environment, since an increase in pH renders the compounds volatile (mousy taint is indeed described as a taste rather than a smell). Production of these compounds seems to be limited to the heterofermentative LAB (*O. oeni* and some species of *Lactobacillus*) (Costello et al., 2001). A wine with a viscous and thick texture is referred to as "ropy" because of the presence of excess exopolysaccharides such as β-D-glucan. In this context, the production of exopolysaccharides is almost exclusively because of *Pediococcus* growth in wine (Bartowsky, 2009).

Biogenic amine (BA) formation results from the decarboxylation of the corresponding amino acids by the action of microorganisms. Many bacterial genera are able to decarboxylate amino acids. This reaction is thought to favor growth and survival in acidic media, since it induces an increase in pH. In wine, several amino acids can be decarboxylated; as a result, histamine, tyramine, putrescine, cadaverin, and phenylethylamine are usually found, the first three being the most frequent (Lonvaud-Funel, 2001). The presence of these compounds is considered by some authors a fundamental parameter to the detriment of alcoholic beverages, for both

sensory problems and consumer health issues (Marques et al., 2008). Since winemaking involves the growth of lactic acid bacteria for malolactic fermentation, biogenic amines may occur. However, not all bacterial strains are able to produce them. Since the early 2000s, it has been possible to detect the presence of undesirable histamine-producing strains by PCR test or DNA probe based on the presence of the gene encoding histidine decarboxylase, ornithine, and/or tyrosine decarboxylase. This problem usually arises in spontaneous MLF, where an unknown LAB population carries out the fermentation, whereas the absence of these genes is increasingly required as a selection criterion for selected starters (Lonvaud-Funel, 2001).

1.1.4 Current Knowledge on Malolactic Fermentation: Managing the Process

1.1.4.1 Physicochemical Parameters Affecting MLF Development

Various studies have reported many factors that influence the occurrence of LAB and MLF in wines. In addition to oxygen and CO_2, Henick-Kling (1993) listed carbohydrates, amino acids, vitamins and minerals, and organic acid content, as well as the alcohol level, pH, SO_2, the method of vinification, and interrelationships between LAB and other wine microorganisms to be the most influential factors to affect LAB growth. Much other research work over the last 20 years has deepened the impact of these factors, providing to winemakers an interpretation key to understanding MLF problems and some tools to manage the MLF process (Alexandre et al., 2004; Bauer and Dicks, 2004; Lerm et al., 2010; Liu et al., 2016; Terrade and de Orduña, 2009). Indeed, if some of the parameters affecting MLF feasibility are not easy to change (grape variety, alcohol or potential alcohol, pH, malic acid content), many others can be managed by the winemaker in order to minimize risks of stuck or sluggish fermentations (SO_2, yeast strain chosen for AF, temperature, nutrients to be added). Table 1.1 summarizes the main physicochemical factors affecting MLF and their potential impact, according to recent studies.

1.1.4.2 Use of Starter Cultures

Winemakers started to recognize the benefits of inoculating grape must or wine with commercial starter cultures of LAB to ensure the successful completion of MLF and to reduce the risks associated with spontaneous MLF in the 1980s (Davis et al., 1985). Potential risks include the presence of unidentified/spoilage bacteria that can produce undesirable or off-flavors, a delay in the onset or completion of MLF, the production of biogenic amines and the development of bacteriophages—all of which contribute to a decrease in the quality of the wine (Bauer and Dicks, 2004; Jaomanjaka et al., 2016; Lerm et al., 2010). Thus, while MLF can occur spontaneously, more and more winemakers, particularly in New World winemaking regions, prefer to minimize the danger of a failed or sluggish MLF by inoculating with a reliable, commercially available starter culture. By inoculating a commercial starter culture, most of which contain *O. oeni* as the single LAB culture, the winemaker can promote the rapid start and completion of MLF and also encourage a positive flavor contribution to the wine (Bartowsky et al., 2015; Lerm et al., 2010).

