# Biotechnology of Ice Wine Production

Wang Jing\*, Li Min\*, Li Jixin\*, Ma Tengzhen\*, Han Shunyu\*, Antonio Morata\*\*, Jose A. Suárez Lepe\*\*

\*Gansu Agricultural University, Gansu, China; \*\*Technical University of Madrid, Madrid, Spain

#### 1 Introduction

Ice wine is a kind of dessert wine or naturally sweet wine produced from frozen grapes obtained after natural freezing while the grapes are still on the vine. A major difference with other high quality sweet wines, such as Sauternes or Tokaji, is that the grape is not botrytized. Variations include artificial ice wines produced from running must concentrated by cooling. The origin of ice wine probably occurred by chance, when winemakers tried to produce wine from frozen grapes after unsuitable climatic conditions in northern areas at the end of maturity. Ice wine is believed to have been first produced in the Franconia region in 1794 after a vineyard's grapes froze while the monks in charge were waiting for the permission to harvest them. The first fully documented *Eiswein* harvest dates back to 1830, when wine growers left grapes on the vine to freeze, assuming they could be used as an animal fodder. However, once they pressed the grape they realized the quality of the must and the extreme sweetness. The fermentation process must have proved difficult because of the high sugar content and it is quite likely that it was impossible to fully ferment the sugars so that as a result the wine remained sweet, but it is likely that they liked it very much and perceived it as high-quality wine.

Ice wines are produced in Canada, Germany, Austria, Switzerland, and China. They are known as ice wines in English, *Eiswein* in German, 冰酒 (Bīngjiŭ) in Chinese, *Vin de glace* in Luxembourg, and *vi de gel* in Catalan. The greatest producers are Germany and Canada. Ice wines are really expensive because 3–4 kg of grapes are used to produce a small 375 mL bottle, equating to a glass per vine or a drop per grape berry; however, the magic conjunction of a strongly concentrated must in sugar acidity and aromatic compounds together with a complex and delicate fermentation process produce an enological jewel.

In many countries, ice wines are strongly connected with heroic viticulture, extreme climates, and even big slope mountain viticulture, making vineyard management and soil conditioning really difficult. But there are also connections to protect excellent natural environments with sustainable/organic viticulture.

#### 2 Market

Currently, the main global producer is Canada (8500 hL, 2015) and the main world market is China. Canadian ice wines generally come from Ontario's Niagara Peninsula and the Okanagan Valley in British Columbia, but also Nova Scotia and Quebec (www. winesofcanada.com). Year after year, Canada's wineries are awarded gold medals throughout the world for their quality ice wines and Canadian winemakers strive to produce better wines, challenging themselves and each other to produce this liquid gold. Only ice wine approved by the appellation of origin system called Vintners Quality Alliance (VQA) is allowed to be produced in Canada and several requirements set by the VQA must be met, which includes the prohibition of artificial freezing and sugar addition during the entire ice wine ("icewine" in the VQA terminology) production process (Setkova et al., 2007). Thus, one of the most important things is to distinguish real ice wines from the fraudulent products that have begun to emerge in the market recently. The main market for Canadian ice wines is China with sales of more than 65,000 L, followed by the United States of America and South Korea with about 22,000 L (Agriculture and Agri-Food Canada, 2010). The price of Canadian ice wines in China ranges from €30 to more than €400 in premium categories.

Due to the lower yield of grapes and the difficulty of processing, ice wines are more expensive than table wines. They are sold in half-bottles, with the average price being around \$45 per bottle. Currently, with a gradually increased understanding of ice wine, the Chinese market is steadily expanding. It has been reported that China has overtaken France as the world's largest consumers of red wine in 2014, having consumed 1.86 and 1.80 billion bottles of red wine, respectively, and considering the special preference for dessert wines, it is probable that China is also the world's main consumers of ice wines.

Production of ice wine in China is slightly higher than 700,000 L, about 40% of the global production. The price of ice wines ranges from €49 to €92 for 375 mL bottles in the Gansu province and from €21 to €115 in Changyu (Golden Ice Wine Valley, Liaoning province). Chinese ice wine history is not more than 20-years old and as such its exportation is not fully developed. In Gansu, Qilian exports ice wine to Japan and Malaysia, but only a very limited amount of about 1000 L. Changyu Golden Ice Wine Valley has exported to Germany, Australia, the United Kingdom, the United States, and other European countries, but the export volume is also very limited, just 3000–5000 L each year.

## 3 Regions for Ice Wine Production

There are few places suitable for producing ice wine in the world, traditionally only three countries including Germany, Austria, and Canada. The main ice wine producing countries are Germany and some regions in Austria, Ontario in Canada, Niagara Peninsula, and British Columbia's Ken Root Valley (Fig. 10.1).

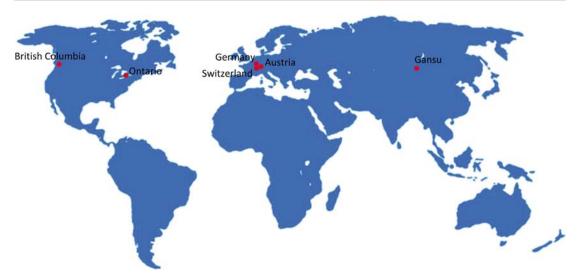


Figure 10.1: Countries and Regions Producing Ice Wines.

Germany is the home of ice wine, originating in the Franconia region. At present, the main regions that produce ice wine in Germany are Pfalz, Mosel, Rheingau, and Rheinhessen. Although Germany is the home of ice wine, it cannot produce it year after year. Eiswein is part of the *Prädikatswein* quality category in German wine classification. The *Pradikat* level is determined by the level of sugars in the grape at harvest, and chaptalization is not permitted. Grapes used must be of a *Beerenauslese* quality. The main difference between Canadian and German ice wines is in the varieties of grapes, with Riesling being one of the most important for the production. Also, German generally has lower alcoholic volumes with 6%-9% (v/v) being the most common.

Ontario (Canada) has been producing ice wine since 1972 with three VQA wine appellations—Niagara Peninsula, Prince Edward County, and Lake Erie North Shore; the Niagara Peninsula is also divided into 10 subappellations. The Inniskillin winery produced its first vintage in 1984, the first commercial production of Canadian ice wine, but in 1972, a German immigrant made ice wine at Okanagan using Riesling grapes. In 2015, Canadian ice wine production was 850,000 L (Wine Country Ontario, 2015).

Switzerland ice wines are produced in Valais among Europe's highest vineyards, the plots range in altitude from between 650 and 1150 m. Swiss production is very small but is of high quality.

Austria is also famous for its ice wine production, and has four ice wine producing areas including Vienna, Wachau, Styrie, and Burgeland. Wachau is the most popular ice wine producing area in Austria.

In recent decades, China has developed its ice wine producing regions. There are several ice wineproducing regions, which includes the Qilian Mountain in Gaotai (Gansu Province), Huanren and Tonghua in Liaoning Province, Meri Snow Mountain's Lancang River Valley in Yunnan, and Yili, Xinjiang. The two most famous ice wine producing regions are the Qilian Mountain in Gansu and Huanren in Liaoning. The Qilian producing region, located in the central area of Hexi Corridor in Gansu Province at northern latitude 47 degrees, is flat, and the elevation is 1400 m. Area of 300 Ha is devoted to ice wine production in Gansu. It has a very extreme climate, with very cold temperatures in winter (reaching  $-25^{\circ}$ C), making it necessary to cultivate the vineyard using buried viticulture. At  $-20^{\circ}$ C, the permanent wood of the vine dies and it is necessary to protect the plants against extreme cold. For this purpose, plants are within a trench below ground level and the plant is covered by earth over the colder months of winter (Fig. 10.2).





Figure 10.2: Vineyard at Qilian Employing Buried Viticulture Techniques (Gansu province, China).

There is enough sunshine, the average daily temperature is 14.9°C, the climate is dry, the annual precipitation ranges from 66.4 to 104.4 mm, the relative humidity of sandy soil is 52%, heat changes fast, and water from the Oilian Mountain is used for irrigation. This production region uses the Qilian Mountain as a natural protective screen. The region has optimal conditions for organic production because of the low incidence of pests and rot diseases due to very low environmental humidity and extreme temperatures in winter. As there is no disease and industrial pollution, it is a favorable region for ice wine development, and the annual production of ice wine is 330,000 L. The Huanren ice wine producing region in Liaoning is located near northern latitude 41 degrees, known as the Liaoning Huanlonghu Region, it has a plantation area that is 380 m high, has fertile soil, the vineyard is on a gentle slope, the summer is hot but there is a large temperature difference between the day and night, the winter is cold but not dry. As such, it has three ideal conditions "a cold climate, water, and sunshine" to develop the grape, and is called the golden ice valley by wine experts. The region produces 400,000 L wine annually.

Michigan has been turning frozen grapes into ice wine since 1983. New York's Finger Lakes region also produces ice wines in the United States of America. The climate is not always ideal for ice wine. Some places get cold enough a few years in a decade, but this is not generally the case. The production is quite small but the wines are of a very good quality and come with a high price tag. In 2016, President Obama served Grand Traverse County at an official reception dinner at the white house. It is necessary for temperature to be at  $-8^{\circ}$ C to collect frozen grapes for Michigan ice wines.

Luxembourg also produces Vin de glace. Quality labels Vin de Glace (also Vendanges Tardives and Vin de Paille) were regulated in 2001 following very explicit quality criteria, such as sugar concentration, varieties, and handmade harvests. The harvest of the grapes for this wine occurs at around 6 a.m., following a night of frosty temperatures with a minimum of  $-7^{\circ}$ C. Several hours of this low temperature are needed one night before the grapes can be gathered. This is why, the harvest of the grapes for this wine is only possible in December or even in January. Under the control of the Wine Growing Institute (Institut vini-viticole, IVV), three domains harvested grapes: Alice-Hartmann (Wormeldange), Schumacher-Lethal (Wormeldange), and Madame Aly Duhr (Ahn) (Grand Duchy of Luxembourg official site, 2016).

