CHAPTER 9

Amarone: A Modern Wine Coming from an Ancient Production Technology

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Contents

Abstract

Amarone wine is a renowned dry red wine produced in Valpolicella (Verona, Northern Italy). It is made from local grapes varieties (Corvina, Rondinella, and Molinara) that are slowly dried under natural conditions during the fall into winter.

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After the postharvest drying, carried out for several weeks in dedicated lofts called *fruttaio*, the grapes are vinified: crushed, given prefermentative cold maceration, undergo alcoholic fermentation on the skins, malolactic fermentation, and finally maturation. The partially dried grapes are traditionally crushed during the second half of January to February. Because cellar conditions are unfavorable for either alcohol or malolactic fermentation, selected microbial cultures (yeasts and malolactic bacteria) are often necessary to correctly manage fermentation. The progress of both fermentation processes needs constant surveillance. During maturation conducted in vessels or wooden containers (tonneau in durmast oak), clarification and stabilization lead to improvements in quality. Product specifications require that Amarone not be bottled before the wine has been aged for 2 years (Anonymous (2010). Disciplinare di produzione dei vini a denominazione di Origine Controllata e Garantita "Amarone della Valpolicella". *Gazzetta Ufficiale della Repubblica Italiana*. Serie generale n. 84. April 12). Amarone achieved its DOCG (Controlled and Guaranteed Denomination) status in 2010.

I. HISTORY

Amarone is an important red wine produced in Valpolicella, an area close to Verona, in north-eastern Italy. Other red wines are produced in the region, but Amarone is the most important. It has become renowned worldwide because of its unique attributes, deriving from its special production process. It is based on partially dried grapes, similar to sweet, *passito*-style wines. However, it is distinct in its current form. Being dry, it competes with other, prestigious, dry red wines produced internationally.

Amarone has its origin based on procedures used in producing sweet, *passito*-style wines. The process has an ancient heritage in the region surrounding Verona. The first written record of its production dates back to the sixth century AD, in documents by Cassiodoro. He was a minister to Theodoric, king of the Goths, who had chosen Verona as his capital. Cassiodoro described an *Acinatico* (a sweet *passito*-style wine, currently referred to as *Recioto*) as

> a sweet wine red as purple or white as lilies, fragrant, noble, dense.

He also noted the ancient technology by which it was produced:

> in the autumn grapes are chosen in the domestic bowers, hung up by the bottom tip, then conserved in jars and in ordinary repositories. They hardened during time, do not liquefy, useless humors are

> exuded, and the grapes become sweet. This goes on until December, until winter begins, and wine becomes new when in all the wine cellar it is already old.

The technology is probably even more ancient than the sixth century.

Wine produced by crushing and fermenting dried grapes produces, as in the past, well-structured red wines. They are typically characterized by high residual sugar contents and, in the Veneto region, they are termed *Recioto* wine.

In the past, sweetness was largely restricted to fruit (fresh or dried) and honey. Correspondingly, any sweet food or beverage would have been both rare and precious. Therefore, a sweet wine from Verona made it desirable, appreciated, and sought after. In addition, its unique and distinctive sensory attributes made Recioto special.

In view of the fundamental lack of understanding about the nature of fermentation, and a deficiency in technical equipment, it is surprising that favorable coincidences permitted the production of a stable, sweet wine. Drying the grapes for about 2–3 months produced a must enriched in sugar content. This feature complicated the initiation of alcoholic fermentation by yeasts. In addition, incomplete metabolism of all the fermentable sugars can lead to stuck fermentation, and the likelihood of microbial spoilage or refermentation when the wine is bottled. Moreover, the prolonged drying process retarded the start of fermentation into winter (January). Low cellar temperatures limit yeast activity, resulting in slow growth. The consequence was the production of a distinctively scented, sweet wine of unique character.

Occasionally, to the disappointment of Recioto producers, fermentation spontaneously went to completion, resulting in a dry wine. This was a mystery, but occurred primarily when the winters were mild. The resulting product was described as bitter, rather than, as usual, sweet. Considering its sensory characteristics and structure, the wine could indeed have been referred to as ''very bitter''—in Italian, *amarone*. Nonetheless, it was tasty, without off-flavors, heady, flavorful, but, unfortunately, not sweet.

Over the years, tastes have changed, thus the development of a new appreciation for dry, Amarone wines. In addition, with the widespread availability of sugar, sweet foods, and beverages were no longer special. Amarone's original and pleasant combination of flavors, almost voluptuous and seductive, led to unexpected appreciation among consumers worldwide. Recioto is still appreciated, but it is now relegated to the status of a rare enologic product, having lost its former commercial importance.

Due to its special sensory attributes, Amarone has progressively become the preferred style made from partially dried grapes in Veneto.

