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Chapter 16

YEASTS ASSOCIATED WITH THE PRODUCTION OF DISTILLED ALCOHOLIC BEVERAGES

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Abstract

Distilled alcoholic beverages are produced firstly by fermenting sugars emanating from cereal starches (in the case of whiskies), sucrose-rich plants (in the case of rums), fructooligosaccharide-rich plants (in the case of tequila) or from fruits (in the case of brandies). Traditionally, such fermentations were conducted in a spontaneous fashion, relying on indigenous microbiota, including wild yeasts. In modern practices, selected strains of *Saccharomyces cerevisiae* are employed to produce high levels of ethanol together with numerous secondary metabolites (eg. higher alcohols, esters, carbonyls etc.) which greatly influence the final flavour and aroma characteristics of spirits following distillation of the fermented wash. Therefore, distillers, like winemakers, must carefully choose their yeast strain which will be very important in providing the alcohol content and the sensory profiles of

spirit beverages. This Chapter discusses yeast and fermentation aspects associated with the production of selected distilled spirits and highlights similarities and differences with the production of wine.

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16.1 Introduction

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast, in particular the species *Saccharomyces cerevisiae*, plays a vital role in the production of all alcoholic beverages (see Figure 16.1) and the selection of suitable yeast strains is essential not only to maximise alcohol yield, but also to enhance beverage sensory quality.

Fig 16.1 here

The yeast species that dominates in the production of worldwide distilled spirits is *S. cerevisiae*, and the specific strains of this species employed in fermentation exert a profound influence on spirit flavour and aroma characteristics. For large-scale beverage fermentations, as in brewing, winemaking and distilled spirit production, pure cultures of selected strains of *S. cerevisiae* are typically employed (Walker 1998). These strains are either sourced and cultivated *in house* or supplied for direct inoculation from yeast producing companies. In smaller-scale (artisanal) processes, spontaneous fermentations may be allowed to occur that rely on indigenous microorganisms (wild yeasts and bacteria) present in the raw materials and in the production facility. For example, this would be typical in small distilleries in Mexico (for Tequila and Mezcal production) and in Brazil (for Cachaça production). In some types of alcoholic beverage fermentations, non-*S. cerevisiae* yeasts may be employed either as starter cultures, or occur naturally. For example, *Schizosaccharomyces pombe* is found in molasses fermentations for rum production, and *Kluyveromyces marxianus* strains are employed in cheese whey fermentations for the production of white spirits such as vodka and gin. Table 16.1 summarizes different yeast species encountered in alcoholic beverage fermentations.

Table 16.1 here

This Chapter will focus on the zymology aspects pertaining to distilled spirit production and will compare and contrast fermentation processes for both wine and spirits.

16.2 Yeasts in production of cereal-based spirits

Distilled spirits that employ cereals as their starting raw materials include: whisky (e.g. Scotch whisky), whiskey (e.g. Irish and American), vodka, gin and shochu (see Table 16.1). The cereals in question are predominantly barely, wheat, rye, maize, rice and sorghum. The starting carbohydrate in all cases is starch which cannot be fermented directly by *S. cerevisiae*. This glucose polysaccharide requires pre-hydrolysis to simple sugars prior to yeast fermentation, and this contrasts markedly from winemaking where fermentable sugars (glucose and fructose) are readily available in the grape berries and in the subsequent must. Other salient differences exist between wine and distilled cereal spirits. For example, the alcohol content of bottled spirits is approximately 3-4 fold higher in finished wines (e.g. a typical whisky would have an alcohol concentration of 40% v/v, whereas a typical table wine would be 12% v/v). Yeast strains employed for spirits and for wine are also different and the following discussion covers fermentation aspects of distilled spirits from cereals, with a special emphasis on whisky production processes. Figure 16.2 outlines the major categories of global whiskies.

Fig 16.2 here

One of the best selling spirit drinks in the world is Scotch whisky which is produced by fermentation of an infusion of malted barley and other cereals with strains of *S. cerevisiae* and matured over time in oak barrels (Russell and Stewart 2014; Walker and Hill 2016). There are two main types of Scotch whisky: malt whisky and grain whisky. Blended Scotch whisky is a mix of these types. Malt whisky is produced using malted barley as the cereal and enzyme source, and the fermented wash is distilled in copper pot stills. Grain whisky is produced using wheat or maize as the predominant cereal, with a small proportion (e.g. 15%) of malted barely as a source of amylolytic enzymes, and the fermented wash is distilled continuously in large “Patent” or “Coffey” stills. In the UK, Scotch Whisky has had a legal definition since 1909 (recognised by the EC in 1989) and the current (2009) Scotch Whisky Regulations define five categories of Scotch Whisky: *Single Malt, Single Grain, Blended, Blended Malt* and *Blended Grain.* Blended Scotch whisky is typically a mix of malt and grain whiskies, with some blends having a much a 50 individual malt and grain whiskies. These Regulations state that “Scotch Whisky”:

a. Has been distilled at a distillery in Scotland from water and malted barley (to which only whole grains of other cereals have been added) all of which have been -
    (i) processed at that distillery into a mash;
    (ii) converted at that distillery into a fermentable substrate only by endogenous enzyme systems; and
    (iii) fermented at that distillery only by the addition of yeast;
b. Has been distilled at an alcoholic strength by volume of less than 94.8%.
c. Has been matured in oak casks not exceeding 700 litres for a period not less than 3 years.

Whisky processes involve the production of a sugary solution called *wort*, which is generated following the enzymatic extraction of maltose and other sugars from an aqueous mash of barley malt grist (as in the case of malt whisky) or an aqueous mash of malt and other cereals (as in the case of grain whisky). The saccharification of cereal starch is accomplished by amylolytic enzymes present in malted barely. For Scotch whisky production, exogenous (commercial) enzymes are not permitted, but for grain neutral spirit (GNS) destined for vodka or gin production, application of such enzymes is permitted. After cooling, the wort is then fermented with selected strains of *S. cerevisiae* to produce wash at around 8% v/v ethanol. For malt whisky, fresh alcohol distillate is produced following two batch distillations as shown in Fig 16.3.