Other countries with notable ice wine industries include Croatia, Slovenia, Slovakia, the Czech Republic, Hungary, and Romania (Schreiner, 2001). In warm regions, altitude can play a role in making ice wines, even though some areas are hot and dry, the Spanish winery Altolandon, with vineyards at 1100 m (3600 ft.) made an ice wine with the Petit Manseng variety in January 2014 after temperatures dropped to  $-8.5^{\circ}$ C.

# 4 Varieties and Harvesting Conditions

Because of the high residual sugar and alcohol content in ice wine, there is a real need for high acidity to balance taste to avoid cloying, so traditional ice wines are generally made from aromatic and acidic grape varieties. Although cold weather conditions result in an absolute loss in acidity (potassium tartrate crystallization), juice concentration due to ice formation could provide sufficient acidity) (Jackson, 2008). The aromatic varieties often used for white ice wine are Vidal Blanc, Riesling, Silvaner, Gewürztraminer, Italian Riesling, etc., while Cabernet Franc, Merlot, Syrah, Cabernet Sauvignon, etc., for red ice wines.

Traditional ice wines are made from aromatic and acidic white Riesling, Gewürztraminer, Vidal Blanc, and Sylvaner varieties. In Canada and the United States, Vidal Blanc (*Vitis vinifera* Ugni blanc × Rayon d'Or 4986), a white hybrid grape variety is widely used. It is Canada's leading grape variety. It is a late-ripening variety with great cold hardiness. The clusters can hang on the vine after frost, until almost 30% of its weight is lost due to dehydration. It can be harvested more than 40 days after reaching maturity. Vidal Blanc is a thick skin variety so it is more protected than more sensitive *V. vinifera* against the cold temperatures before harvest. Vidal Blanc is readily available, inexpensive to grow, and easy to manage.

For red grape varieties, Cabernet Franc is used frequently in Canada for ice wine production bit Merlot, Pinot noir, and even Cabernet Sauvignon varieties can also be used. The Niagara-on-the-Lake region was the first to produce Syrah ice wine in 2004. They also started with Sangiovese in 2007.

Austrian red ice wine is made with Zweigel (St. Laurent × Blaufränkisch), which ensures large and heavy clusters hang on the vine for a long time after reaching maturity. White ice wine is made from Scheurebe, Grüner Veltliner, Riesling, Gewürztraminer, and Welschriesling.

The ice wine in China's Huanren producing region is mainly made from white Vidal grapes and red Beibinghong grapes. The ice wine in the Qilian producing region is mainly made of white Vidal and Italian Riesling grapes, and red Merlot grapes (Fig. 10.3). Grapes are frequently harvested at night, and squeezed and fermented in low temperatures.

Beibinghong was selected from the hybrid of the female parent Zuoyouhong, which is the hybrid of (*V. amurensis* Zuoshaner and *V. vinifera* Tchervine muscat) F1 × *V. amurensis* 74-1-326, and the male parent 86-24-53, which is the hybrid of (*V. amurensis* 73040 and *V. vinifera* Ugni blanc) F1 × *V. amurensis* Shuangfeng in 1995 (Song et al., 2008). This cultivar is resistant to extreme cold and serious fruit diseases: downy mildew, white rot, and anthracnose (Liu and Li, 2013). Because of its strong resistance to cold, earth covering treatment, which is common in buried local viticultural practice in Northeastern China for international varieties (such as Vidal, Riesling, and Cabernet Franc), is not required.





Figure 10.3: Frozen Merlot Grapes at Quilian (Gansu, China).

Therefore, the vinicultural management of Beibinghong is less labor intensive. Moreover, Beibinghong berries have relatively thick skins and high acidity, which is similar to cultivars typically used in ice wine production. In recent years, Beibinghong, widely cultivated in the Changbaishan region (Jilin province) and the Huanren region (Liaoning Province), was the most popular cultivar for red ice wine production (Yi-Bin et al., 2016).

Some vinicultural practices will increase many key aroma compounds in ice wine. After thinning at veraison, the volatile compounds in Vidal and Riesling ice wines increased significantly (Bowen and Reynolds, 2015a,b). Delaying the harvest of Riesling grapes increases many key aroma compounds in ice wines, such as: 1-octen-3-ol, ethyl benzoate, ethyl octanoate, cis-rose oxide, and  $\beta$ -ionone (Khairallah et al., 2016), and ethyl isobutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-octen-3-ol, 1-octanol, cis-rose oxide, nerol oxide, ethyl benzoate, ethyl phenylacetate,  $\gamma$ -nonalactone, and  $\beta$ -damascenone in Gewürztraminer (Lukić et al., 2016). Early harvesting produces higher concentrations of esters and aliphatic compounds.

Birds are a big problem for ice vine plots because the delayed harvest makes the sweet grapes really attractive to them. Moreover, at these times, seeds used as food by birds became scarce so they eat bunches of the fruit, destroying the entire crops. This is normally controlled by covering canopies with thin nets. Wild boars may also jeopardize bunches in some regions. Environmental conditions can affect the quality of grapes; wind, rain, hail, or even warm temperatures during harvest make the collection of frozen grapes or grapes at suitable temperature (below  $-7^{\circ}$ C) impossible. Pests and mold can also degrade grape quality, making it difficult to produce high quality ice wines.

Harvesting can be done using mechanical vine harvesters or by picking grapes by hand (Fig. 10.4). Manual harvesting aids in the selection of bunches on the plot with the ability to reject those affected by pests or rotting. A mechanical process is useful to harvest at night when low temperatures are a priority. It is also faster and lower in cost.





Figure 10.4: Frozen Vineyards and Harvests by Hand at Huanren (Liaoning province) and Qilian (Gansu province, China).

# 5 Pressing Technology and the Wine-Making Process

For sugar solutions, a temperature of around  $-20^{\circ}\text{C}$  is necessary to completely freeze water and sugar. When temperatures are in the range between -8 and  $-15^{\circ}\text{C}$ , water is frozen as pure ice crystals, and the resulting liquid is highly concentrated syrup of sugar with little water. So, if the grapes are frozen at this temperature, the syrup can be removed by pressing with a high concentration degree (25–42°Brix), with most of the water remaining in the pomace as ice crystals. Normally, ice wines are pressed in hydraulic basket presses and it

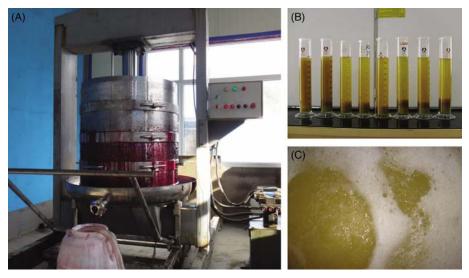


Figure 10.5: (A) Pressing of frozen merlot grapes, (B) Settling of syrupous white must, and (C) Must fermentation.

takes a long time (several hours) because of the hardness of the frozen grape (Fig. 10.5A). It is beneficial to use prechilled presses to keep grape temperature. Pneumatic presses are not frequently used even when it is the most suitable technology for conventional white wines, because it is difficult to extract must at the working pressure range of these presses, usually below 2 bar. Ontario presses at T<sup>a</sup> between -8 and -12°C, and Germany not higher than  $-7^{\circ}$ C. It is usually said that one grape yields a single drop of ice wine must. The yield of pressing is about 10%–15%, which produces sugar concentrations that are generally higher than 25°Brix (Table 10.1). The pressure can reach up to 95 bar and frequently higher than 20 bar. Pressing takes more than 2 h and grapes must remain frozen. After pressing, water remains in the pomace as ice crystals. Pressing process also concentrate acids (malic and tartaric), reaching acidity values frequently higher than 10 g/L.

The wine-making process has several key steps (Fig. 10.6), such as pressing because it is necessary to keep temperatures below -7 to  $-8^{\circ}$ C during the process to keep water in the pomace and to obtain a very concentrated must. Settling is a very slow process due to the syrupous and thick consistency of must because of the high concentration levels of sugar (Fig. 10.5B). Also, fermentation is a very difficult stage that is prone to sluggish fermentations because of the high concentration of sugar and extreme osmotic pressure (Fig. 10.5C). After fermentation, the next steps include typical stabilization processes and bottling—usually in small half bottles or even in 200 mL batches.

## 5.1 Cryoconcentration

Some wine producers try to emulate the natural ice wine traits by freezing grapes or musts. Defenders of this technique in warm countries or regions like California, Australia, New

Table 10.1: Main regional regulations in ice wine production.

Country	Region	Production (L)	Maximum Temperature at Harvest (°C)	Must Yield (%)	Juice Concentration (°Brix)	Varieties	Residual Sugars (g/L)	Acidity (g/L)	Maximum Volatile Acidity (g/L)	Ethanol [% (v/v)]
OIV	region	(-)	-7	(/0)	>25	Variotics	(8/ -)	(8/-)	2.1	>5.5
_						NA /la ite a			2.1	
Austria					29	White Grüner Veltliner Red Zweigelt			2.1	>5.5
Canada	Ontario (three appellations: Niagara Peninsula, Prince Edward County, and Lake Erie North Shore)	850,000	-8	10–15	35-38	White Vidal Riesling Chardonnay Gewurztraminer Red Cabernet Franc	180-320 Average 220	>10		8-13
China	Gansu	330,000	-8	15	35-38	White Vidal Italian Riesling Red Merlot	146-180	8.1-9.2	1.1-1.5	11.5-11.8
	Huanren	400,000	-8	15	33	White Vidal Red Beibinghong	140–180	8.0-10.0	0.8-1.5	11.0

Germany	Mosel Rheingau Rheinhessen Pfalz	45,000	-7		>28	White Riesling Red Merlot Pinot noir	>100 >250	>10	2.1	>5.5
Luxembourg	Moselle			10	35-40 Minimum 28	White Riesling Pinot Blanc Pinot Gris	>125		1.8	
Switzerland	Valais		-12		39	Red Pinot noir Eyholzer Roter (Hibou)				12
United States	Michigan and the Finger Lakes (NY)	5,000ª	-8	<20	36-40	White Vidal Riesling Red Cabernet Franc				12.5

<sup>&</sup>lt;sup>a</sup>6 Michigan Wineries in 2002.