As such, the technical details of its production have become the focus of scientific research. Its ancient traditions are now being expressed in modern form.

II. PECULIARITIES OF AMARONE WINE

A. Production area

The vineyards dedicated to the production of Valpolicella, Recioto, and Amarone are the same. They are localized among the hills of Valpolicella to the northeast and northwest of Verona (Fig. 9.1).

The northwest hills are within the classic Valpolicella area. The mountains are defined by natural boundaries: the northern limit consisting of the Lessinia Plateau, from which three small valleys start (Negrar, Marano, and Fumane). They flow into a hilly landscape and vanish into Pescantina, where the Adige River delimits the southern limit. To the east, hills line ranges from Parona to Montecchio. This is a well-defined border, almost a protective wall, and a natural boundary that is similar to the opposite one: the steep slopes of Mount Pastello to the west.

FIGURE 9.1 Geographical map depicting Verona City, the Garda Lake (Lago di Garda), the Adige river and the three main enological areas: Bardolino area to the west (Bardolino D.O.C. and Bardolino Classico), Valpolicella to the north, Soave area to the east. The Valpolicella area is divided into two distinct regions, one of them is the classic area of Valpolicella (Valpolicella Classico).

The eastern part of the production area is outside the classic zone. It starts in the Pantena Valley, in the northern part of the eastern districts of Verona, and ends in the eastern Illasi Valley. This area is bordered to the north at the Lessinia Plateau and to the south by Road 11, between Verona and Vicenza.

The Valpolicella region is characterized by a mild, temperate climate, as testified by the presence of olive groves up to 300 m above sea level. The northern side is protected by the Lessinia Plateau and the southern region is open and wide.

In winter, the temperatures are lower in the plains than on the hills, and they rarely fall below 0 °C; summer temperatures range between 25 and 30 °C during the day and between 18 and 20 °C at night.

Rainfall varies from an average of about 850 mm/year in the lowland, to an average of 1200 mm/year at the summit of the higher hills. Although there are marked annual variations, rain is usually rare during the winter, being more frequent in spring and autumn.

The most important and frequent winds in winter are the Bora, which blows from the northeast, and the south wind, which blows from the southeast. In summer, beneficial and characteristic breezes blow from south to north. These breezes are important in facilitating the accumulation of aromatic compounds in grapes during ripening by generating desirable temperature fluctuations.

The soils of Valpolicella are linked to its geologic history, starting approximately 100 million years ago when limestone deposits were formed. This is the origin of the precious pink marble of Verona. Subsequently, another important limestone of Verona, known as Prun stone, was laid down. In the Tertiary, massive volcanism disrupted the geologic continuum, producing the dark colored rocks, locally referred to *toari*, that are evident in hilly regions. The emergence of the land from the sea, about 20–25 million years ago, gradually lead the development of deep valleys, shaped by ice during the Quaternary, and the formation of Garda Lake and Adige Valley. Finally, the erosive action of the Adige River and repeated flooding allowed the formation of wide terraces of fluvioglacial sediments favorable to grape cultivation.

The presence of many valleys, the proximity of Garda Lake and various vineyard orientations to sun exposure has generated areas with specific microclimates. These have favored grape growth and the production of high-quality grapes.

B. Grapevine cultivars

Three indigenous grape varieties are typically grown in the region: Corvina, Rondinella, and Molinara. Their characteristics possess attributes facilitating production of the traditional red wines of the region, that is, Valpolicella, Recioto, and Amarone.

In addition to the principal varieties, other cultivars are grown. However, for various reasons they are less preferred by local growers. Although largely neglected by modern winemakers, they are desirable sources of biodiversity. They represent the genetic heritage of the region. Their preservation is a prerequisite to the development of new wine styles. For example, although Oseleta is low yielding, its distinctive character could contribute to the creation of wines of great structure, aging potential, and value. Other examples of the indigenous heritage include Forsellina, Rossignola, Dindarella (Pelara or Quaiara), Cabrusina (Montanara), and Pomella.

The following provides a brief description of the most widely grown cultivars in the region.

Corvina is the most important red grape in Verona and surrounding regions, representing the dominant cultivar used in the production of virtually all red wines in the region. It is essential to Amarone production. It gives the wine a powerful structure, but surprising smoothness. The vine is strong and provides an abundant and consistent yield. It is moderately resistant to the diseases and climatic difficulties of the region. It is characterized by low fertility of its basal buds. Maturation is typically late, producing pyramidal, compact, and medium-sized bunches with lateral wings (Fig. 9.2). The fruit has fragile skin and is easily damaged. This leads to sensitivity to infection by *Botrytis cinerea* during the postharvest drying period.

Corvinone was previously classified as a clone of Corvina, but is currently recognized as a distinct variety. The varieties are distinguishable, except by experts, only by its producing larger grapes. It also differs in possessing basal buds of relatively good fertility. It grows best on hills and in soils of low fertility. Its grape clusters are large and react well to handling and during the drying process.