Fig 16.3 here

The spirit fraction typically has an alcohol concentration of 63-70%v/v and is matured in oak barrels for a minimum of 3 years (but often for 10-15 years) to impart characteristic flavour, aroma and taste to the spirit (Russell and Stewart 2014; Bryce and Stewart 2004; Murray 2017). "Single malt" Scotch whisky is such whisky produced only from malted barley and from a single distillery. Grain whisky distillations employ continuous Coffey (or Patent) stills comprising a rectifier and an analyser to produce spirit at a strength of 94.5% v/v alcohol. Grain whiskies are mainly used for blending with malt whiskies (Lea and Piggott 2003).

Regarding the yeasts used for whisky production, fermentations are conducted by specific strains of *S. cerevisiae* which convert mash sugars into ethanol, carbon dioxide and numerous secondary fermentation metabolites that collectively act as flavour congeners in the final spirit (Walker and Hill 2016). Yeast strain selection for whisky production is therefore critically important in dictating the organoleptic qualities of the final product. The same is true for wine yeasts. The fermentable sugars extracted following cereal mashing are predominantly maltose and maltotriose, in contrast to glucose, fructose and sucrose in wine musts. An important distinction between beer and whisky production is that in whisky wort preparation, because the wort is not boiled, starch degradation processes do not stop when the wort leaves the mashing vessel. Consequently, residual malt enzymes continue their amylolytic activity in fermentation. This has similarities with the Simultaneous Saccharification and Fermentation (SSF) processes typically found in fuel alcohol plants that process maize (Walker 2011).

In Scotch whisky processes, where no exogenous enzymes are allowed, maltodextrin molecules (small branched oligosaccharides) in the wort may be utilised by some whisky yeast strains. For example, a widely used Scotch whisky yeast strain, named "M type" (thought to be a hybrid of *S. cerevisiae* and *S. cerevisiae* var *diastaticus*) possesses limited starch-debranching amylolytic activity (Watson 1993). Whisky yeast strains have been described as "*maltose* + *oligosaccharide type*" to reflect their properties in rapidly fermenting maltose, maltotriose and other oligosaccharides. In contrast, other beverage yeasts have been described as "*sucrose* + *maltose type*" to reflect fermentation of sucrose, glucose and maltose (Jones 1998). The ability to efficiently and completely ferment maltotriose is an important distinguishing characteristic of Scotch whisky yeasts. Table 16.2 outlines the main desired attributes of distiller's yeast, compared with wine yeasts.

Table 16.2 here

Spontaneous fermentations are no longer conducted in modern whisky distilleries that use freshly propagated or commercially supplied pure-cultured strains of *S. cerevisiae*. This yeast may be supplied in compressed (cake) form, liquid (cream) yeast, or in dried form (Walker and Hill 2016). The pitching rate (inoculum) is generally 0.5-2x10^7 cells/mL. Unlike breweries or wineries, whisky fermentations are typically allowed to proceed for 2-3days without precise temperature control. Wort pH in a whisky fermentation will start at pH5-5.5 and will fall to pH4.2-4.5 at the end of fermentation. Yeast viability at the end of fermentation is very low due to the combination of low pH, temperatures >30°C, and high final ethanol concentrations. These factors exert considerable physiological stress on yeast (Walker and van Dijck 2006).

Whisky-distillers (and oenologists), unlike brewers, do not recycle yeast. Fermented wash including yeast is distilled resulting with concomitant destruction of yeast cells. This necessitates the supply of freshly propagated yeast from separate commercial yeast organisations (Jones 1998). The specifications for Scotch whisky yeast strains include yeast cell viability, bacterial count and moisture content. (Korhola et al. 1989). Some Scotch whisky distilleries formerly supplemented their distiller's yeast with a small proportion of spent brewer's yeast. The presence of brewers' yeast provides flavour benefits in terms of final spirit quality (Korhola *et al.*, 1989) and in controlling fermentation pH due to pyruvic acid uptake (McGill 1990).

In addition to the selected distillers strain of yeast to initiate fermentation, various wild yeasts such as non-distilling strains of *S. cerevisiae, Pichia membranefaciens, Torulaspora delbrueckii* and *Candida* species may also be present in whisky fermentations. Although such yeasts are potentially problematic in affecting fermentation progress, their levels are usually kept low due to the dominance of the main production yeast strain of *S. cerevisiae.* Another wild yeast, *Dekkera bruxellensis* (anamorph *Brettanomyces bruxellensis*), which is a common contaminant in wine fermentations, may play an important role in cereal fermentations for distilled spirits production. For example, Passoth *et al.* (2007) found that *D. bruxellensis* dominated wheat-based distillery fermentations following out-competition of the *S. cerevisiae* starter cultures.

Due to the lack of a pre-fermentation boiling stage (as in brewing) and due to the non-aseptic processes employed, other microorganisms, notably bacteria, are present during cereal fermentations. For example, the importance of lactic acid bacteria has been shown in grain whisky processes (van Beek and Priest 2003); in wheat-based processes (Passoth *et al*. 2007); in corn mash-processes (Thomas et al. 2001; Smith 2017) and in rice-based processes (Watanabe et al. 2007). In a wheat distillery, Passoth *et al.* (2007) proposed that *Lactobacillus vini* contributed, with yeast, to an "ethanol-producing consortium". In Scotch malt whisky fermentations, limited bacterial activity plays an important role in flavour development of the final spirit. In particular, whisky producers recognise positive influences of lactic acid bacteria including *Lactobacillus plantarum* and *L. fermentum* during the latter stages of fermentation. Lactic acid bacteria (LAB) can produce lactic and acetic acids, which become esterified to ethyl lactate and ethyl acetate, respectively. These compounds impart sweet, fruity, creamy, and pineapple-like flavours to the spirit. *Lactobacillus* spp. can also produce γ-dodecalactone which imparts a "sweet and fatty" characteristic to the spirit. In other cereal-based distilled spirits such as "sour-mash" bourbons, lactic acid bacterial growth is encouraged during certain process steps to depress wort pH and impart desired flavour congeners to the final distillate (Smith 2017).