Source: From Wine Country Ontario, 2015. Available from: http://winecountryontario.ca/media-centre/icewine; Bowen, A.J., 2010. Managing the quality of ice wines. In: Reynolds, A. (Ed.), Managing Wine Quality, first ed. Woodhead Publishing, Cambrige, UK, pp. 523–552 (Chapter 18); Commission Regulation (EC), 2008. No 423/2008. Off. J. Eur. Union 8; OIV, 2003. Definition of Ice Wine. Resolution OENO 6/2003. International Organization of Vine and Wine. Paris, France; Robinson, J., 2001. Available from: http://www.jancisrobinson.com/articles/icewine-worth-the-money-and-the-hassle.

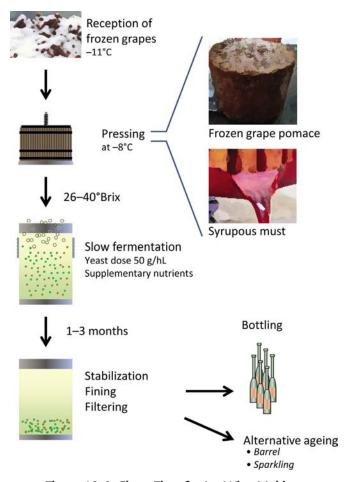


Figure 10.6: Chart Flow for Ice Wine Making.

Zealand, Spain, and even Argentina, say that the way in which the grape is frozen is not of importance and that the artificial process using cooling technology also allows the preservation of grapes from microbial alterations and pests. However, traditional producers say that not only the cold is necessary but that grapes mature under these aggressive conditions to develop the full aromatic complexity of a traditional ice wine.

## 6 Fermentation and Biotechnologies

The ecology of grapes under ice wine harvesting conditions shows a prevalence of *Aureobasidium pullulans* and *Rhodotorula glutinis* (Alessandria et al., 2013). Also, spontaneous fermentations were mainly driven by *Hanseniaspora uvarum*, *Metschnikowia fructicola*, and *Saccharomyces cerevisiae* at the midend of the fermentation process.

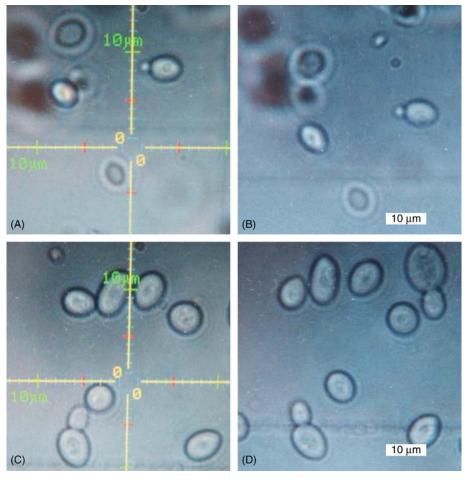


Figure 10.7: Saccharomyces cerevisiae fermenting concentrated must at 38°Brix (A-B) and running must at 22°Brix (C-D). Smaller size, lower turgency, and less budding percentage.

Ice wine fermentation is a complex and difficult process due to the high sugar content in the majority of the regions, with typical levels reaching higher than 30°Brix. There are reports that Riesling juice cannot reach 10% (v/v) of ethanol if the initial sugar concentration is higher than 42°Brix (Pigeau et al., 2007). Moreover, a sugar concentration of above 52.5°Brix could make the must unfermentable. During the making of ice wine, yeast cells are under very limitative conditions. It is frequent to observe smaller sized cells than those in normal wines (22–23°Brix), and osmotic pressure also affects yeast by reducing budding percentage and plasmolysis can be observed in the shape of the cells (Fig. 10.7). Buds are smaller, and take more time to evolve into adult cells, slowing the fermentation rate.

When yeasts are under hyperosmotic stress due to very high sugar concentrations, they produce glycerol and accumulate it inside the cytoplasm to balance external osmotic pressure. It also means a higher excretion of glycerol to the fermentative media (Fig. 10.8) that usually reach 10 g/L. The over production of acetic acid is another typical consequence of hyperosmotic stress with values ranging from 0.8 to 2.3 g/L.

Fermentation of must from frozen grapes takes a long time (>6 weeks, sometimes several months) and values of some fermentative intermediates are normally higher than for conventional wines (Fig. 10.9). Cell growth is inhibited by hyperosmotic conditions in standard must fermentations (24–26°Brix), the cell counts can easily overcome log 8 CFU/mL,

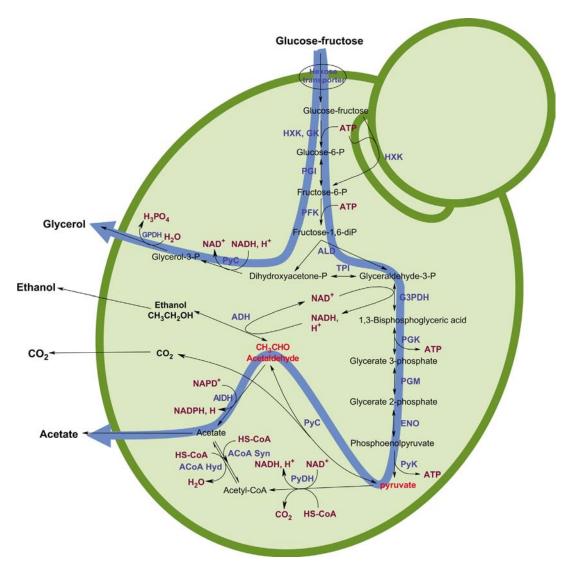


Figure 10.8: Biochemistry of the High Production of Glycerol and Acetic Acid Under Hyperosmotic Conditions.

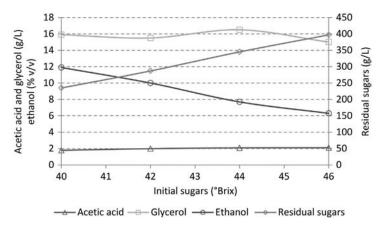


Figure 10.9: Fermentative Production of Acetic Acid, Glycerol, Ethanol, and Residual Sugars

Depending on the Initial Sugar Concentration.

Adapted from Pigeau, G.M., Bozza, E., Kaiser, K., Inglis, D.L., 2007. Concentration effect of Riesling ice wine juice on yeast performance and wine acidity. J. Appl. Microbiol. 103, 1691–1698.

however, ice wine musts (38–40°Brix) are frequently around or below log 7. The lag phase is considerably longer and the fermentation rate is slower than for table wines.

Acetic acid, glycerol, and total acidity formation standardized to metabolized sugar strongly correlate to must concentration (Pigeau et al., 2007). The fermentation of 38°Brix must increases acetic acid and glycerol production 8.4- and 2.7-folds, respectively (Pigeau and Inglis, 2007). Fig. 10.10 shows value ranges of acetic acid and glycerol depending on initial sugar concentration in must.

Acetaldehyde concentration reaches values of four times higher than normal wines during the first week, but later the concentration is lower due perhaps to the high volatility of such molecules and its metabolization by aldehyde dehydrogenases. The expression of several mitochondrial aldehyde dehydrogenases has been observed and it can also affect the formation of glycerol-3-phosphate. Production of acetic acid means more than an 80% increase in total acidity (Pigeau et al., 2007). The content of ethyl acetate in ice wines is frequently higher than in traditional wines because of the levels of volatile acidity (Lukić et al., 2016).

Typical prolonged lag phases and sluggish fermentations of highly concentrated musts in ice wine production mean that it may take several months to obtain the suitable alcohol volume. The use of stepwise rehydration protocols increases ethanol concentration levels and improves yeast viability making the fermentation process safer and shorter (Fig. 10.11). The procedure includes several acclimatization steps from more diluted juice to higher concentrate media to adapt yeast metabolism and cell envelops to extreme osmotic conditions (Kontkanen et al., 2004). The use of warm temperatures makes the yeast membrane and cell

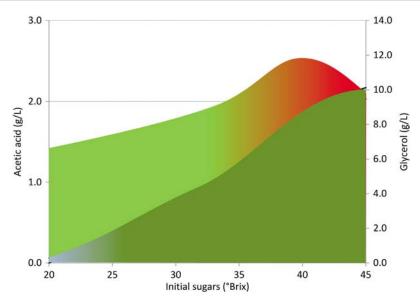


Figure 10.10: Range of acetic acid (grey line) and glycerol (black line) in high sugar fermentations. Adapted from Pigeau, G.M., Bozza, E., Kaiser, K., Inglis, D.L., 2007. Concentration effect of Riesling ice wine juice on yeast performance and wine acidity. J. Appl. Microbiol. 103, 1691–1698 and Inglis, D., 2012. Torulaspora delbrueckii and ice-wine fermentation: the start of a winning combination. XXIIIes Entretiens Scientifiques Lallemand. Monestier, France.

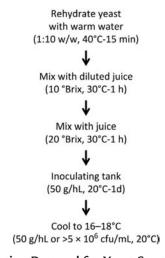


Figure 10.11: Acclimatization Protocol for Yeast Starters to Ferment Ice Wines.

Adapted from Kontkanen, D., Inglis, D.L., Pickering, G.J., Reynolds, A., 2004. Effect of yeast inoculation rate, acclimatization, and nutrient addition on icewine fermentation. Am. J. Enol. Viticult. 55, 363–370.

wall more flexible to pressure adaptation. Under these conditions, it is possible to get higher yeast counts and better-adapted cells to the fermentation of highly concentrated musts. When acclimatized yeasts are used at 0.2 g/L, the alcoholic volume increases slightly, however, when double inoculation dose (0.5 g/L) and stepwise acclimatization are used together, it is easier to reach 10% (v/v) of ethanol (Kontkanen et al., 2004).