FIGURE 9.2 A typical grape bunch of the Corvina variety.

Rondinella is valuable mainly due to its fruit quality and consistent yield. Moreover, it grows sufficiently well on clayey soils. The cultivar also has the advantages of showing noticeable resistance to many pathogens as well as environmental stress, such as cold and drought. Its resistance to fungal infection makes it particularly suitable to surviving the prolonged drying process. It also donates complex aromas and flavors.

Molinara produces fruit with a thick waxy layer covering the berries. This donates a whitish countenance reminiscent of a dusting with white flour. This feature probably is the origin of its name, *molinara*, being a dialectic term for ''miller'' in Verona. Molinara produces a crop that is both abundant and consistent. It has pyramidal, medium to long bunch with lateral wings, mature late in the season, and are highly resistant to rot. Its wine possesses a delicate fragrance, is slightly bitter, and possesses desirable acidity.

The varietal composition of Amarone (Anonymous, 2010) stipulates that the content of Corvina can vary from 40% to 80%; Corvinone can replace Corvina by up to 50% and Rondinella can vary from 5% to 30%. Up to 15% can come from other local grape varieties (each within a limit of 10%).

With the cooling of Europe between the fourteenth and eighteenth centuries, as a result of the Little Ice Age, the vine cultivation moved to lower altitudes and has a never returned to its original sites, partly due to social and economic reasons (Maroso and Varanini, 1984). This largely accounts for the origins of the ancient grapevine varieties in Verona, along with factors such as varietal transplantations from east to west, domestication of local wild vines, and the pivotal role of Verona along the ancient trading routes. Indication of the common ancestry of many Verona's cultivars has been shown by the work of Vantini *et al.* (2003), Imazio *et al.* (2006), and De Mattia *et al.* (2008). Varieties such as Corvina, Oseleta, Rondinella, and Dindarella all appear to originate from a mountainous zone just behind Valpolicella. Confirmation of the role of proto-domestication of the grapevines in Western Europe and gene introgression has been provided by Imazio *et al.* (2006) and Scienza and Masi Technical Group (2006). Varieties such as Denella, Dindarella, Oseleta, Pelara, and Rossignola are considered to derive from wild grapevines by domestication (Mattivi *et al.*, 1990; Scienza *et al.*, 1990).

C. Production of Amarone wine

1. Grape selection

The product specifications of Amarone require that the grapes possess a potential alcohol content of at least 11° (v/v). Only healthy and uniformly ripe grapes are permitted. For this, experienced grape harvesters collect only sun exposed clusters, undamaged by pests, disease, or mechanical injury. This is required for the grapes to survive the extended drying period without spoilage. Depending on the vintage, this may constitute between 30% and 60% of the crop. The grapes are harvested only when dry (to promote proper drying) and avoid subsequent spoilage.

The harvested clusters are subsequently subjected to additional inspection and selection (in the vineyard and winery) to cull out all but the healthiest clusters or portions thereof. These are placed in single layers on wooden, fruit-drying racks, called *arele*, or in modern plastic boxes. These are transferred for storage and partial drying for several weeks in dedicated lofts (Fig. 9.3), called *fruttaio*.

2. Postharvest grapes drying

The grapes are slowly dehydrated in *fruttaio*, large breezy lofts, where air is continuously exchanged. Dehydration can last up to 120 days (Paronetto, 1991), depending on the physical and chemical characteristics of the grapes and prevailing climatic conditions. Product specifications require they not be crushed until the subsequent conditions have been

FIGURE 9.3 A traditional grapes drying loft located in Mazzano (Valpolicella area).

realized: December 15 has passed and their sugar content has reached a potential alcohol content of 14% (v/v). The grapes are usually crushed when they have lost 40% of the original weight (Barbanti *et al.*, 2008; Ferrarini *et al.*, 2005), except product specifications take precedence. This usually results in most winemakers crush partially dried grapes in January (Paronetto, 1991). In some vintages, excessive weight loss has been reported, for example, in 1999, weight loss of 59% for Corvina, 55% for Corvinone, 66% for Rondinella (Barbanti *et al.*, 2008). This may inhibit the initiation or completion of alcoholic fermentation, obviating the production of dry Amarone.