The choice of the distillers strain, together with contributions from other microorganisms, will play an important role in dictating the final flavour and aroma characteristics of cereal-based distilled spirits. In addition to the main fermentation metabolites, ethanol and carbon dioxide, numerous secondary fermentation compounds will be produced that act as important flavour congeners in the final spirit. Table 16.3 summarises the principal flavour congeners in distilled spirits, and these include: fatty acids and their esters (e.g. ethyl caprate, ethyl laurate), organic acids (e.g. succinic acid), higher alcohols or "fusel oils" (e.g. n-propanol, isoamylalcohol), aldehydes (e.g. acetaldehyde) and vicinal diketones (diacetyl).

Table 16.3 here

Many flavour-active compounds are produced by yeast in reactions between alcohols and acyl CoA molecules but some esters, notably ethyl lactate, are linked to the presence of lactic acid bacteria. Ester production during fermentation is linked to the relative abundance of the corresponding alcohols and acyl CoAs, with ethyl acetate being the predominant ester produced, with isoamyl acetate at lower concentrations. The latter has a much lower flavour threshold than ethyl acetate and contributes a fruity (banana) aroma to beverages. Another yeast-derived group of flavour congeners important in distilled spirits (and in wine) are the fusel oils. Their concentration levels in beverages are linked to the levels of corresponding amino acids in the fermentation medium (for example, phenylalanine stimulates phenylethanol production leading to a rose-like aroma). In distilled spirits these compounds, if controlled within certain limits, are beneficial contributors to the aroma characteristics of distillates.

Further information on the origin of different flavour congeners in distilled spirits can be found in: Lea and Piggott (2003), Walker and Hughes (2010), Russell and Stewart (2014), Goodall *et al*. (2015) and Walker *et al*. (2017).

16.3 Yeasts in the production of *Agave* and and *Dasylirion*-based spirits

Agaves or *magueye*s (*Agave*) and *sotoles* (*Dasylirion*) are succulent plants that have great biological, ecological and economic importance. They belong to the family

Asparagaceae, subfamilies Agavoideae and Nolinoideae, respectively, which are endemic to America. During pre-Hispanic times the integral use of these plants was so important, that the survival and cultural development of several Mesoamerican civilizations could not be explained without their existence (Fiore *et al*. 2005; Lappe-Oliveras *et al*. 2008). Different *Agave* and *Dasylirion* species have long been exploited for the production of distilled alcoholic beverages, which are produced by distillation of the fermented must of several species, and are generically known with the names *mezcal* or *sotol*, respectively (Lappe-Oliveras *et al*. 2008).

In Mexico, a great diversity of agaves (175 species) exists with around 50 species being used in the production of *mezcal*. From these, 28 are frequently employed in artisanal production and, 14 in larger-scale commercial processes. The final product may come from the exploitation of a single agave species, or from the combination of several ones (Aguirre and Eguiarte 2013; Torres *et al*. 2015). Thus, the distinctive characteristics of *mezcal* spirit beverages depends on: the agave species or mixture of species used; the growth conditions and maturity of the plants, as well as from the cooking, fermentation, distillation and aging processes. Together, these factors give rise to a great diversity of *mezcales*. Some of them, such as *tequila*, *bacanora* and *mezcal*, are widely known, have national and international recognition and are protected by their appellation of origin (AO). However, most *mezcales* are regional products, not known outside their place of origin and do not have an AO that protects them.

The genus *Dasylirion* consists of 20 species, 16 of them endemic, which grow in several states of Mexico (Reyes-Valdes *et al*. 2012). *Sotol* is produced in the states of Chihuahua, Coahuila and Durango from several *Dasylirion* species, mainly *D. durangense.*

16.3.1 Distilled Agave spirits: Tequila, mezcal, bacanora, raicilla and sotol

Tequila, Mezcal, Bacanora, Raicilla and sotol are specific names of distilled beverages obtained from different *Agave* and *Dasylirion* species (Table 16.4). Tequila is the famous spirit beverage classically associated with Mexico. The word *tequila* derives from the Nahuatl *tequillan*, from *tequitl* = tribute, work or employment and *tlan* = place, meaning a place of tribute or in which work is done. However, this word may also be associated with the *Ticuilas* a tribe who lived in the hillside of the extinct volcano Tequila, in Jalisco. The word *mezcal*, *mescal or mexcal* derives from the Nahuatl words *metl* = maguey or agave, and *ixcaloa* = to roast, and means roasted agave. In pre-Hispanic Mexico the agave plants had several uses (food, fodder, medicine, construction material, textiles, and soap, among others) and for centuries they have been used to produce alcoholic beverages.

Table 16.4 here

With the introduction by the Spaniards of the Philippine and Arab stills, in the second half of the sixteenth and the beginning of the seventeenth centuries, respectively, the distilled agave beverages originated and over time took their present form (Cedeño, 2003). However, recently some researchers based on strong archaeological and ethnographic evidence have proposed that distillation is not of mestizo origin, but pre-hispanic, which has caused great controversy that remains to be elucidated.

The following contribution will present the different processes of elaboration of agave spirits with special emphasis on yeasts species and their contribution to the characteristics of the final product.