To increase the quality of ice wine fermentation yeast, selection and breeding are powerful tools for obtaining strains or hybrids with improved performances that are better adapted to the osmotic stress of high concentrated musts. The hybridization of a robust Saccharomyces cerevisiae wine strain with S. bayanus with a strong fermentative power and low production of volatile acidity has been used to reduce its contents during ice wine fermentation (Bellon et al., 2015). The yeast strain also produces variations in the wine's aromatic profile but it is also strongly influenced by interactions between the yeast effect, vintage, and variety (Crandles et al., 2015).

The choice of yeast strain has also been found to significantly affect the accumulation of acetic acid, glycerol, reduced-sulfur odor, and color (Erasmus et al., 2004). Compared with using S. cerevisiae, spontaneous fermentation in Cabernet Franc ice wine is unique in its production of geranyl acetone and ethyl benzoate. Using S. bayanus in Cabernet Franc ice wine produces the highest concentrations of 15 compounds including 4 alcohols, 7 esters, furfural, hexanoic acid, TDN, β-damascenone (Kinga et al., 2015), and highest concentrations of 2-methyl-1-butanol, isoamyl acetate, and ethyl decanoate in Riesling ice wines (Crandles et al., 2015). This demonstrates that different yeast species produce unique compounds or higher concentrations of specific compounds relative to other treatments (Kinga et al., 2015).

The addition of nutrients, especially to increase yeast assimilable nitrogen, is normally carried out by dosing diammonium phosphate or yeast hydrolysates. Also, yeast fragments release unsaturated lipids and vitamins that help yeast to grow and to make fermentation, even under extreme conditions, easier. Some commercial yeast activators have been described because of the positive role it plays in ice wine fermentations or in problem musts or stuck fermentations: GO-FERM (Lallemand, Montreal, and Canada) is a rehydration nutrient containing pantothenate, biotin, magnesium, zinc, and manganese (Kontkanen et al., 2004).

Ice wines are frequently found in 375 mL bottles because of its high price. In bottling, refermentations and alterations can be controlled by the addition of sulfur dioxide and sorbate. In Canada, average ice wine values were 180 and 140 mg/L, respectively (Soleas and Pickering, 2007). The addition of SO<sub>2</sub> can be diminished by using lysozyme to control bacterial growth (Chen et al., 2015). In ice wines, sulfite content ranges 2–3 times the average values found in table wines because of the high amount of residual sugar, helping to avoid refermentation in the bottle by yeast, but also bacterial developments. Lysozyme, an enzyme able to hydrolyze the peptidoglycan layer of a bacteria cell wall, reduces the viability of Gram-positive bacteria. Its use is allowed in oenology to control or delay malolactic

fermentation and bacterial alterations. So, the use of lysozyme, together with  $SO_2$  and sorbate, produce synergistic effects that largely help to control bacterial developments at lower  $SO_2$  values (Chen et al., 2015).

# 7 Use of Non-Saccharomyces

The use of non-*Saccharomyces* yeasts is a trend in current oenology (Morata and Suárez-Lepe, 2016; Suárez-Lepe and Morata, 2012), with some species, such as *Torulaspora delbrueckii*, which behave as an osmotolerant, becoming an interesting alternative to *S. cerevisiae*. *T. delbrueckii* (Fig. 10.12A–B) is a low producer of acetic acid and ethyl acetate, when it is used in ice wine fermentation whose volatile acidity is near 0.4 g/L, reaching a final alcoholic degree of 9% (v/v). When the same yeast is used in sequential fermentation with *S. cerevisiae*, it is possible to obtain 10% (v/v) of ethanol, which is more suitable for ice wine production, and volatile acidity remains below 0.6 g/L (Inglis, 2012). Moreover, the control of osmolality in *T. delbrueckii* can be managed by the production of glycerol and arabitol (Lucca et al., 2002), perhaps facilitating a better adaptation to hyperosmotic substrates. The production of some interesting aroma compounds, such as 2-phenylethyl acetate (rose petals), 3-ethoxy propanol (solvent and blackcurrant), and diacetyl (buttery and nutty) can be enhanced with many *T. delbrueckii* strains, helping to improve the sensory profile (Loira et al., 2014) (Table 10.2).

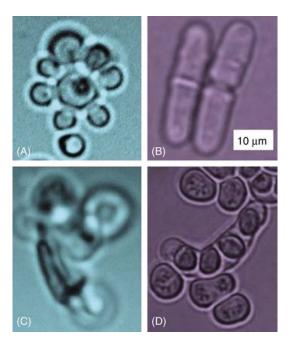


Figure 10.12: Optical Microscopy of T. delbrueckii.

(A) Budding cell, (B) conjugation and *Schizosaccharomyces pombe*, (C) reproduction by bipartition, and (D) lineal sporulation with four asci.

Species	Brand	Producer	Nitrogen Requirements	Alcohol Tolerance [% (v/v)]	Volatile Acidity	Sugar Tolerance
T. delbrueckii	Level2 TD Viniflora PRELUDE	Lallemand CHR Hansen	Moderate Moderate	10 9	Low Low	High High
L. (Kluyveromyces) thermotolerans	Viniflora CONCERTO	CHR Hansen	Moderate	10	Low	Medium
Schizosaccharomyces pombe	ProMalic	Proenol	Very low	13	High	High

Table 10.2: Some non-Saccharomyces yeasts used or with potential applications in ice wine production.

http://www.enolviz.com/modulos/pdfs/productos/levaduras/28/699118\_PRELUDE.nsac.pdf; http://www.lallemandwine. com/products/catalogue/product-detail/?range=9&id=54; http://www.proenol.com/files/editorials/ProMalic\_FT204-09\_PT.pdf?u=.

Schizosaccharomyces pombe (Fig. 10.12C–D) reduces malic acid levels by maloalcoholic fermentation (Suárez-Lepe et al., 2012), and could prove interesting in achieving a better acidity balance in some ice wines. Malic acid degradation is very efficient in S. pombe because of the existence of a specific malic acid carrier **mae1p**, and also because malic enzyme is cytosolic nonmitochondrial like in S. cerevisiae (Saayman and Viljoen-Bloom, 2006).

Many S. pombe strains can reach more than 13% (v/v) of ethanol in table wines, so can be used as single inoculum to make complete fermentations in ice wines. The only problem is the high production of acetic acid, frequently near 1 g/L in table wines (Benito et al., 2012), but this has never been studied in highly concentrated musts. Also, acetate production can be controlled by means of mixed or sequential fermentations with S. cerevisiae (Loira et al., 2015). The peculiar metabolism of S. pombe increases the amount of pyruvate during fermentation, affecting the formation of stable pyranoanthocyanins, such as vitisin A and vitisin A derivatives. These pigments behave as very stable pigments in enological conditions and can improve color and color persistence of red ice wines (Morata et al., 2012). Furthermore, nitrogen requirements of S. pombe are very low and it has been proposed as a biotechnology to reduce biogenic amines due to low yeast assimilable nitrogen needs and the metabolization of malic acid by maloalcoholic fermentation reducing the development of lactic acid bacteria (Benito et al., 2012).

Lachancea (Kluyveromyces) thermotolerans has been described as a low acetate producer (Comitini et al., 2011) and is able to produce +L-lactic acid (Gobbi et al., 2013). Moreover, L. thermotolerans is able to ferment until it reaches 10% (v/v) of ethanol, a property that makes it really interesting in ice wine production. Additionally, it has been reported that an increase in both glycerol and 2-phenylethanol levels is associated with S. cerevisiae (Gobbi et al., 2013).

Non-Saccharomyces offer new tools to control volatile acidity in ice wines and at the same time they are able to increase aromatic complexity and there is a possibility of other improvements in biological ageing and stability with some species.

# 8 Ageing of Ice Wines

# 8.1 Ageing on Lees

Ageing on lees (AOL) is a biological ageing technique consisting of keeping wines in contact with lees (Fig. 10.13) for several months/years to increase mouth feel by the release of yeast polysaccharides and mannoproteins and to improve aromatic complexity by some yeast metabolites or because of the formation of new aromatic compounds. Lees are the death cells that have fermented the wine. The AOL of ice wines can improve structure, increasing the integration and smoothing of acidity. Moreover, it is possible to obtain a more complex sensory profile by the formation of new aromatic compounds with notes of baked goods and yeast.

AOL starts with the autolysis process of yeast-by-yeast enzymes that starts to depolymerize cell envelops. In this process, not only cell wall constituents but also cytosolic contents are released in wines. Yeast cell walls are formed by chitin, glucans, and mannoproteins. The cell wall is 30% of the yeast's dry weight, polysaccharides represent approximately 85%, and proteins 15% (Nguyen et al., 1998). Yeast cell wall polysaccharides increase mouthfeel, reduce astringency and bitterness, improving wine softness and density.

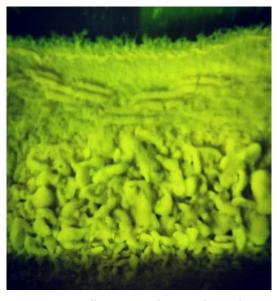


Figure 10.13: Yeast Lees Sediments During Bottle Ageing on Lees (AOL).

Moreover, AOL is reductive protecting fruitiness and it probably helps to preserve variety aromas in ice wines. Yeast cell walls contain glutathione, a tripeptide with strong antioxidant properties located mainly in its thiol groups. Glutathione is currently used by many winemakers to control oxidations and minimize levels of SO<sub>2</sub>, which are higher in sweet wines than in dry ones.

Yeast autolysis is a very slow process but is also strain dependent, so selection of suitable strains can help speed it up. The production of pure yeast biomasses of a single yeast culture selected for its specific positive properties for AOL improves the microbiological application of this technique (Suárez-Lepe and Morata, 2006). Moreover, cocktails of several yeast species/strains can be used for AOL processes to increase the amount of polysaccharide released and to increase aromatic complexity.