Drying is usually associated with two independent events. The grapes loose moisture, concentrating substances such as sugars, polyphenols, and aromatic compounds. In addition, a portion of the grapes succumb to attacks by *B. cinerea*. Depending on the environmental conditions, the fungus can infect the grape (Fig. 9.4), inducing either a ''noble rot'' (Fig. 9.5) or ''gray rot'' (Fig. 9.6). There are, respectively, desirable and destructive consequences of infection. Fungal invasion usually develops during the early stages of drying process, when the moisture level of the grapes are at their highest and climatic conditions are more favorable (Barbanti *et al.*, 2008; Ferrarini *et al.*, 2007a,b). Each grape cultivar is characterized by its own susceptibility to *Botrytis*: Corvina being easily

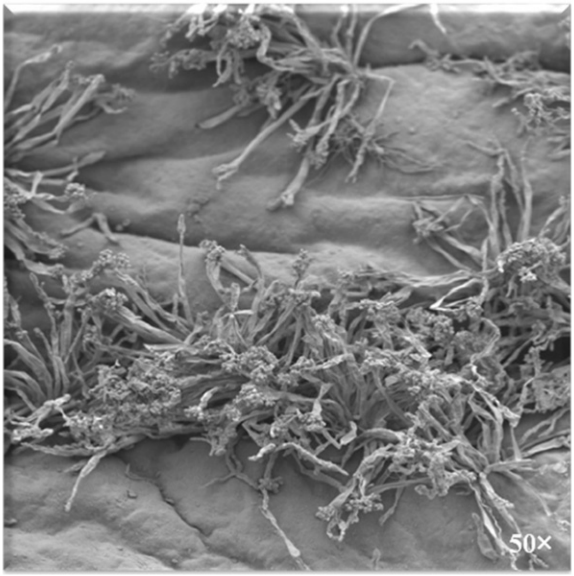

FIGURE 9.4 A detail of the surface of a grape berry attacked by *Botrytis cinerea* rot observed by means of the Scanning Electron Microscopy (Torriani *et al.*, 2009).

FIGURE 9.5 Berries of the Corvina variety attacked by noble rot (Torriani *et al.*, 2009).

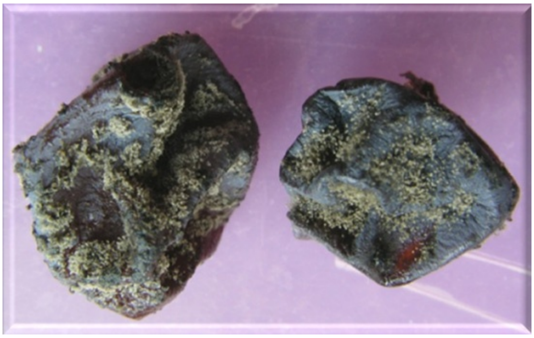

FIGURE 9.6 Berries of the Corvina variety attacked by grey rot (Torriani *et al.*, 2009).

attacked; Rondinella being relatively resistant, while Molinara's susceptibility being moderate (Paronetto, 1991).

Noble rot is favored by the cold temperatures and drying breezes associated with storage in *fruttaio*. When noble rot occurs, the hyphae of the mold create microscopic injuries on the surface of the berries, enhancing water loss and dehydration. The fungus metabolizes sugars and acids and promotes the production of chemical compounds including glycerol, acetic acid, and various enzymes, resulting in characteristic chemical changes (Ribéreau-Gayon *et al.*, 2006). Although the berry surface is damaged by fungal hyphae, spoilage by other microorganisms is rare (Corte *et al.*, 2001).

To limit *Botrytis* action, and the development of a destructive gray mold, the lofts are closed during rainy and humid weather. The open structure of trays also plays an important role in limiting the inception of gray mold by facilitating air flow in and around the grape clusters. Moreover, the wood of the trays acts to absorb moisture that may condense on the grapes or exudates from the grapes. Nonetheless, wooden racks are being replaced by perforated plastic boxes. They have the advantages of being more easily cleaned and sanitized, favor hygienic condition in the drying lofts, reduce the risk of *de novo* infections (Paronetto, 2008).

Traditionally, drying lofts have been built in windy locations, especially on hill tops. However, better and more consistent humidity control can be achieved with modern instrumentation, such as commercial air and heat exchangers.

To preserve both the traditional characteristics provided by natural drying but provide better control, environmental conditioning is used only when necessary. This system, termed NASA (natural super assisted drying) by the Masi Winery is being used in some of its lofts. The computer control unit contains a database of climatic data from positive vintages that can be compared with existing conditions. If the current data do not divergent significantly from those in the database, the attic is left under natural conditions. However, if the difference becomes marked, the computer initiates condition to bring the attic to an ideal situation, while closing the windows (Paronetto, 2008; Scienza and Masi Technical Group, 2006).

3. Wine production

After partial dehydration, the grapes are transformed into wine: crushing, maceration, alcoholic fermentation, malolactic fermentation, and finally aging. This occurs during the winter, usually beginning in the second half of January and February, depending on the duration of the previous drying step. Because of the cold weather, these impose distinctive requirements and consequences on both the technical progress and the final qualitative results.