16.3.2 Tequila categories, types and production process

The most famous agave spirit from Mexico is undoubtedly Tequila. This alcoholic beverage is obtained from distillation of the fermented must of *Agave tequilana* Weber var. azul; it is produced in the territory of AO which includes all the state of Jalisco and some regions of the states of Guanajuato, Michoacán, Nayarit and Tamaulipas (Table 16.4). The principal characteristics of this agave are: a high concentration of a complex mixture of highly branched fructooligosaccharides containing principally β (2-1) linkages (Mancilla-Margalli and López 2006) which are used as reserve carbohydrate by the plant, low fiber content, and the presence of some chemical compounds like terpenes, which contribute to the flavour and taste of the final product. The elaboration process is subject to the Mexican Official Regulation NOM-006-SCFI-2012, which recognizes only two tequila categories: "*Tequila* 100%" obtained exclusively from sugars of *A. tequilana* var. azul, and "*Tequila*", produced using 51% of agave sugars and 49% from other sugar sources (sugar cane, molasses or hydrolyzed corn syrup). In each category there are five types of tequila: *tequila blanco* (silver) without maturation; *tequila joven* or *oro* (gold) containing permitted additives (oak extract, glycerin, sugar syrup) and colours (generally caramel colour); *tequila reposado* (aged) matured at least two months in white oak barrels (this being the most popular kind of tequila); *tequila añejo* (extra aged) and *tequila extra añejo* (ultra aged) matured for 1 or 3 years in white oak barrels, respectively. According to the Consejo Regulador del Tequila (CRT) (Tequila Regulatory Council) in 2017 the global production of tequila was 271.4 million liters. "*Tequila* 100%" represented more than 56% of the total production, and more than 80% of the annual production was exported. By mid-2018 there were 1450 registered tequila brands produced in

235 Mexican distilleries certified by the CRT, which is the council that verifies the compliance of the Official Mexican Regulation.

The process of elaboration begins with the harvest of 7 to 9 year old agave plants (Cedeño, 2003). As with many other agave plants, *A. tequilana* var. azul is rich in carbohydrates, mainly highly branched fructans and neo-fructans (Mancilla-Margalli and López 2006; Waleckx *et al*. 2008). The complete plant is cut down, the leaves are removed leaving the stem and the leaf bases, what is called the head or *piña*. The heads are cooked in brick ovens heated by steam injection for 36-48 h at 100°C; then the steam injection is suspended and the agave pieces are left in the oven for two more days to complete the cooking process. Nowadays, in most distilleries, brick ovens have been replaced by steel autoclaves to increase efficiency. The main objectives of the cooking process are: 1) To accomplish hydrolysis of fructans into simple sugars, mainly fructose, glucose and sucrose, which are easily fermented by yeasts; 2) To facilitate the milling operation and the extraction of sugars, since during cooking the agave plants acquire a soft texture 3) To generate some important chemical compounds (e.g. fusel oils) which determine the sensorial characteristics of the final product; some of these are produced by caramelisation and Maillard reactions (Cedeño 2003).

The cooked agave is milled to extract a sweet must which contains a high concentration of fructose and other fermentable sugars. In some distilleries, the milling process is still done with rudimentary mills (*tahona*), such as those used in the elaboration of *mezcal*. Nowadays, the mills used in the tequila industry are similar to those used in the sugarcane industry. In the last 15 years a new milling/cooking technology has been developed to extract fructans or fructose with hot water using diffusors. This technology is applied to crude crushed agave or to wash the agave fibers improving the efficiency of the sugar extraction.

The fermentation process will be discussed in detail in the Section 16.3.4. Most distilleries use stainless steel fermentation tanks (whose capacity ranges from 2,000 to 120,000 L) although some still employ wooden tanks. The fermentation wort of "*Tequila* 100%" is comprised solely of agave must, with an initial sugar concentration between 4-10% v/v, depending on the amount of water added during milling. For "*Tequila*" other sugars are added, which are previously dissolved and mixed with the agave must to obtain an initial sugar concentration of 8-16%, depending on the sugar tolerance of the yeast strain that will be used in the fermentation process. The pH of agave must oscillates around 4.5, needing no adjustment. Wort formulation is based on the composition of raw materials and the nutritional requirements for yeast growth and fermentation. Generally, a nitrogen source (urea, ammonium sulphate, ammonium phosphate) and salts (magnesium sulphate) can be added. The fermentation wort may be left to ferment spontaneously or in some distilleries selected *S. cerevisiae* strains are employed (Gschaedler *et al.* 2004).

Distillation involves the separation and concentration of the alcohol from the fermented wort. Tequila is obtained after two consecutive differential distillations in copper or stainless steel stills. Some distilleries also use rectification columns. During the first distillation the fermented must is split into three different products: a light product (head), a tail product (or vinasses) which is discarded and a slop cut product called *ordinario* (with an ethanol content of 20-30% v/v) which is subjected to a second distillation to obtain a distillate or spirit with around 55% v/v ethanol (Prado Ramirez *et al.* 2005).

Finally, Tequila can be matured in different ways, depending on the type of tequila to be obtained. According to the norm, this process can only be carried out in the region of AO. The regulation specifies that the maturation tanks have to be made of oak or holm oak wood with a maximum capacity of 600 L. Prior to bottling, Tequila is filtered. In the case of "*Tequila* 100%" the product has to be bottled in the region of AO, "*Tequila*" can be exported in bulk outside of Mexico, but when bottled, it must be labelled with the legend Made in Mexico or Product of Mexico.