The use of non-Saccharomyces yeast has been recently described as a tool to improve AOL processes (Kulkarni et al., 2015; Suárez-Lepe et al., 2012), speeding it up, and reducing the time needed for autolysis. When osmophilic yeasts with specific configurations of doublelayer cell walls, such as S. pombe or S. ludwigii are used, the possibilities presents to increase polysaccharide contents in a shorter time is a technical advantage (Palomero et al., 2009). At the same time, it is possible to improve mouthfeel given the large average size of their polysaccharides compared to S. cerevisiae.

The use of AOL in ice wines can help to improve the aromatic complexity and flavor balance and, at the same time, protect the aroma compounds from oxidation. Moreover, AOL can soften the acidity making a better-balanced wine. AOL can be applied in a barrel or steel tank. Several biotechnologies can be used, from the simple use of lees from fermentation to the production of pure lees of a specific yeast strain or even the use of non-Saccharomyces yeasts.

## 8.2 Sparkling Ice Wines

First sparkling ice wine was made in Canada in 1998, by Inniskillin Company using the Charmat Method in stainless steel tanks. At sensory level, the integration of CO<sub>2</sub> bubbles reduces sweetness and helps to improve integration and freshness. To make sparkling wines, there are main technologies for traditional bottle fermentation and Charmat process.

In the first technique, the most traditional one, partial fermentation of the sugar must be done in the bottle reaching the desired CO<sub>2</sub> pressure, after fermentation the typical AOL stage improves mouthfeel and aromatic complexity. As AOL take a long time to develop yeast autolysis and promote the formation of aromatic compounds, a period longer than 9 months is frequently recommended to reach suitable expression. After fermentation and AOL, it is necessary to remove lees to obtain a clean and clear product (disgorging). Dosing, capping, riddling, disgorging, and corking in the usually small quantities of 375 mL bottles is labor intensive. However, a lengthy AOL time produces a better integration of CO2 with fine

bubbles, a creamy texture, and a complex AOL flavor. A second possibility is the Charmat Method in which fermentation is carried out on a bigger scale in steel pressure tanks, where the sparkling wine is settled and later transferred to bottles under isobaric conditions. The Charmat Method is easier and has lower costs compared with traditional bottle fermentation. In both techniques, yeasts undergo a very difficult fermentation process because of the high osmotic and CO<sub>2</sub> pressures.

#### 8.3 Oak Aged Ice Wines

Also available in the market are ice wines aged in French and American oak barrels for a variable time (4–9 months) for added complexity. Barrel ageing also means an evolution in color, with it being typical for these white ice wines to turn into golden or honey tones. The great natural acidity is softened by barrel aging, but at the same time, the high acidity of ice wines protects them during the long ageing period and helps to maintain structure and balance. The complex aroma evolves from fruity notes to reach greater complexity and depth and also improves with a creamy vanilla flavor. Simultaneous use of lees and oak aging together with agitation (*Bâtonnage*) will result in a smooth buttery wine with toffee characteristics. During ageing, temperatures must remain below 15°C and there should be high humidity levels. Bottled ice wine should have a long life if kept at a suitable temperature in the absence of light and vibrations.

## 9 Analytical Controls

Due to its relatively high price, the authenticity of ice wine is becoming a real issue in wine production. Ice wines are produced according to strict production standards and regulations. According to German law, Eiswein is classified under Qualitätswein mit Prädikat (QmP). Frozen grapes for ice wine production must be harvested at the minimum sugar concentration level and should be between 110 and 128° Oechsle (26–30°Brix). The minimal alcohol by volume content is 5.5% and the maximum quantity of total SO<sub>2</sub> allowed is 400 mg/L. In Canada, ice wine is regulated by the VQA. A VQA regulation states that frozen grapes for ice wine production must be harvested at the minimal level of sugar must be 35°Brix equivalent to 153.5° Oechsle. According to the VQA, ice wines in Canada must have at least 7% alcohol, a concentration of acetic acid not above 2.1 g/L and residual sugars must be 125 g/L (100 g/L in British Colombia). Austrian law dictates that Eiswein must have 25° Klosterneuburger Mostwaage (KMW). Under Luxembourg legislation, Vin de Glace must have 120° Oechsle (28.1°Brix) (Mencarelli and Tonutti, 2013). According to the National Standard of the People's Republic of China, the alcohol content should be between 9% and 14% by volume and residual sugar must be above 125 g/L in 

\*\*Time December 125 g/L in \*\*Time December

Analytic measurements are a necessary approach in ice wine production for product monitoring and quality control. OIV recognized and published many analysis methods, and some countries consider that the methods of analysis shall prevail as reference methods for the determination of the analytical composition of the wine in the context of control operations. Many vine-growing countries have introduced its definitions and methods into their own regulations (OIV, 2015). In fact, the fast and unambiguous identification of potential markers, suitable to qualitatively differentiate the crops from various areas and farming systems, is a crucial requirement in trade and regulations. Conclusive analysis is a matter of highly reliable and objective instrumentation (Setkova et al., 2007).

#### 9.1 GC Applications

Today, Gas Chromatography (GC) and GC-MS with specific sample preparation are widely used for the detection and quantification of the organic molecules and also organometallic species in ice wine, especially for the analysis of both volatile and semivolatile compounds. Setkova et al. (2007) utilized a headspace solid-phase microextraction-gas chromatographic-time-of-flight mass spectrometric (SPME–GC–TOF-MS) method for the analysis of volatile and semivolatile components of ice wine from Canada and the Czech Republic. The entire method for ice wine analysis did not exceed 20 min per sample (Table 10.3). An odor assessment of 25 Canadian and German ice wines was carried out using a GC olfactory detector system (SGE International Pty.

Table 10.3: Optimized conditions of the analytical method for the determination of volatile and semivolatile components of the ice wine aroma.

SPME						
Ice wine sample volume	3 mL in 10 mL headspace vial					
Salting out	1 g NaCl					
Sample incubation conditions	45°C, 5 min					
SPME fiber and mode	DVB/CAR/PDMS 50/30 µm; headspace					
Sample extraction conditions	45°C, 5 min					
Sample agitation speed (incubation/extraction)	500 rpm/500 rpm					
Fiber desorption conditions	260°C, 2 min					
GC-TOF-MS						
GC column	RTX-5 (10 m × 0.18 mm I.D., 0.2 μm)					
Injection mode	Split less					
GC oven program	40°C (30 s); 50°C/min to 275°C (30 s)					
Carrier gas, mode, and flow	Helium at constant flow, 1.5 mL/min					
Transfer line temperature	275°C					
GC run time	5.7 min (last wine component eluted at 4.45 min)					
Mass analyzer	High-speed time-of-flight					
Ionization type/energy	EI/70 eV					
Ion source temperature	200°C					
Detector voltage	1700 V					
Mass fragments collected	35-450 m/z					
Data acquisition rate	50 spectra/s					

SPME, Solid-phase microextraction.

Ltd, Ringwood, Australia). The end of the GC column was connected to a variable outlet splitter set to deliver flow to the mass selective detector and the olfactory detector port at a ratio of 30:70. This aroma port was fitted to vacant detector housing, and the tubing passing through the oven wall was heated with oven temperature air by means of an air venturi (Cliff et al., 2002). Other researchers have been analyzing ice wine volatiles using a GC equipped with a Carbowax column (Cliff and Pickering, 2006; Nurgel et al., 2004). GC-MS has also been used to determine amino acid. Ice wine stored for 24 years contained 0.9% p-Proline, 6.4% p-Glx, 3.0% p-Asparagine, and 1.5% p-Alanine determined by GC-MS-MS (Ali et al., 2010).

#### 9.2 LC Applications

High performance liquid chromatography (HPLC) methods are widely used for the analysis of many nonvolatile compounds in ice wine. The technique is suitable for sugar determination and fast detection glucose and fructose in ice wine. Two types of sugars in six types of ice wine were determined by HPLC with evaporative light-scattering detector. The samples were analyzed on a NH<sub>2</sub> chromatographic column (5  $\mu$ m  $\times$  4.6 mm  $\times$  25 cm). The flowing phase was acetonitrile: $H_2O = 80:20$  and the flow was 0.8 mL/min. The temperature in column and detector was 40°C and the injection volume was 20 µL (Wang et al., 2014). HPLC also has been used for the determination of phenolic compounds in ice wine. Li et al. (2016) determined phenolic compounds in two types of ice wine using the HPLC-MS-MS method. For anthocyanins, the mobile phase was composed of (A) 6% (v/v) acetonitrile containing 2% (v/v) formic acid, and (B) 54% (v/v) acetonitrile with 2% (v/v) formic acid. The gradient elution was as follows: 10% B for 1 min, from 10% to 25% B for 17 min, isocratic 25% B for 2 min, from 25% to 40% for 10 min, from 40% to 70% for 5 min, and from 70% to 100% for 5 min, with a flow rate of 1.0 mL/min. The injection volume was 30 μL and the detection wavelength was 525 nm. The column temperature was 50°C. The MS conditions were as follows: electrospray ionization, positive ion model; nebulizer, 35 psi; dry gas flow, 10 L/min; dry gas temperature, 325°C; scan, 100–1000 m/z. For the nonanthocyanin phenolic compounds, the mobile phase was comprised of (A) 10% (v/v) acetic acid, and (B) 90% (v/v) acetonitrile containing 10% (v/v) acetic acid. The elution gradient ranged from 5% to 8% B for 5 min, from 8% to 12% B for 2 min, from 12% to 18% for 5 min, from 18% to 22% for 5 min, from 22% to 35% for 2 min, from 35% to 100% B for 2 min, 100% B for 4 min, and from 100% to 5% B for 2 min with a flow rate of 1.0 mL/min. The injection volume was 2 μL and the detection wavelength was 280 nm. The column temperature was 25°C. The MS conditions were as follows: electrospray ionization, negative ion model; nebulizer, 35 psi; dry gas flow, 10 L/min; dry gas temperature, 325°C; scan, 100–1000 m/z. Also, glucose, fructose, and malic acid could be monitored by LC equipped with a UV detector set to 210 nm and a refractive index detector.