O. oeni is still the main species used in the many commercial starter cultures available today. Most of the cultures are prepared with single strains or a mixture of

TABLE 1.1
Main Factors Affecting MLF Effectiveness

Factor	Effect on MLF	Mainly Cross Linked with	Ref.
Temperature[a]	Temperature affects growth rate, length of the lag phase, and maximum population of malolactic bacteria. Optimal growth rate of *O. oeni*, in absence of ethanol, is around 25°C. Optimum growth at 10%–14% alcohol is between 18°C and 20°C.	Ethanol	Bauer and Dicks, 2004
Ethanol	Ethanol decreases optimal growth temperature of LAB; ethanol tolerance is decreased at elevated temperatures due to membrane fluidity. Generally *O. oeni* strains are able to survive and proliferate in 10% ethanol, concentrations > 14% (v/v) inhibit *O. oeni* growth. Ethanol tolerance in LAB is strain dependent in *Oenococcus* and *Lactobacillus* species.	Temperature, SO_2	Bauer and Dicks, 2004; Henick-Kling, 1993; Lerm et al., 2010
pH	Wines of pH 3.3 and above generally exhibit few problems, whereas at lower pH, difficulties may arise. *O. oeni* usually represents the dominant species in wine below pH 3.5. At higher pH *Lactobacillus* and *Pediococcus* spp. may survive and grow. pH influences SO_2 impact on MLF.	SO_2	Bauer and Dicks, 2004; Henick-Kling, 1993
SO_2[a]	Antimicrobial activity of SO_2 strongly affects growth of LAB cells and influences malolactic activity. pH has crucial effect on the form of SO_2 present. The lethal level of molecular SO_2 for most wine LAB is <0.3 mg/L (corresponding, e.g., to 5 mg/L free SO_2 at pH 3.2 and 13% ethanol, 15 mg/L free SO_2 at pH 3.6 and 13% ethanol). To a lesser extent, also, bound inhibits MLF (bound SO_2 at 30 mg/L delays the growth of LAB, bound SO_2 > 50 mg/L reduces malolactic activity by 50%). Some yeast strains are capable of producing rather large amounts of SO_2 during AF.	pH, yeast	Bauer and Dicks, 2004; Henick-Kling, 1993; Krieger and Silvano, 2016; Lerm et al., 2010
Malic acid	L-malate stimulates growth and biomass production by *O. oeni* up to 3 g/L. Higher concentrations may show inhibitory effect. At low pH, L-malate is metabolized at a high rate, whereas carbohydrate metabolism proceeds very slowly.	pH	Bauer and Dicks, 2004; Krieger and Silvano, 2016; Lerm et al., 2010

(*Continued*)

TABLE 1.1 (CONTINUED)
Main Factors Affecting MLF Effectiveness

Factor	Effect on MLF	Mainly Cross Linked with	Ref.
Nutrients[a]	Depending on strains, some amino acids (4 to 9) are essential for growth, while others are required for optimum growth of *O. oeni*. Nicotinic acid, riboflavin, pantothenic acid, and either thiamine or pyridoxine are necessary vitamins, manganese an essential ion. Yeast can consume these nutrients; yeast autolysis releases nutrients that stimulate LAB growth and malolactic activity.	Yeast	Krieger and Silvano, 2016; Terrade and de Orduña, 2009
Yeast strain[a]	Inhibitory and stimulatory effects differ between strains: AF with SO_2 producing yeast strain results in wine inhibitory to MLF and medium chain fatty acid production by yeast negatively affects LAB growth and reduce ability to metabolize malic acid. Antagonism between yeast and LAB during alcoholic fermentation may be also explained by nutrient depletion and production of specific inhibitory peptides. New nontargeted studies recently provided insights into other molecules belonging to classes of oligopeptides, carbohydrates, amino acids.	Nutrients, SO_2	Alexandre et al., 2004; Bauer and Dicks, 2004; Lerm et al., 2010; Liu et al., 2016
Phenolic compounds	Polyphenolic compounds impact the growth of bacteria in wine, but research results are very confusing, with some studies describing the phenolic compounds as activators, and others as inhibitors. In a recent study, the addition of grape tannins and red wine extracts to a white wine showed a positive influence on the growth, survival, and malolactic activity of *O. oeni* strains; a 3-O-galloyl esterase and gallate decarboxylase have been found in *L. plantarum*. Results showed different malolactic behaviors in relation to wine phenolic compositions for *O. oeni* and *L. plantarum*. Diversity was found within each group.		Chasseriaud et al., 2015; du Toit et al., 2011

[a] Factor that the winemaker can manage during the vinification process.

two or three strains. Nowadays, many commercial starter cultures are available to induce malolactic fermentation; most consist of strains of lactic acid bacteria, which have a high malolactic activity and a high tolerance of low pH and high ethanol content. These starter cultures have been commercialized in various forms, including fresh, frozen, and lyophilized cultures (Gonzalez et al., 2011; Munoz et al., 2011). Since the introduction of malolactic starter cultures for improving the induction of MLF, there has been considerable research and development aimed at optimizing inoculation strategies and strain selection to further enhance MLF efficiency.