16.3.3 Mezcal, Bacanora and Sotol categories, types and production process

For the elaboration of others spirits obtained from *Agave* and *Dasylirion* species, the general stages of the processes are the same as in Tequila. The main differences between Tequila and the different types of Mezcal are the species of agave used as raw material, and that the elaboration process of Mezcal is more artisanal than the Tequila process. It is important to highlight that the word *mezcal* is the generic name of all the agave distilled beverages, which are produced in 26 of the 32 states of Mexico (Aguirre and Eguiarte 2013; Torres et al. 2015). This word refers also to a spirit with AO whose elaboration process is subjected to the Mexican Official Regulation NOM-070-SCFI-2016). The region of AO includes the states of Durango, Guerrero, Oaxaca, San Luis Potosi, Zacatecas and some regions of Guanajuato, Michoacán, Puebla and Tamaulipas (Table 16.4). The regulation stipulates that mezcal is a distilled alcoholic beverage, 100% maguey or agave obtained by distillation of the fermented juices, extracted from mature cooked heads of different agave species (*A. angustifolia*, *A. cupreata, A. durangensis, A. inaequidens, A. maximiliana, A. potatorum*, *A. salmiana*, among others) (Fig. 16.4a) harvested in the territory of AO. Three categories are recognized *Mezcal*, *Mezcal Artesanal* (*Artisanal Mezcal*) and *Mezcal Ancestral* (*Ancestral Mezcal*); each one with six classes.

i) *Blanco o joven* (white or young), without any further processing

ii) *Madurado en vidrio* (matured in glass); Mezcal stabilized in a glass container for more than 12 months

iii) *Reposado* (aged); matured from 2 to 12 months in wooden containers of any size, form and capacity

iv) *Añejo* (extra aged); matured more than 12 months in wooden containers with less than 1000 L capacity

v) *Abocado con* (doomed with); added with authorized ingredients to give flavour, such as maguey´s worm, damiana, lemon, honey, orange, among others

vi) *Destilado con* (distilled with); Mezcal must be distilled with ingredients to incorporate flavours, such as turkey or chicken breast, rabbit, *mole*, fruits, among others

Fig 16.4 here

In 2017 the global production of *mezcal* reported by the Consejo Regulador del Mezcal was 3,986,221 L, 88 % of *Mezcal Artesanal*, with Oaxaca being the largest producer (87.0%). In recent years Mezcal has achieved national and international recognition, and is the second most consumed agave distilled beverage in the country after Tequila.

Bacanora and *sotol* are other agave spirits with government official recognition and AO. The Mexican Official Regulation NOM-168-SCFI-2004 specifies that Bacanora is only produced in the state of Sonora from *A. angustifolia*. Sotol is produced in the states of Chihuahua, Coahuila and Durango. The Mexican regulation NOM-159-SCFI-2004 recognized two categories: "*Sotol 100% puro*", obtained exclusively from sugars of the *Dasylirion* spp. and "*Sotol 51%* or *sotol*", produced using 51% of *Dasylirion* sugars and 49% from other sugar sources. Four types of Bacanora and Sotol are allowed: *blanco* (silver), *joven* u *oro* (gold), *reposado* (aged) and *añejo* (extra aged) with the same description as the different tequila types.

Outside these areas with AO other agave spirits are produced. *Raicilla* is elaborated in the Western region of Jalisco with different agave species; another spirit is produced in Southern Jalisco with different varieties of *A. angustifolia.*

After harvest of the raw material (wild or cultivated) the mezcal elaboration process is similar to the one described for tequila, although there are modifications since in general it is a more artisanal process (Fig. 16.4). The raw agave heads (Fig. 16.4b) are usually cooked in pit ovens filled with stones, heated with wood and covered with earth, to impart a smoked flavour to the product, or it can be done in brick ovens or steel autoclaves, as in some distilleries of San Luis Potosí and Zacatecas (Figs. 16.4c, 16.4d). The milling of the cooked agave heads is commonly done in a rudimentary mill or *tahona* (Figs. 16.4e, 16.4f). In San Luis Potosí during the milling process water is added, and the juices are collected by gravity. In Oaxaca and Guerrero all the crushed agave is used in the fermentation process added with some water. In some parts of Guerrero and Michoacán milling is still carried out with wood or steel mallets and the juices are collected in a *canoa*, a hollow-log fermentation container (Kirchmayr *et al.* 2017).

A wide variety of fermentation vessels are used: round holes carved directly in the ground, rectangular wooden crates buried in the ground, wooden vats with 1,000 L capacity (Fig. 16.4g), rectangular stone or brick tanks with 3,000-10,000 L capacity (Fig. 16.4h), or stainless steel tanks as those used in the Tequila industry. In general, the fermentation process is carried out spontaneously with the microbiota present in the must, and lasts 1 to 10 days depending on the temperature, region and weather conditions. The fermented must is distillated twice in rudimentary equipment as Philippine-type stills (whose advantage is that they allow the production of spirits from a small amount of agave, and that they can be disassembled and transported quickly) or metal stills (Fig. 16.4i). In others distilleries the classic Arab-type still with serpentine is used (Fig. 16.4j).

16.3.4 Yeasts and fermentation aspects

Yeasts identified in natural fermentations

Few research papers deal with the identification and characterization of the yeasts involved in the fermentation process of the different agave spirits. Lachance (1995) firstly reported the yeast communities present in a Tequila distillery, where a natural fermentation was undertaken. Due to the cooking step carried out to hydrolyse fructans into fermentable sugars, the yeasts found on fresh agave plants (*Clavispora lusitaniae*, *Kluyveromyces marxianus, Metschnikowia agaves,* and *Pichia membranifaciens*) differed from those found on cooked agave, fresh must and crushing equipment (*Candida* spp*., Hanseniaspora vineae*, *P. membranifaciens, S. cerevisiae* and *Torulaspora delbrueckii*). During fermentation, a succession of different yeast species was observed. During earlier fermentation stages a rich mixture of species was detected including *Dekkera bruxellensis*, *Hanseniaspora guilliermondii, H. vinae*, *K. marxianus*, *P. membranifaciens, T. delbrueckii* as secondary yeasts and, *S. cerevisiae* (three biotypes) as dominant species. During the progression of the fermentation the heterogeneity of species diminished and, at the end of the fermentation the maltose-positive non-flocculent *S. cerevisiae* biotype became dominant (Table 16.4). Gschaedler *et al*. (2004) reported the isolation of different yeast species in 13 tequila distilleries: *Candida magnoliae*, *Hanseniaspora uvarum, H. vinae Issatchenkia orientalis* and *Kluyveromyces lactis* (Table 16.4). Karyotype analysis of seven *S.cerevisae* isolates showed six different profiles indicating the existence of a wide genetic heterogeneity within this species (Table 16.3).