After crushing, the stalks are removed and the juice left in contact with grape skins and seeds (pomace) to carry out maceration. During this stage, substances, mainly tannins, anthocyanins, and aromatic compounds begin to dissolve and accumulate in the juice. The low temperatures during prefermentative maceration promote a distinctive kind of maceration. The cold temperatures prevent the metabolism of the indigenous yeasts and other microbes. Thus, maceration initially occurs in unfermented juice. This situation enhances the extraction of the fresh fruity aromas typical of Corvina and Rondinella—being characterized as cherry and marasca cherry.

In the meanwhile, yeasts adapt themselves to the cold conditions and begin alcoholic fermentation. Fermentation is slow but regular, usually carried out by *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The fermentation has to be kept under constant surveillance as the low temperatures, combined with the high sugar content, may lead to premature and unwanted fermentation cessation, even when selected cultures of yeasts are added.

The fermentation usually is complete within 30 or 40 days. During this period additional anthocyanins are extracted from pomace and the color stabilizes due to polymerization between the anthocyanins and tannins. Winemakers call this process pomace ''maturation''. Moreover, due to the increasing alcohol concentration, many other metabolites are extracted from the pomace. In addition, yeast metabolites contribute to the aromatic complexity of the wine.

Usually, after alcoholic fermentation, the wine undergoes malolactic fermentation, induced primarily by *Oenococcus oeni.* Not only can this lactic acid bacterium convert L-malic acid into L-lactic acid but also it is involved in many other transformations fundamental to Amarone quality.

Finally, Amarone undergoes a period of maturation in wooden barrels. Their dimensions may differ, depending on the winemaker's preference. This period is vernacularly termed ''aging''. During maturation Amarone undergoes stabilization that involves clarification, precipitation, and chemical transformation that improve the wine's quality. Product specifications require that Amarone cannot be bottled until 2 years of aging.

D. The sensory characteristics of Amarone wine

Amarone is undoubtedly a distinctive, high-quality wine. The inherent attributes of the grapes, combined with the actions of noble rot, a prolonged cold prefermentative maceration, and the unique fermentation conditions produce a highly concentrated, alcoholic, intensely colored, powerful but smooth, with overripe and stewed fruit flavors, particularly cherry aromas. Other descriptors that have been used to characterize the Amarone fragrance include reminiscences of walnut, toasted hazelnuts, red fruit, and sometimes citrus jam, licorice, coffee, and cocoa. Its velvet body is sometimes severe and rude, often soft, with sweet nuances, but never cloying. It is also characterized by a long finish.

III. ALCOHOLIC FERMENTATION AND MACERATION

The environmental condition under which fermentation occurs is very different from those of traditional autumnal winemaking, and imposes clear difficulties. The temperature of the must is often about 5 °C, or lower and its high sugar content jointly impede the onset and conduct of fermentation.

During traditional winemaking in October, endogenous yeasts are very active and their action may need to be limited by appropriate additions of sulfur dioxide. Usually spontaneous fermentation begins with apiculate yeasts of the genus *Hanseniaspora*. However, their extended action is desirable due to their ability to produce large quantities of volatile acids. Fortunately, they are sensitive to the alcohol they produce, as usually they do not survive (or at least do not metabolize) above ethanol concentrations of about 4%. Apiculate yeasts are followed by the more efficient and more useful *S. cerevisiae*. In the case of very rich musts, it may be succeeded by *S. bayanus* that ferments most of the remaining fermentable sugars. In addition, there may be a succession of different strains of these species, each contributing with their own distinctive and subtle attributes to the wine.

In Amarone musts, the yeast flora is small and qualitatively different from that of normal musts. In some studies, the yeast *S. bayanus* var. *uvarum* has been reported. This yeast is highly tolerant to low temperatures, as can grow in Amarone must even under the most difficult winter conditions. For this reason, it was generally considered, both from a technical and a sensorial aspect, a specific and distinctive microorganism in the Amarone fermentation (Dellaglio *et al.*, 2003; Naumova *et al.*, 2011; Tosi *et al.*, 2009b).

Alcoholic fermentation usually begins, albeit slowly, by yeast specialized to grow at low temperatures and they metabolize most of the sugars into alcohol. Subsequently, other strains more resistant to the increasing alcohol content prevail and finish the fermentation process.

The sequence of strains following one another may produce distinctive results, depending on their individual characteristics and on prevailing conditions. Occasionally, these consequences may be undesirable, contributing to off-odor production and inducing color loss by adsorption. This is particularly a problem when fermentations are prolonged.

In order to better manage winemaking and avoid defects, the use of selected starter yeast strains, suited to specific conditions, are a standard feature of modern in winemaking. These strains belong to the same yeast species usually found in spontaneous Amarone fermentations: *S. cerevisiae* and *S. bayanus*.