## 9.3 FT-MIR Applications

Using Fourier transform-middle infrared spectrometry (FT-MIR), it is possible to analyze wine and must within a 90 s timeframe simultaneously on a significant number of important enological parameters, such as alcohol content, relative density, extract, sugar-free extract, refraction, conductivity, glycerol, total phenols, reducing sugar, fructose, glucose, sucrose, total acid, pH value, volatile acid, total SO<sub>2</sub> and tartaric acid, malic acid, lactic acid, and citric acid. Sample preparation is usually easy, fast, and cheap, which includes decarbonation and cleaning by centrifugation or filtration. FT-MIR is a secondary analytical method that first needs to calibrate the instrument against the chemical reference methods for the different components (Table 10.4). Once the system has been cleaned and zeroed, it is a good practice to perform the standardization procedure before running calibration samples. Some companies have applications to transfer calibrations developed on the unit to be transferred to another standardized instrument (Patz et al., 2004).

Table 10.4: Concentration range in wines, average, and correlation  $(R^2)$ .

Parameter	Range	Average	R > 2a
Alcohol (vol.%)	7.4-14.0	11.4	0.982
Alcohol (g/L)	58.7-110.7	90	0.975
Relative density (20/20)	0.9908-1.0940	1.0021	0.999
Extract (g/L)	19.8-238.1	42.7	0.999
Sugar-free extract (g/L)	14.7-55.6	22.2	0.859
Conductivity (µS/cm)	1150-3230	1879	0.948
Glycerol (g/L)	5.20-27.80	7.85	0.983
Total phenol (mg/L)	134-2260	570	0.959
TEAC (mmol/L)	2.5-30.9	7.1	0.920
Sugars			
Fructose (g/L)	0.0-165.7	14.9	0.998
Glucose (g/L)	0.2-63.5	6.5	0.996
Sugar before inversion (g/L)	1.5-220.8	23.1	0.998
Sugar after inversion (g/L)	1.5-234.7	23.8	0.998
Acidity and organic acids			
Total acid (g/L)	3.72-14.10	6.14	0.973
pH	2.49-3.99	3.37	0.834
Volatile acid (g/L)	0.14-1.41	0.44	0.768
Tartaric acid (g/L)	0.8-3.3	2	0.423
Malic acid (g/L)	0.0-6.6	2.3	0.811
Citric acid (g/L)	0.0-2.3	0.32	0.487
SO <sub>2</sub>			
Total SO <sub>2</sub> (mg/L) (Tanner-Brunner)	32-588	120	0.703
Total SO <sub>2</sub> (mg/L) (photometry)	7–415	86	0.843
Free SO <sub>2</sub> (mg/L)	0-58	20	0.120

<sup>&</sup>lt;sup>a</sup>Averaged from validation with two independent data sets (A and B).

#### 9.4 Texture

As texture analysis is a fast and low-cost analytical technique, it can also be favorably applied in oenology as a routine tool for monitoring wine grape quality. Among the different mechanical parameters measurable, berry skin thickness and hardness are indices that reflect anthocyanin extractability and dehydration kinetics with adequate reliability (Rolle et al., 2012). Universal testing machines are those currently used in texture tests applied in grape studies and provide precise measures of force, time, distance, and deformation (Rolle et al., 2010). In grapes for ice wines, this technique can report information on frozen grape extractability.

# 10 Sensory Quality

### 10.1 Visual Appearance and Color

Appearance is very important for a wine. Sight is the first of our senses to be used in wine tasting. The eyes introduce a wine, providing an initial reference point, such as clarity, transparency, sediment, brilliance, color, bubbles, and fluidity. Meanwhile, sight can also affect the sensitivity of smell and taste. A glass of quality ice wine should be clear, bright, transparent, shiny, and reflective. Depending on its depth of color, a limpid red ice wine might not be transparent, but transparency is necessary for white ice wine.

Clarity is closely related to taste. A cloudy wine with particles in suspension will directly and adversely affect one's taste bud sensory qualities. The wine will be masked by a screen of impurities and the flavor will be distorted. A cloudy wine never tastes right; it is rough and lacking in harmony. Furthermore, haze is always a sign of spoilage for consumers. An appropriate level of wine clarity implies the level of visible impurities under analysis does not exceed the threshold of what is considered acceptable, because there is no such thing as absolute limpidity in wine. A clear wine becoming hazy once again is quite natural, regardless of whether it's been cleared by natural settling, or by a clarification procedure. Some fragile clear wines can become cloudy when exposed to air, while others can be similarly affected by light, cold, heat, or microbiological changes, all of which can detract from the wine's clarity and quality. It is only a stable wine that can harmoniously develop all its qualities as it ages. As long as the wine remains unstable, it is susceptible to various changes in clarity, known as cases.

In general, a glass of ice wine will reduce fluidity due to a high viscosity, which is largely dependent on the sugar, ethanol, glycerol, and soluble polysaccharide content. This is discernible only at unusually high sugar (15 g/L fructose or 5 g/L glucose) and/or alcohol (10%–15%) content or when the glycerol content exceeds 25 g/L (Jackson, 2009), and ice wine just meets this condition in terms of sugars. At and above this level, viscosity could reduce the perceived intensity of astringency and sourness of wine (Smith and Noble, 1998). In addition, ice wine will form more rivulets than dry wine due to capillary action.

The color of a wine may provide some indication of a wine's body, its age, health, and maturity. Generally, color is related to characteristics, a certain color indicates a certain type of wine. A white ice wine with a yellowish-golden color makes us recall a cluster of overripe grapes with yellow skins and a sweet fruity taste. Such a special golden color probably results from the joint effects of juice concentration, caftaric acid oxidation, and the release of catechins on freezing (Jackson, 2008).

#### 10.2 Aroma Profile

Ice wine has distinctive aromatic characteristics, varying from peach, pear, fig, green apple, raisin, dried apricot, citrus, pineapple, litchi, mango, to violet, honey, and caramel, depending on its origin, grape variety, and vinification. Young ice wine has a lively fruity flavor, the sensory profile evolves into honey, caramel, and dried fruit notes upon aging. The complex flavor of ice wine may be a cause of the following: using aromatic white varieties, aromatics adequately accumulate during the slow maturity of the berry, high sugar content enriched yeast fermentation to produce more alcohol and esters, besides, being aged in barrel or aged on lees can show more complex or evolved aromas.

Canadian white ice wine is known for its honey, lemon, apple, and tropical fruits, such as mango, fig, pawpaw, pineapple, lychee, dried apricot, and smoky, cinnamon fragrance, balanced with the proper acidity levels. Its sensory profile evokes dried apple, jam, and caramel when aged in barrels, and finishes with the smell of oranges. Aroma profile of red ice wine is a mix of strawberry, cream, spice, and mild herbs (Kinga et al., 2015). Sparkling ice wine has a fragrance of nectarine, apricot, lemon, honey, and the bubbles are finer than sparkling Brut wine.

German Eiswein (Qualitätswein mit Prädikat) are ice wines of beerenauslese concentration, made from grapes collected and pressed while frozen to concentrate sugar, acidity and extract, it is a truly special wine with a singular concentration of fruity acidic freshness and sweetness. Some of the wines present nutty and oily notes. Riesling is, without doubt, Germany's most highly esteemed grape variety, and also the main variety for Eiswein. Riesling is relatively cold hardy so the fruits can mature slowly to form its elegant and delicate aroma, and maintain high acidity in the pulp (Zhan, 2010). Riesling Eiswein is truly special with a singular concentration of fruity acidic freshness and sweetness, full of green lemon, pear, apple, peach, cherry (jam), chamomile, honey, raisin, dried apricot, syrup, cinnamon, and caramel aromas. Some of them have nut and oily notes (Parker, 2012).

Austria Zweigel red ice wine has a rose appearance with a fresh, sweet but not cloying taste. White ice wine made from Scheurebe, Grüner Veltliner, Riesling, Gewürztraminer, and Welschriesling show freshness and sweetness, that is, well balanced with the high acidity, full body, similar with Germa Eiswein.

Beibinghong ice wine is deep purple in color, has a special fruity aroma of *V. amurensis*, and sweet, honey, roast caramel, and is typically full-bodied with a lasting finish. Sensory profiles of Vidal Blanc ice wine in China depend on the region, and in the Jilin province is full-body,

sweet, balanced, refreshing, and has a lasting finish, while on the contrary, in the Liaoning province it is tart, watery and light (Table 10.5).

Luxembourg Riesling ice wines show a typical golden-yellow color. They have complex and concentrated aromas of dried flowers, lemon grass, and pineapple. Later, you are left with a fresh and powerful mouthfeel—large and generous mineral freshness with citrus hints in a perfect balance.

Sensory profiles of some typical varieties used in the elaboration of ice wines share common aspects but also remarkable specificities. Vidal Blanc has an intense character that is enhanced by the freezing process, resulting in an ice wine with a typical flavor profile that is greatly appreciated by the market. It is typical to find honey and peach aromas, as well as pineapple, apricot, and butterscotch in ice wines made with the Vidal grape. Gewürztraminer ice wines show terpene, floral, pungent, and ripe fruit aroma series (Lukić et al., 2016). Riesling ice wine provides a medium-bodied taste full of sweet apricot, peach, apple, citrus, and grapefruit and the aromas of honey and sweet grape. Fruit aroma is well balanced with a suitable and stimulating acidity. Cabernet Franc produces a wine with a bright red ruby tone and the powerful aroma of fresh strawberries. Sensory features and pairing of main ice wine varieties are as:

- Riesling (*V. vinifera*) is Germany's leading grape variety, a white grape known for its characteristic "transparency" in flavor. Aroma varies from sweet apricot, peach, apple, citrus, grapefruit, floral to mineral substance. The color will change to deep golden yellow through aging, accompanied by a more complex flavor of ripe fruit and honey. Its fine aroma is well balanced with a suitable and stimulating acidity. Riesling Eiswein can be paired with fish, chicken, shell fish, and soft cheese (Zhan, 2010).
- Vidal Blanc is an ideal variety for ice wine making. Matured fruit can form a mix fruity of orange, pineapple, grapefruit, apricot, and honey. When aged in barrel, it looks like a golden liquid with a complex aroma and delicate mouthfeel, is full bodied with a long finish. "Vidal Blanc" ice wine can be paired with fried goose liver, fruit, complex flavored cheese, ice cream, chocolate, etc. (Zhan, 2010).
- Gewürztraminer (*V. vinifera*), a white grape, has high sugar content and a relative low acidity level. It is famous for its high density complex aroma, varying from tropical fruit, such as banana, mango, lemon, litchi, peach to rose, honeysuckle, peony, violet, geranium, locust, verbena, gingersnap, lilac, cinnamon, even musk (Zhan, 2010). "Gewürztraminer" ice wines show terpene, floral, pungent, and ripe fruit aroma series (Lukić et al., 2016). It can be paired with meat sauces, cheese, foie gras, and venison, goes especially well with Asian food like Sichuan cuisine, Indian Curries, Vietnamese, and Thai cuisines (Zhan, 2010).
- Sylvaner (*V. vinifera*, Traminer × Osterreichisch-Weiβ), a white grape, has a unique aroma like "Riesling" but relative low acidity. With the fragrance of soil, basil, cotton, fern, ripe fruit, pale in color, "Sylvaner" ice wine can be paired with seafood, and asparagus (Zhan, 2010).