For Mezcal, studies have been published dealing with the mycobiota present during the natural fermentation stage in different production regions of Mexico. A great diversity of yeasts was found in all studies, especially in the initial fermentation stages, where several common genera and species were recognized (Table 16.4). For example, in mezcal from Yucatán, Lappe *et al*. (2004) reported *Candida parapsilosis*, *Cl. lusitaniae*, *Debaryomyces hansenii*, *I. orientalis*, *K. marxianus*, *Millierozyma farinosa, Meyerozyma caribbica*, *My. guilliermondii*, *Ogataea angusta*, *P. membranifaciens*, *Rhodotorula* spp., *Rh. mucilaginosa*, *T. delbrueckii* and *Wickerhanomyces anomalus* at the beginning of the fermentation. As fermentation progressed they also observed a dramatic reduction in yeast heterogeneity, probably due to the fermentation conditions, until the end of the fermentation, stage in which *K. marxianus* was the dominant species. During fermentation of Mezcal from Oaxaca, Andrade-Meneses and Ruiz-Terán (2004) identified species of the genera *Candida, Hanseniaspora, Rhodotorula* and *Saccharomyces.* In two other mezcal factories from the same region, Kirchmayr *et al*. (2017) reported a great diversity of non-*Saccharomyces* species, stating great differences in yeast diversity between factories and production years (Table 16.4). In this study, the coexistence of non-

Saccharomyces populations (*Kluyveromyces*, *Torulaspora* and *Zygosaccharomyces*) with *S. cerevisiae* until the end of fermentation was described.

In two studies of mezcal from San Luis Potosí produced with *A. salmiana* subsp. *crassispina*, a low yeast diversity was detected during fermentation. Escalante-Minakata *et al*. (2008) identified the yeasts *Cl. lusitaniae, K. marxianus* and *Pichia fermentans;* and the bacteria *Zymomonas mobilis* subsp. *mobilis*, *Z. mobilis* subsp. *pomaceae*, *Weissella cibaria*, *W. paramesenteroides*, *Lactobacillus farraginis*, *L. kefiri*, *L. plantarum* and *L. pontis*, highlighting the participation of a mixed microbial culture in Mezcal fermentations. Verdugo-Valdez *et al*. (2011) described species of four additional yeast genera (*Candida, Kazachstania, Saccharomyces, Torulaspora* and *Zygosaccharomyces*) (Table.16.4). As the presence of growth inhibitors (e.g. saponines) has been reported in the agave species used in this region, the raw material might have a direct impact on the presence and survival of some yeast species.

In mezcal from Tamaulipas, species of non-*Saccharomyces* (*Candida*, *Clavispora*, *Kluyveromyces*, *Meyerozyma*, *Pichia*, *Torulaspora*, *Yamadazyma*, *Zygosaccharomyces*) and *Saccharomyces* (*S. cerevisiae*) were detected in the early stages of the fermentation (Table 16.4). Only *T. delbrueckii* and *S. cerevisiae* persisted until the end of the process.

In two Mezcal distilleries from Durango, Páez-Lerma *et al.* (2013) identified the same yeast biota (*H. uvarum*, *K. marxianus*, *P. fermentans, S. cerevisiae, Saturnispora diversa* and *T. delbrueckii*) at the beginning of the fermentation. As fermentation progressed the yeast diversity decreased and at the end of both processes only *S. cerevisiae* or *S. cerevisiae* and *T. delbrueckii* were recovered, respectively.

Kirchmayr *et al*. (2014) described the microbial consortia found in three mezcal distilleries in Michoacán. Besides species of the genera *Kazachstania,* *Kluyveromyces, Meyerozyma, Pichia, Saccharomyces, Torulaspora* and *Zygosaccharomyces*) which changed clearly between factories the authors also described the presence of high populations of lactic acid bacteria during these fermentations (Table 16.4).

In fact, the high abundance of bacterial populations has been detected in several of the mentioned studies, e.g. Tequila and Mezcal from San Luis Potosí, Oaxaca, Guerrero and Tamaulipas, in which different species of *Lactobacillus*, *Leuconostoc, Oenococcus* and *Weissella*, as well as acetic acid bacteria have been reported. Acid fermentation has been observed parallel to the alcoholic fermentation carried out mainly by yeasts and in some cases also by *Zymomonas mobilis* (Escalante Minakata *et al*. 2008; Narváez-Zapata *et al*. 2010, Kirchmayr *et al*. 2017).

For Bacanora, *S. cerevisiae* was reported as the predominant yeast during natural fermentation processes carried out in different municipalities, although several non-*Saccharomyces* yeasts species of the genera *Candida, Dekkera, Meyerozyma, Millerozyma, Ogataea, Torulaspora* and *Rhodotorula* were also identified (Table 16.4). For Raicilla and Sotol, few studies have been conducted to characterize the microbial diversity present during fermentation. In both beverages, *K. marxianus* and *S. cerevisiae* have been reported, in addition to *Cl. lusitania* for the former and, *Dekkera* spp., *Kloeckera* sp., *Hanseniaspora* sp., *Zygosaccharomyces* spp. for the latter.