One of the major considerations has been to determine the optimal time point for inoculation. Starter cultures can be co-inoculated with yeast (at the beginning or towards the end of alcoholic fermentation) or inoculated sequentially (after alcoholic fermentation). Generally, relative to sequential inoculation, co-inoculation reduces overall vinification time. This has important consequences for the wine industry: Speeding up vinification rate leads to more rapid wine stabilization and reduces the risk of spoilage (Bartowsky et al., 2015). Indeed, grape must is an environment more suitable than wine for microbial growth because it does not contain some of the limiting factors; in these conditions, better adaptation and activity of malolactic bacteria are expected. The obstacles to microbial activity, in particular ethanol and eventually some yeast-produced molecules, accumulate gradually during alcoholic fermentation, allowing time for bacterial biomass adaptation and ensuring a greater chance of survival for lactic acid bacteria (Guzzon et al., 2016).

Nonetheless, there has been some reluctance by industry to adopt co-inoculation as a practice. One possible concern for using this tool is that *O. oeni* is heterofermentative. This means that under certain conditions, one of the products of its sugar metabolism is acetic acid. Thus it might be assumed that *O. oeni* has the potential to produce wines with elevated volatile acidity. Considering that the consumption of sugar and malic acid can occur simultaneously, the bacteria utilized in this kind of fermentation must be tailored specifically to avoid spoilage phenomena associated with the consumption of sugar by lactic acid bacteria via heterolactic fermentation. It has been demonstrated by several works that, at least under winemaking conditions and with careful management of fermentations, *O. oeni* survives better and does not produce acetic acid when grown in grape juice at low pH (Zapparoli et al., 2015a,b). Under these conditions, indeed, it preferentially utilizes organic acids (malic and citric acids) rather than sugars (Henick-Kling, 1993).

There has been growing interest internationally in studying and industrially using co-inoculation in the production of many red and some white wines. Co-inoculation strategies have been found to benefit production of a wide range of grape varieties providing reliable malolactic fermentation (as reviewed by Bartowsky et al., 2015), as well as in different contexts of harsh situations including high alcohol (Zapparoli et al., 2009) and low pH (Guzzon et al., 2013, 2016; Knoll et al., 2012; Pan et al., 2011), and to be appreciated for their aromatic contribution to the final product, as discussed in Section 1.1.3.2. As the pH increases, there is a shift to a preference for sugar utilization of *Oenococcus oeni*, thus increasing the risk of acetic acid accumulation in case of early inoculation (Bartowsky et al., 2015). However, in many spontaneous MLFs in wines exhibiting a pH over 3.5, *Pediococcus damnosus* and certain unknown *Lactobacillus* strains may dominate, creating the risk of organoleptic defects. The application of proper microbiological selection criteria to wines in this category has

recently led to the isolation of bacterial strains capable of inducing a quality MLF in high-pH wines. In this context, one of the emerging trends in the application of malolactic fermentation is to use alternatives to *O. oeni* as starter cultures, with a particular interest in *Lactobacillus plantarum*. This bacterium is homofermentative for hexoses such as glucose; it will produce only lactic acid and not acetic acid when it metabolizes glucose, thus eliminating any potential risk of producing volatile acidity.

In addition, *L. plantarum* has a preference for malate as an energy source at low pH, making it suitable for MLF in a cofermentation or even prealcoholic fermentation, when it can begin decarboxylating malate before a yeast starter culture has been added (Bartowsky et al., 2015; du Toit et al., 2011). From a sensory point of view, *L. plantarum* produces a broader range of extracellular enzymes, including glycosidases and esterases, than *O. oeni*. These enzymes potentially play an important role in the development of wine sensory properties through the release of flavor molecules; thus *L. plantarum* may enhance wine sensory properties more largely than *O. oeni* (Bartowsky et al., 2015; du Toit et al., 2011). Last but not least, the wide genetic diversity of the *Lactobacillus* spp. and in particular of the *plantarum* species makes it interesting for recent projects aiming at enhancing regionality of wine through MLF (Iorizzo et al., 2016; Spano and Capozzi, 2011; Testa et al., 2014). Indeed, recent studies have shown how microbial activity is an integral part of regionally distinct wine characteristics (terroir), an important aspect of wine production and consumer appreciation. Wine production and grape and wine microbiota present regionally defined patterns associated with vineyard and climatic conditions and are objects of increasing interest (Bokulich et al., 2016) thanks to the high-throughput sequencing technologies that are providing the greatest advance to this sector of research. So, research has been conducted on regional microbial isolates and their potential application in winemaking, since grape resident microbial diversity forms an untapped reservoir of indigenous bacteria strains and may be primarily considered in an MLF starter selection scheme, and new findings are likely to be expected in this field.