Table 10.5: Sensory properties of ice wine produced by several companies in China.<sup>a</sup>

			Ice Wine				
Region	Province	Company	Style	Varieties	Aroma	Color	Mouthfeel
Northeast	Jilin	Tonghua Wine Industry	White	Vidal blanc	Floral, fruity, tropical fruit,	Yellow-golden	Sweet, vinous, balance,
		Co., Ltd			honey		refresh, long finish
			Red	Beibinghong	Dried fruit, honey, dried	Ruby-red	Sweet, balance, refresh,
					grape		long finish
		Qingshanyuan Wine Industry Co., Ltd	Red	Beibinghong	Fine	_	Vinous
		Xuelan Wine Industry	Red	Beibinghong	_	_	_
		Co., Ltd	White	Vidal blanc	_	_	_
		Tonghua Zilong Wine Industry Co., Ltd	White	_	Apricot, pineapple, honey, tropical fruit	-	Sweet, fat, balance, long finish
			Red	Shuanghong	Honey, peach, pineapple, black berry, fruity	Ruby-red	-
			Red	Beibinghong	Grape, fruity, nut	Garnet	Soft, balance, bouquet
	Liaoning	Sun Valley Ice Wine Industry Co., Ltd	White	Riesling	Floral, fruity, delicate, fine, complex	Yellow-golden	-
			White	Vidal blanc	Pineapple, mango, apricot, peach, honey, sweet melon	Yellow-golden	Tart, watery, light
			White	Pinot blanc	Complex, fine, long finish	Yellow-golden	Refresh, sweet, balance
		Wunv Mountain Milan	Red	Beibinghong	-	_	_
		Liquor Company	White	Vidal blanc	_	_	_
		Zhangyu Golden Ice Valley	White	Vidal blanc	Fruity, pineapple, honey,	Yellow-golden	Sweet, fine, delicate,
		Wine Industry Co., Ltd			tropical fruit		full-body, long finish
Northwest	Xinjiang	Yizhu Wine Industry Co.,	Red	_	_	_	-
		Ltd	White	Vidal blanc			
	Gansu	Qilian Wine Industry Co.,	White	Italian Riesling	Fruit, honey, apple, fruity,	Pale yellow-	Mellow, fat, bouquet,
		Ltd		Semillon	complex, fine, harmony	golden	typical
			Red	Merlot	-	Ruby-red	Sweet, vinous, long finish
	Ningxia	Hangsheng Xixia King	White	Riesling	Lemon, rose	Yellow-golden	Sweet, vinous, special
		Liquor Company	White	Gewürztraminer			
		Florian Wine Co., Ltd	White	Chardonnay	Fruity	Straw-yellow	Sweet, mellow
			Red	Pinot nior	Fruity	Ruby-red	Sweet, mellow

<sup>-,</sup> Not mentioned.

<sup>&</sup>lt;sup>a</sup>Data from the company's website.

Cabernet Franc (V. vinifera), a red grape variety, produces a wine with a bright red ruby tone
and the powerful aroma of fresh strawberries. It's frequently used in Canada for ice wine
production but Merlot, Pinot noir, and even Cabernet Sauvignon can also be used. The Niagaraon-the-Lake region was the first to produce Syrah ice wine in 2004 and Sangiovese in 2007.

Ice wines show a complex aroma formed by terpenes, furans, acetals, esters, etc. (Lukić et al., 2016). When aromatic profiles by stir bar sorptive extraction-gas chromatography-olfactometry-mass spectrometry (SBSE-GC-O-MS) of both table and ice wines made from Riesling and Vidal blanc varieties, higher concentrations of more aroma compounds were observed in the second ones (Bowen and Reynolds, 2012). The highest odor activity was observed in: β-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate; these molecules were above threshold in Riesling and Vidal ice wines. Also in ice wines of these varieties, Crandles et al. (2015) measured odor activity values higher than unit in: Linalool, *cis*-rose oxide, 1,1,6-trimethyl-1,2-dihydronaphthalene, β-damascenone, β-ionone, ethyl cinnamate, *p*-vinyl guaiacol, and decanal.

Ice wine is quite refreshing, despite the high amount of residual sugars, due to high acidity levels (normally >10 g/L). Many people describe it as a delicious dessert in a glass, and it is quite common for a small 375 mL bottle to be enjoyed by 6–8 people.

Ice wine usually has a medium to full body, with a prolonged finish. Its aroma is frequently evocative of peach, pear, dried apricot, honey, citrus, figs, caramel, green apple, raisins, and a hint of violet on both the nose and palate, etc., depending on the variety (Fig. 10.14).

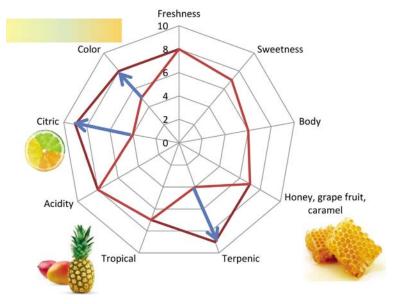


Figure 10.14: Sensory Profile of Typical White Ice Wines. Intensity of some descriptors is variable according to grape variety.

The perfume of tropical and exotic fruits, such as pineapple, lychee, or mango is quite common, especially with white varietals. Moreover, its sensory profile evolves according to harvest times, with more complex late harvest ice wines being more frequent. On the other hand, nonconventional ice wines: sparkling, aged in barrel, aged on lees, can show more complex or evolved aromas according to the specificities of the ageing process. Barrel ageing increase notes like butterscotch and brown sugar and it further improves body and achieves a more rounded mouth feel.

### 11 Conclusions

Ice wines are produced in only a few special regions worldwide, ideal climatic conditions are necessary for good grape maturation together with a requirement for frozen grapes at harvest times. Furthermore, although ice wines are produced on small scale compared with traditional still wines, they are in a high price bracket and they enhance the prestige of wine-making regions able to create these wines. As ice wine making is a hard process, new biotechnological improvements can help oenologists to improve fermentation quality and safety. Lot of improvements and trends are being incorporated to ice wine technology: the use of nontraditional varieties in ice wine technology allowing new sensory profiles or better resistance against the cold, pests, or diseases, new ageing technologies, such as sparkling ice wines, barrel-aged ice wines, and so on, open new possibilities to develop very interesting products. Finally, ice wine is a magic synthesis of extreme climate conditions, human tradition, and knowhow, and extremely adverse fermentations that makes a really beautiful wine.

## **Acknowledgments**

We would like to express our gratitude to Chen Yangxiong, enologist of Gansu Qilian Winery Co. Ltd., for kindly providing us the photos used in Figs. 10.2, 10.3, 10.4, and 10.5.

## References

- Agriculture and Agri-Food Canada, 2010. Available from: http://www5.agr.gc.ca/resources/prod/Internet-Internet/ MISB-DGSIM/CB-MC/PDF/5320-eng.pdf.
- Alessandria, V., Giacosa, S., Campolongo, S., Rolle, L., Rantsiou, K., Cocolin, L., 2013. Yeast population diversity on grapes during on-vine withering and their dynamics in natural and inoculated fermentations in the production of ice wines. Food Res. Int. 54, 139-147.
- Ali, H.S.M., Pätzold, R., Brückner, H., 2010. Gas chromatographic determination of amino acid enantiomers in bottled and aged wines. Amino Acids 38, 951-958.
- Bellon, J.R., Yang, F., Day, M.P., Inglis, D.L., Chambers, P.J., 2015. Designing and creating Saccharomyces interspecific hybrids for improved, industry relevant, phenotypes. Appl. Microbiol. Biotechnol. 99, 8597–8609.
- Benito, S., Palomero, F., Morata, A., Calderón, F., Suárez-Lepe, J.A., 2012. New applications for Schizosaccharomyces pombe in the alcoholic fermentation of red wines. Int. J. Food Sci. Technol. 47, 2101-2108.