It is clear that during the initial phases of the fermentation of agave distillates from different regions of Mexico, a great yeasts diversity is present that influences the quality and sensory properties of the final product. Yeast diversity tends to diminish towards the end, as also shown in other spirits and in wine fermentations, although several non-*Saccharomyces* yeasts persist during the whole process. Besides *S.*

cerevisiae, the dominant and persistent species, *K. marxianus* and *T. delbrueckii* were reported in most of the aforementioned studies; while *Cl. lusitaniae*, *M. guilliermondii*, *P. membranifaciens* and *Z. bailii* only in some The remaining yeast genera and species were only sporadically isolated. It is clear that the yeast species involved in agave must spontaneous fermentations are variable, and no Mezcal has the same microbial diversity profile, which makes these beverages unique.

Fermentation development

The general practice in the production of Mezcal, Raicilla, Bacanora and Sotol is the spontaneous or natural fermentation of the must by the microbiota present in the substrate. In the Tequila industry, few companies maintain the natural fermentation because the microbial consortium produces a wide diversity of volatile compounds that contribute to the sprit flavour and bouquet, despite the lower productivity of ethanol. In some distilleries, mainly in the Tequila industry, the wort is inoculated with *S. cerevisiae* commercial strains (fresh baker's yeasts or dried yeast for wine, beer, or rum production). This practice is not the most appropriate because these yeasts are adapted to other substrates different from agave must and could have a negative impact on the sensory profiles of the final product. Another option is to use yeast strains isolated from natural fermentations which is deemed the most appropriate practice (Gschaedler *et al*. 2015). When a starter culture inoculum is used, the selected yeast strain (maintained on agar slants, lyophilized or in liquid nitrogen) is propagated in a medium with the same composition as the agave wort. The inoculum is scaled up with continuous aeration to produce enough volume to inoculate 5 or 10% of the final volume of the fermentation tank. Depending on the yeast strain, populations from 100-300 x 10^6 cells mL^{-1} are normally achieved at the onset of fermentation (Cedeño 2003). Tequila fermentation starts when the formulated must is poured in the fermentation tanks with or without yeast inoculation. Normally the temperature of the wort at the beginning of the fermentation is around 30°C, increasing during the process and can exceed 40°C. This has a negative effect on the yeasts, so the strains employed in the process require to be temperature tolerant. Using yeast inocula, the fermentation time ranges from 12 h in the fastest process to three or four days in the slowest. Without inocula the fermentation lasts from two days to one week. The time of fermentation has an important impact on the generation of the volatile compounds – these are lower in a fast fermentation compared with a slow fermentation. The rate of the fermentation depends mainly on the yeast strain, medium composition (sugar concentration at the beginning of the fermentation, nutriment supplementation), operating and weather conditions. Ethanol production can be detected from the beginning of the fermentation and, depending on the yeasts involved and the initial sugar concentration, ethanol concentrations reach 4-9% v/v at the end of the process (Gschaedler *et al.* 2015). In order to increase fermentation yields, the use of enzymes to convert residual agave polymers into fermentable sugars has been reported (Cedeño, 2003). Arrizon and Gschaedler (2002) showed the possibility to achieve high fermentation efficiency (above 90%) at high initial sugar concentration or when an additional nitrogen source is added during the exponential growth phase of the yeast.

Several studies have focused on the use of non-*Saccharomyces* yeasts (alone or in co-culture with *Saccharomyces*). Díaz-Montano *et al.* (2008) compared, at laboratory scale, the fermenting behaviour in *A. tequilana* juice of 5 yeasts strains: 3 of *S. cerevisiae* and 2 of *Hanseniaspora*. This study highlighted major differences between the three *S. cerevisiae* strains, especially in the production of volatile compounds, and the disadvantage of *Hanseniaspora* strains to achieve complete alcoholic fermentation, although these strains produced large amounts of esters. Valle-Rodríguez *et al*. (2012) showed that supplementation of the agave juice with certain specific amino acids allowed *H. vinae* to complete the fermentation. González-Robles *et al*. (2015) explored the use of mixed cultures of *Saccharomyces*/*Hanseniaspora* observing the contribution of the latter in aromatic profiles of Tequila. Amaya-Delgado *et al*. (2013) carried out fermentations of *A. tequilana* juice at an industrial scale using two non-*Saccharomyces* yeasts (*P. kluyveri* and *K. marxianus*) with a fermentation efficiency higher than 85 % and an interesting production of volatile compounds, mainly esters. This behaviour was confirmed at laboratory scale by Segura-García *et al*. (2015).

Tequila fermentations are generally carried out in open tanks, allowing evaporation of alcohol and carbon dioxide to alleviate yeast stress. Nowadays, some distilleries use cooling systems to reduce alcohol evaporation and to keep fermentation temperature tolerable for yeasts (Gschaedler *et al*. 2015).

Finally, it is important to mention that during Tequila fermentation lactic acid bacteria from the genus *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* are also present and, *Acetobacter*, an acetic acid bacterium may appear in old fermented worts (Cedeño 2003). These bacteria may have a positive impact on the generation of volatile compounds, as has been reported in whiskey, cider, and wine fermentations. However, if the bacterial populations are too large, they could affect the ethanol yield and produce some undesirable compounds.

In the case of Mezcal, Bacanora and Raicilla fermentations, these are generally carried out with the agave fibers present (except for Mezcal from San Luis Potosi), increasing the fermentation time. A general characteristic of these processes is that they are completely spontaneous, without any control of the key parameters of fermentation, such as sugars concentration or temperature. In San Luis Potosi, the fermentation of *A. salmiana* subsp. *crassispina* juice is carried out without the agave fibers, with low initial sugar concentration and is generally induced by the addition of a mixed inoculum containing low counts (>1 x 10^6 cells mL^{-1}) of non-*Saccharomyces* yeasts and bacteria. Verdugo Valdez *et al*. (2011) observed a short fermentation (around 30 h), with low residual sugars concentration and yeast population between 10-15 x10^6 cells mL^{-1}. The amount of ethanol and volatile compounds were relatively low and were directly related to the initial sugar content of the agave must. From Oaxaca, where spontaneous fermentation is generally carried out with agave fiber, Kirchmayr *et al*. (2017) published a study about Mezcal production process in two different distilleries, in two consecutive years. Fermentation kinetics and volatile compound generation differed markedly between the studied processes (all of them spontaneous), mainly due to the lack of control during the fermentation and changes in the ambient condition (mainly temperature). This study pointed out great differences in the initial sugar concentration and total yeast populations (that ranged from 12 to 180 x10^6 cells mL^{-1}). Low fermentation efficiencies were observed due to high concentration of residual sugars at the end of the fermentation. Nevertheless, this study also demonstrated that the employment of wild inocula in the fermentation of cooked agave juice may be a good practice in order to increase alcoholic fermentation efficiency.