Starter strains have two main advantages. They conduct fermentation without problems, permitting the process to proceed in a predictable and controllable manner. In addition, they generate faint but perceptible sensorial differences. This permits the producer to highlight particular nuances in quality important to a more personalized wine style. Thus, biotechnology is providing tools that enable winemakers to customize the winemaking process toward a desired goal.

Together with fermentation, both the prefermentative and simultaneous maceration influences the supply of essential yeast nutrients and substrates for their enzymatic transformation. The release of nutrients from the pomace is also under the influence of heat and alcohol, generated by yeast metabolism.

Phenolic compounds, anthocyanins and tannins, are considered among the most important substances extracted from the grape pomace.

They influence the depth and stability of the wine's color and donate much of its overall structure and body. Other significant compounds extracted are various polysaccharides (such as pectins), aromatic compounds, and mineral elements (Ribéreau-Gayon *et al.*, 2006). The contribution of these varies depending on the presence of the different parts of the grape (skins, seeds, stems) and their maturation. Regrettably, some extracts may have unfavorable or even negative consequences on wine quality. For example, the concentration of substances in grape tissues detrimental to wine quality increases as grape quality diminishes. Thus, optimal maceration refers to maximizing the extraction of desirable constituents and minimizing the release of those considered undesirable.

Grape maceration can be described as a succession of phenomena comprising (Ribéreau-Gayon *et al.*, 2006):

- The extraction and solubilization of various constituents. It is promoted by the enzymatic and mechanical breakdown of plant tissues, especially the skin.
- The dispersion of extracted substances in the must wine, following the equilibrium laws for their different phases.
- The absorption of the extracted constituents on solid surfaces (stems, pomace, and yeasts), some of which may be redissolved.
- The modification of the extracted constituents as they can interact with one another, with alcohol, or other metabolic by-products or are involved in oxidation–reduction (redox) reactions. This can lead to major compositional changes resulting in stabilization or loss.

These phenomena can occur at various rates, depending on the duration and temperature of maceration, the presence of sulfur dioxide and oxygen, and on mechanical operations. In fact, during fermentation, the ''cap,'' consisting of a layer of the solid components of the grapes (skins and pips), is pushed upward by carbon dioxide gas released by yeast metabolism. In a process termed *rimontaggio* or pumping over, the must is drained from the bottom of the vessel and pumped over the top of the cap by hose. In a process termed *follatura* or cap punching, the cap is plunged down into the fermenting must using special equipment.

In the production of Valpolicella, a wine made with Corvina and Rondinella grapes low in tannin content, it is not desirable to extend the maceration period as it can risk the extraction of off-flavors from the grapes such as the herbaceous type, mainly referred to hexanols. Although Amarone and Valpolicella wines are made of the same grapes, their composition is influenced by the drying process. As a consequence, fermentation occurs during the coldest months of the year, resulting in the presence of atypical fermentative conditions. The use of prefermentative cold maceration also has a influence in favoring the extraction of fresh fruit aromas and more specifically those of cherry and marasca cherry that are typical descriptors in tasting sessions of Corvina and Rondinella (Paronetto, 1991). This enhances the presence of a more pronounced aromatic expression of the grapes' aroma.

However, the risk of undesirable herbaceous flavors affecting the aging potential of Amarone is limited because dehydration helps to reduce their presence in skins and stalks; there are no scientific investigations in this field but generally local oenologists attribute this observation to the chemical–physical change of the stalks from green-herbaceous to brown-wooden. In addition, infection by noble rot contributes to the production of characteristic sensations to the wine (Tosi *et al.*, 2009a; Usseglio-Tomasset *et al.*, 1980). Botrytized wines are renowned for a wide range of aromas, evoking citrus, dried fruits, and honey. The analysis of the aromatic components of wines with different percentage of botrytized grapes showed differences in some high-impact odorous compounds such as some acetates and esters, phenylacetaldehyde, benzaldehyde, vanillin, 4-terpineol, 1-octen-3-ol, sherry lactones (Tosi *et al.*, 2009a).

Following prefermentative maceration an extended maceration occurs during alcoholic fermentation. It favors anthocyanin extraction and color stabilization by reaction with tannins. Moreover, alcohol contributes both directly and indirectly to the wine's aromatic complexity by enhancing the volatility of other aromatic compounds and in the generation of additional flavorants. During this period, the producer can decide on when to terminate maceration (by pressing) or adjust its consequences by adjusting the temperature, pumping over, etc.