- Bowen, A.J., Reynolds, A.G., 2012. Odour potency of aroma compounds in Riesling and Vidal blanc table wines and ice wines by gas chromatography-olfactometry-mass spectrometry. J. Agric. Food Chem. 60, 2874–2883.
- Bowen, A.J., Reynolds, A.G., 2015a. Aroma compounds in Ontario Vidal and Riesling ice wines. II. Effects of crop level. Food Res. Int. 76, 540-549.
- Bowen, A.J., Reynolds, A.G., 2015b. Aroma compounds in Ontario Vidal and Riesling ice wines. I. Effects of harvest date. Food Res. Int. 76, 550-560.
- Chen, Kai, Han, Shun-yu, Zhang, Bo, Li, Min, Sheng, Wen-jun, 2015. Development of lysozyme-combined antibacterial system to reduce sulphur dioxide and to stabilize Italian Riesling ice wine during aging process. Food Sci. Nutr. 3, 453-465.
- Cliff, M.A., Pickering, G.J., 2006. Determination of odour detection thresholds for acetic acid and ethyl acetate in ice wine. J. Wine Res. 17, 45-52.
- Cliff, M., Yuksel, D., Girard, B., King, M., 2002. Characterization of Canadian ice wines by sensory and compositional analyses. Am. J. Enol. Viticult. 53, 46-53.
- Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M., 2011. Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with Saccharomyces cerevisiae. Food Microbiol. 28, 873-882.
- Crandles, M., Reynolds, A.G., Khairallah, R., Bowen, A., 2015. The effect of yeast strain on odour active compounds in Riesling and Vidal blanc ice wines. LWT-Food Sci. Technol. 64, 243-258.
- Erasmus, D.J., Cliff, M., van Vuuren, H.J.J., 2004. Impact of yeast strain on the production of acetic acid, glycerol, and the sensory attributes of ice wine. Am. J. Enol. Viticult. 55, 371–378.
- Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M., 2013. Lachancea thermotolerans and Saccharomyces cerevisiae in simultaneous and sequential co-fermentation: a strategy to enhance acidity and improve the overall quality of wine. Food Microbiol. 33, 271–281.
- Grand Duchy of Luxembourg official site, 2016. Available from: http://www.luxembourg.public.lu/en/ actualites/2016/01/25-vinglace/index.html.
- Inglis, D., 2012. Torulaspora delbrueckii and ice-wine fermentation: the start of a winning combination. XXIIIes Entretiens Scientifiques Lallemand. Monestier, France.
- Jackson, R.S., 2008. Wine Science, third ed. Elsevier Publishing, Amsterdan, Bostonpp. 526–527, Chapter 9.
- Jackson, R.S., 2009. Wine Tasting—A Professional Handbook, second ed. Elsevier Publishing, Amsterdan, Bostonp. 45, Chapter 2.
- Khairallah, R., Reynolds, A.G., Bowen, A.J., 2016. Harvest date effects on aroma compounds in aged Riesling ice wines. J. Sci. Food Agric. 96, 4398-4409.
- Kinga, S., Reynolds, A.G., Bowen, A.J., 2015. Effect of yeast strain on aroma compounds in Cabernet francice wines. LWT—Food Sci. Technol. 64, 227–235.
- Kontkanen, D., Inglis, D.L., Pickering, G.J., Reynolds, A., 2004. Effect of yeast inoculation rate, acclimatization, and nutrient addition on icewine fermentation. Am. J. Enol. Viticult. 55, 363–370.
- Kulkarni, P., Loira, I., Morata, A., Tesfaye, W., González, M.C., Suárez-Lepe, J.A., 2015. Use of non-Saccharomyces yeast strains coupled with ultrasound treatment as a novel technique to accelerate ageing on lees of red wines and its repercussion in sensorial parameters. LWT—Food Sci. Technol. 64, 1255–1262.
- Li, J.C., Li, S.Y., He, F., Yuan, Z.Y., Liu, T., Reeves, M.J., Duan, C.Q., 2016. Phenolic and chromatic properties of Beibinghong red ice wine during and after vinification. Molecules 21, 431.
- Liu, L., Li, H., 2013. Review: Research progress in amur grape, Vitis amurensis Rupr. Can. J. Plant Sci. 93, 565–575.
- Loira, I., Vejarano, R., Bañuelos, M.A., Morata, A., Tesfaye, W., Uthurry, C., Villa, A., Cintora, I., Suárez-Lepe, J.A., 2014. Influence of sequential fermentation with Torulaspora delbrueckii and Saccharomyces cerevisiae on wine quality. LWT-Food Sci. Technol. 59, 915-922.
- Loira, I., Morata, A., Comuzzo, P., Callejo, M.J., González, C., Calderón, F., Suárez-Lepe, J.A., 2015. Use of Schizosaccharomyces pombe and Torulaspora delbrueckii strains in mixed and sequential fermentations to improve red wine sensory quality. Food Res. Int. 76, 325–333.
- Lucca, M.E., Spencer, J.F.T., Figueroa, L.I.C., 2002. Glycerol and arabitol production by an intergenic hybrid, PB2, obtained by protoplast fusion between Saccharomyces cerevisiae and Torulaspora delbrueckii. Appl. Microbiol. Biotechnol. 59, 472-476.

- Lukić, I., Radeka, S., Grozaj, N., Staver, M., Peršurić, D., 2016. Changes in physico-chemical and volatile aroma compound composition of Gewürztraminer wine as a result of late and ice harvest. Food Chem. 196, 1048-1057.
- Mencarelli, F., Tonutti, P., 2013. Ice wine. Sweet, Reinforced and Fortified Wines: Grape Biochemistry, Technology and Vinification. John Wiley & Sons, Ltd., Chichester, West Sussex, UK, pp. 301-302, Chapter 20.
- Morata, A., Suárez-Lepe, J.A., 2016. New biotechnologies for wine fermentation and ageing. In: Ravishankar Rai, V. (Ed.), Advances in Food Biotechnology, first ed. John Wiley & Sons, Ltd., West Sussex, United Kingdom, pp. 288-289.
- Morata, A., Benito, S., Loira, I., Palomero, F., González, M.C., Suárez-Lepe, J.A., 2012. Formation of pyranoanthocyanins by Schizosaccharomyces pombe during the fermentation of red must. Int. J. Food Microbiol. 159, 47–53.
- Nguyen, T.H., Fleet, G.H., Rogers, P.L., 1998. Composition of the cell walls of several yeast species, Appl. Microbiol. Biotechnol. 50, 206-212.
- Nurgel, C., Pickering, G.J., Inglis, D.L., 2004. Sensory and chemical characteristics of Canadian ice wines. J. Sci. Food Agric. 84, 1675-1684.
- OIV, 2015. In: Compendium of International Methods of Wine and Must Analysis. China Zhijian Publishing House & Standards Press of China Publishing, Beijing. p. 8 (Foreword).
- Palomero, F., Morata, A., Benito, S., Calderon, F., Suarez-Lepe, J.A., 2009. New genera of yeasts for over-lees aging of red wine. Food Chem. 112, 432-441.
- Parker, R.P., 2012. The World's Greatest Wine Estates. Beijing Union Company Publishing, Beijing, pp. 457–475.
- Patz, C.D., Blieke, A., Ristow, R., Dietrich, H., 2004. Application of FT-MIR spectrometry in wine analysis. Anal. Chim. Acta 513, 81-89.
- Pigeau, G.M., Inglis, D.L., 2007. Response of wine yeast (Saccharomyces cerevisiae) aldehyde dehydrogenases to acetaldehyde stress during ice wine fermentation. J. Appl. Microbiol. 103, 1576–1586.
- Pigeau, G.M., Bozza, E., Kaiser, K., Inglis, D.L., 2007. Concentration effect of Riesling ice wine juice on yeast performance and wine acidity. J Appl. Microbiol. 103, 1691–1698.
- Rolle, L., Torchio, F., Cagnasso, E., Gerbi, V., 2010. Evolution of mechanical variables of winegrapes for ice wine production during on-vine drying. Italian J. Food Sci. 22, 143–149.
- Rolle, L., Siret, R., Segade, S.R., Maury, C., Gerbi, V., Jourjon, F., 2012. Instrumental texture analysis parameters as markers of table-grape and winegrape quality: a review. Am. J. Enol. Viticult. 63, 11-28.
- Saayman, M., Viljoen-Bloom, M., 2006. The biochemistry of malic acid metabolism by wine yeasts—a review. S. Afr. J. Enol. Viticult. 27, 113-122.
- Schreiner, J., 2001. Icewine, the Complete Story, first ed. Warwick Publishing, Toronto.
- Setkova, L., Risticevic, S., Pawliszyn, J., 2007. Rapid headspace solid-phase microextraction-gas chromatographic-time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction: method development and optimization. J. Chromatogr. A 1147, 213–223.
- Smith, A.K., Noble, A.C., 1998. Effects of increased viscosity on the sourness and astringency of aluminium sulphate and citric acid. Food Quality Prefer. 9, 139-144.
- Soleas, G.J., Pickering, G.J., 2007. Influence of variety, wine style, vintage and viticultural area on selected chemical parameters of Canadian ice wine. J. Food Agric. Environ. 5, 97–112.
- Song, R.G., Lu, W.P., Shen, Y.J., Jin, R.H., 2008. A new ice-red wine grape variety—Beibinghong. Sino-Overseas Grapevine Wine 4, 19-22.
- Suárez-Lepe, J.A., Morata, A., 2006. New method of aging on lees. Spanish Patent P-200602423.
- Suárez-Lepe, J.A., Morata, A., 2012. New trends in yeast selection for winemaking. Trends Food Sci. Technol. 23, 39-50.
- Suárez-Lepe, J.A., Palomero, F., Benito, S., Calderón, F., Morata, A., 2012. Oenological versatility of Schizosaccharomyces spp. Eur. Food Res. Technol. 235, 375–383.
- Wang, Y.Z., Hu, W.Z., Li, T.T., Ma, K., Jiang, A.L., 2014. Determination of sugars in five types of ice wine by high performance liquid chromatography (HPLC) with evaporative light-scattering detector (ELSD). Sci. Technol. Food Ind. 21, 320-323.

Wine Country Ontario, 2015. Available from: http://winecountryontario.ca/media-centre/icewine.
Yi-Bin, Lan, Xu, Qian, Zhong-Jun, Yang, Xiao-Feng, Xiang, Wei-Xi, Yang, Tao, Liu, Bao-Qing, Zhu, Qiu-Hong, Pan, Chang-Qing, Duan, 2016. Striking changes in volatile profiles at sub-zero temperatures during overripening of 'Beibinghong' grapes in Northeastern China. Food Chem. 212, 172–182.
Zhan, J.C., 2010. Wine Grape Varieties. China Agricultural University, Beijing, China, pp. 85–106, Chapter 4.

# **Further Reading**

Ronald, S.J., 2008. Wine Science, third ed. Elsevier Publishing, Amsterdan, Boston, pp. 526-527, Chapter 9.