1.1.4.3 Control of MLF to Avoid Further Microbial Spoilage

How best to avoid wine spoilage is not always easy to define. Even appropriate hygiene practices and the chemically harsh nature of wine cannot be relied on as a deterrent to unwanted bacteria. As an initial barrier, the high ethanol concentrations (up to 16% v/v) and high wine acidity (pH as low as 2.9) can inhibit development of bacterial populations; however, in wines with lower ethanol concentrations and low acidity (above pH 3.6), it can be challenging to arrest late bacterial growth, since, unlike the treatment of wort in beer brewing, grape must is not pasteurized prior to yeast inoculation (Bartowsky, 2009; Ribéreau-Gayon et al., 2006). Some kinds of risks are easier to avoid than others. For example, injudicious aeration during and/or after the winemaking process can result in the growth and activity of acetic acid bacteria, high volatile acidity, and a vinegary taint in wine. These bacteria are classified into the genera *Acetobacter*, *Acidomonas*, *Gluconobacter*, and *Gluconacetobacter*; of these, *Gluconobacter oxydans*, *Acetobacter aceti*, *Acetobacter pasteurianus*, *Gluconacetobacter liquefaciens*, and *Gluconacetobacter hansenii* are normally associated with grapes and wine, but can be easily avoided by limiting oxygen (Swiegers et al., 2005).

Sulfur dioxide (SO_2) is the key additive for the preservation of wines. Carbonyl and keto compounds in wine can bind to SO_2 and decrease its efficacy, resulting in higher total SO_2 requirements. Increased consumer demand for low sulfite and organic wines poses production challenges if SO_2 binders have not been properly managed during vinification. In this context, the correct management of fermentation stages (alcoholic and malolactic) can be critical to obtain the best ratio between free and bond SO_2. In particular, malolactic fermentation has been known to reduce bound SO_2 levels. Some studies suggest that microbiological wine stabilization 1 week after malic acid depletion is an effective strategy for maximum removal of SO_2 binders while reducing the risk of possible post-ML spoilage by *O. oeni* leading to the production acetic acid and biogenic amines (Jackowetz and Mira de Orduña, 2012; Osborne et al., 2006).

Another point on which MLF management can be important to prevent spoilage is biogenic amine formation. Previous works showed that most of the commercial malolactic bacteria did not produce BA, and that the application of commercial malolactic starters in wines is useful to reduce the BA amounts, since in the inoculated wines BA concentrations were significantly lower when compared with those not inoculated (Lonvaud-Funel, 2001). These results suggest that the use of selected malolactic starters can minimize BA production (Marques et al., 2008). When BA-producing strains are present in indigenous microflora, the winemaker is particularly encouraged to inoculate selected malolactic starters to replace the indigenous microflora. Nevertheless, when the dominance of starter cultures on the indigenous BA-producer microflora is not sufficient, this does not represent the definitive solution. Thus, a recent work reports the selection of autochthonous strains of *Lactobacillus plantarum* able to degrade BA and their suitability to be used as malolactic starter in wine production (Capozzi et al., 2015). This represents one more scenario in which BA could be controlled, in fermented foods, by modulating microbial resources as MLF.

Moreover, MLF management strongly affects the development of *Brettanomyces* during subsequent wine aging. Some studies showed that wines that underwent MLF inhibited the growth of *Brettanomyces*, resulting in a product containing little or no volatile phenols. Wines that did not undergo MLF or that underwent late spontaneous MLF that proceeded slowly allowed proliferation of *Brettanomyces*, resulting in a product containing more volatile phenols (Gerbaux et al., 2009; Nardi et al., 2014). Thus, early inoculation of wine with malolactic bacteria may be a tool for lowering the risk of volatile phenol production. In a recent resolution, the International Organisation of Vine and Wine drafted the "Code of good vitivinicultural practices in order to avoid or limit contamination by *Brettanomyces*" (OIV, 2014). The document attests that if MLF is delayed, the risk of production of volatile phenols increases because *Brettanomyces* can take advantage of the time between alcoholic and malolactic fermentation to multiply, benefiting from the absence of SO_2. Thus, the use of malolactic starters is proposed as a good way to limit *Brettanomyces* development. Moreover, co-inoculation or early sequential inoculation is presented as the best tool to prevent *Brettanomyces* contamination by reducing the lag phase in between AF and MLF, as shown in scientific studies (Coulon et al., 2010; Gerbaux et al., 2009). After malolactic fermentation, it is recommended to eliminate all microorganisms—particularly by adding SO_2.