IV. MALOLACTIC FERMENTATION

At the end of alcoholic fermentation, yeasts begin to die and lactic acid bacteria (LAB) become active. They are responsible for malolactic fermentation, in which L-malic acid is decarboxylated to L-lactic acid by malolactic enzyme. This conversion of a dicarboxylic acid into a monocarboxylic acid deacidifies the wine, softening its taste by converting a relatively harsh tasting malic acid into a softer tasting lactic acid. Moreover, malolactic bacteria contribute to microbial stability by consuming available nutrients on which spoilage organisms might grow. LAB also produces aromatic compounds, such as diacetyl and acetoin, that can modify the wine's aroma, flavor, and mouthfeel (Moreno-Arribas and Polo, 2005). These changes may be desired or not, depending on the kind of wine. Wines that typically undergo malolactic fermentation, notably red wines, are considered to be improved, especially those destined for aging. This also applies to several white wines produced in cold climates. LAB strains isolated from wine may belong to several genera, notably *Oenococcus*, *Lactobacillus*, and *Pediococcus*.

The principal malolactic bacterium is *O. oeni* (Dicks *et al.*, 1995). When the species was still called *Leuconostoc oenos*, Peynaud and Domercq (1968) proposed it be split into two taxa, based on metabolic and physiological criteria. Subsequently, strains have been characterized by ribotyping and macrorestriction profile analyzes. These also suggest the presence of two distinct clusters (Tenreiro *et al.*, 1994). In contrast, several molecular techniques suggest that the species is genomically and phylogenetically homogeneous (Guerrini *et al.*, 2003; Le Jeune and Lonvaud-Funel, 1997; Zapparoli *et al.*, 2000; Zavaleta *et al.*, 1996). Nonetheless, strains may show horizontal gene transfer or bacteriophage insertions (Bon *et al.*, 2009; Borneman *et al.*, 2010; Mills *et al.*, 2005). Relative to Valpolicella, a related wine, up to 13 different strains of *O. oeni* have been isolated and characterized (Torriani *et al.*, 2002).

The natural selection of autochthonous *O. oeni* strains present in grape juice or in the winery facilities may involve adaptation to the unfavorable conditions such as low pH, high-alcohol content (Amarone wines it can reach 16% alcohol), nutritional starvation, high concentrations of sulfur dioxide, low temperatures, and presence of inhibitory compounds such as polyphenols (Lonvaud-Funel, 1999). Generally, the malolactic fermentation is carried out by indigenous bacteria contaminating the grape and cellar equipment, and commences spontaneously. However, spontaneous Malolactic Fermentation can start randomly and a delay in it onset could alter wine quality. Delays may be due to the harsh environmental conditions relative to bacterial survival and growth in wine (Beltramo *et al.*, 2006; Henick-Kling, 1995; Maicas, 2001). Zapparoli *et al.* (2009) have recently studied malolactic fermentation in high-alcohol wines like Amarone. This may lead to the selection of *O. oeni* optimized for the production of Amarone wine.

V. MATURATION IN COOPERAGE (AGING)

At the end of alcoholic and malolactic fermentation, the wine undergoes clarification and stabilization prior to bottling: the phase inappropriately called "aging." This is the final step in winemaking, which involved those operations required to permit the expression of features considered necessary to the wine's overall quality. Nuances that distinguish wine styles also develop.

Traditionally, maturation of red wines generally occurs in barrels or other wooden containers of various volumes. The wood provides special conditions for maturation that are favorable to the development of the wines character (Del Alamo Sanza *et al.*, 2004; Pérez-Coello and Dìaz-Maroto, 2009). These usually include limited oxidation. Wood porosity together with filling and refilling operations ensure both a slow continuous and periodic incorporation of oxygen into the wine. The oxygen favors condensation reactions between anthocyanins and tannin subunits. The polymers contribute directly and indirectly to color stability (resistance to oxidative browning), and the development of astringency and bitterness (Del Alamo Sanza *et al.*, 2004). In addition, tannins and aldehydes, extracted from the wood, are involved in the development of the wine's structure and body.

Maturation in wood also contributes to the wine's overall fragrance. Their nature depends on the type and origin of the wood, on the seasoning (drying) of the wood, on the degree of toasting during barrel assembly, and on their repeat usage (Pérez-Coello and Dìaz-Maroto, 2009).

Aging in oak cooperage encourages the extraction of a series of benzoic and cinnamic phenolics (including vanillin and syringic acid), gallic acid, and coumarins. It also induces modifications in their physical and chemical parameters (Del Alamo Sanza *et al.*, 2004).

Clarification, by the sedimentation of suspended particles and precipitation of salts such a potassium bitartrate, is facilitated by storage in barrels. Their small volume reduces convective phenomena and allows the wine's temperature to cool markedly during the winter, encouraging both phenomena. The precipitation of unstable colloids, that can cause wine turbidity, also occurs during maturation. The precipitates are subsequently removed during racking.

The ambient temperature and humidity of storage also influence the proper course of maturation. During the first year, it is important that the temperature remain sufficiently low (between 5 and 10 °C), to support rapid and complete precipitation. Chemical reactions of condensation of the different families of phenolic compounds are accelerated from 18 to 20 °C. Above 20 °C, growth of spoilage microorganisms can lead to the development of off-tastes and off-odors. During the second year, preferred storage temperatures range from 12 to 15 °C.

After clarification, further modification should be limited. Humidity control is important to regulate water and alcohol loss by avoiding undesirably dry or moist condition in the cellar—low humidity promoting water loss and high humidity favoring alcohol loss through the barrel. Experience has shown that the ideal relative humidity is between 80% and 90%. Clearly, barrel aging is different than that in an inert container of the same size.

Another important phenomenon that occurs during maturation concerns the lysis of the yeast cells at the end of the fermentation. The autolysis of the yeast cells involves a set of reactions, many of which are enzymatic, effecting the degradation of cellular constituents and their release in the surrounding medium. These constituents include proteins, peptides, glycoproteins, amino acids, nucleotides, nucleic acids, vitamins, minerals, and fatty acids (Feuillat, 2003).

The significance of yeast autolysis has been long known in wine production, notably in the production of sparkling wines. Its use in table wines has only recently begun to be seriously investigated. Its promotion is termed *sur lees* maturation. Because of its complexity, an alternative to yeast lees has been proposed. Yeast lysates are obtained by using different cell fractions from dead yeast cells. Depending on their origin, method of production, or suggested use they may be called yeast-cell walls, autolyzed yeast, yeast-cell-based products, yeast polysaccharides, or yeast mannoproteins. Of these, the most studies are mannoproteins. They can interact with phenolic compounds, absorbing anthocyanins, and binding with the tannins; adsorb undesired substances, such as short-chain saturated fatty acids (C8, C10, C12) and ochratoxin; stimulate malolactic fermentation; interact with aromatic compounds, either enhancing or diminishing their perception; and limit the precipitation of potassium bitartrate and turbidity inducing proteins, acting as a ''protective colloids''. Because of their potentially beneficial and detrimental effects it is important to systematically monitor the results during the maturation period when the wine may remain in contact with yeast lees.

Although many of these phenomena are not fully understood from a scientifically point-of-view, their potential significance is such that they can no longer be neglected, even if their practical management is not easy.

VI. BIOTECHNOLOGY—NEW POSSIBILITIES FOR AMARONE WINE

The production of Amarone can be better controlled, and probably improved, by developing a deeper understanding of the three basic stages that give the wine its originality and quality attributes.

In particular, aspects requiring further studies include partial dehydration and the consequences of botrytization; the development of solution to problems associated with alcoholic and malolactic fermentation; and a clear understanding of critical processes associated with maturation.

A. Grape dehydration phase

The effect of various thermohygrometric conditions on grape dehydration kinetics has been recently studied by Barbanti *et al.* (2008). Nonetheless, improved ways of controlling this delicate process are always looked for, with the aim to better clarify the role of *Botrytis* in the metabolic changes that occur in the *fruttaio*, and to choose the best conditions to favor the development of noble rot.

Because infection by *B. cinerea* typically is destructive, making the production of a drinkable wine impossible, it has a strong negative image among grape growers and winemakers alike. Most research is directed at limiting if not preventing its growth on grapes. However, its positive role in the form of noble rot produces some of the highest quality white wines, such as Sauternes and Tokay (Doneche, 1993), but its effects in the production of red wines needs to be further explored (Tosi *et al.*, 2009a; Usseglio-Tomasset *et al.*, 1980) and may be valorized.

B. The winemaking process

Producers have adopted the use selected commercial strains of yeasts and LAB to overcome some of the problems associated with fermentation under the harsh conditions of winter. However, to achieve greater sensory individuality and enhance regional character, there is a trend by producers to select strains isolated from their local grapes and individual wineries (Bovo *et al.*, 2005; Malacrinò *et al.*, 2005).

To date, scientific research has not confirmed a relationship between the local microflora and the typicality of the wine. This may be due to the variety of grapes involved and variation in winery practice. Nevertheless, research continues, because, even if the results were limited to features such as cold and alcohol tolerance, the use of selected indigenous microbes will lead to the delineation of higher quality standards, more responsive to the canons of *genius loci* (the particularities of the zone).

1. Aging

The phenomena associated with maturation are extremely important in directing the positive evolution of the wine's quality as well as physicochemical and microbial stability. It was ascertained that wine oxidation enhanced aldehydes formation and the increase of typicality sensory descriptors of Amarone wine. As a consequence, oxidative aging seems to provide the wine with the peculiar typicality which makes Amarone one of the most renowned wines in the world (Fedrizzi *et al.*, 2011; Tosi *et al.*, 2009a).

Although much still needs to be learned, biotechnological advancements complement traditional procedures in the search for improved quality and regional character.

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