There are very few *in situ* fermentation studies of agave distillates. However, in spontaneous fermentations, in which non-*Saccharomyces*, *Saccharomyces*, lactic and acetic acid bacteria participate, although they sometimes have low conversion rates of sugars to ethanol, they do result in beverages with complex sensory profiles that are currently sought by a new type of consumer. Some of the yeasts isolated from these spontaneous fermentations display interesting characteristics, compared with wine strains. Fiore *et al*. (2005) evaluated parameters of technological interest, such as SO_2 and copper resistance, ethanol tolerance and enzymatic activities in *Candida krusei*, *C. magnolia*, *Kloeckera africana*, *K.. apiculata*, and *S. cerevisiae* strains isolated from agave, Sotol and grape must. All agave strains were more resistant to SO_2 than wine strains, and non-*Saccharomyces* agave yeasts were more tolerant to ethanol. This behaviour is not common in non-*Saccharomyces* associated with alcoholic beverages. In contrast, all the non-*Saccharomyces* strains showed similarities in β-glucosidase and β-xylosidase activities, except *C. krusei*. Remarkable characteristics were the β-glucosidase activity of a *S. cerevisiae* strain and β-xylosidase activity of a *C. krusei* strain, both isolated during the fermentation of Sotol carried out with must and fiber. This special condition could have caused the adaptation of the yeasts that would explain these activities.

Production of aromatic compounds

Regarding the process of elaboration of these spirits, the production of volatile aromatic compounds in such beverages is influenced by several factors. These include raw materials, fructan hydrolysis processes (oven, acidic or enzymatic hydrolysis), spontaneous or directed fermentations, distilling systems (still or column) and finally aging (Table 16.5).

Table 16.5 here

Raw materials used in agave distilled beverages play an important role in the development of the sensory characteristics of the final product. Compounds including terpenes, alcohols, esters, and acids derive from the raw materials (Prado-Jaramillo *et al.* 2015). Peña-Álvarez *et al.* (2004) identified diverse terpenes in different agave species used in Mezcal (7 in *A. angustifolia*, 9 in *A. salmiana*) and Tequila production (32 in *A. tequilana*). Arrizon *et al.* (2007) determined the volatile compounds in tequila and raicilla. Sixteen terpenes were found in both beverages mainly: β-myrcene, isocineole, linalool, 1-terpineol, 4-terpineol, citronellol, nerol, geraniol and nerolidol; 15 more were detected only in raicilla, predominantly α-pinene, camphene, limonene, γ-terpinene, ρ-cymene, myrcenol, neryl acetate, geranyl acetate, α-eudesmol. From these, geraniol, linalool, limonene, myrcenol, γ-terpinene, 4-terpineol, were among others are found in the final product. This highlights the impact of the raw material on the sensory profiles of agave distillates (Tables 16.5).

During production of agave distillates, it is necessary to carry out the hydrolysis of fructans, which can be accomplished by a cooking process. During this process agaves heads and/or agave juice are exposed to temperatures between 95°C and 121°C, causing caramelisation and Maillard reactions. Some compounds that have been identified in cooked agave juice are: 5-hydroxymethylfurfural, methyl-2-furonoate and 2, 3-dihydroxy-3,5-dihydro-6-methyl-4 (H) -pyran-4-one (Mancilla-Margalli and López, 2002). Prado-Jaramillo et al.(2015) reported the presence of acids, aldehydes, ketones, esters, and mainly furans (1-furan-2-yl-ethanone, 5-acetoxymethyl-furfural, 5-methyl-furfural, furfural and hydroxymethylfurfural) and terpenes (α-terpineol, Δ-cadineno, linalool, nerolidol, ocimene and γ-terpinene). These are compounds that persist in the distilled and aged beverage (Tables 16.5).

Another compound generated during cooking or the agave fructans hydrolysis stage is methanol, whose concentration is stipulated in the regulations of Tequila and Mezcal. Due to the cooking conditions (high temperatures and low pH) methoxyl groups are released from the pectin present in the agave heads, and methanol is formed in the presence of water. This alcohol is partially separated during distillation (Prado-Ramírez *et al*. 2005).

The most studied stage in agave distillates production is fermentation. Usually, at the beginning of the process a high yeast diversity is present, mainly non-*Saccharomyces* species (of the genera *Candida*, *Hanseniaspora*, *Meyerozyma*, *Kluyveromyces*, *Pichia*, *Torulaspora*, *Zygosaccharomyces*, etc.) which produce a large amount of volatile compounds and flavour congeners that impact in the sensory quality of the agave spirits (Table 16.6). The generation of these compounds depends on different factors, including: agave species; preparation of the must with hydrolyzed agave juice, with or without bagasse fibers; spontaneous or induced fermentation; yeast strain; fermentation conditions (temperature, dissolved oxygen, pH, nitrogen concentration), among others (Arrizon *et al.* 2002, 2006; Arellano *et al*. 2008; Díaz-Montaño *et al*. 2008; Verdugo-Valdez *et al*. 2011; López-Álvarez *et al*. 2012; Moran-Marroquín *et al*. 2011; Amaya-Delgado *et al*. 2013, Segura-García *et al*. 2015, González-Robles *et al*. 2015, Kirchmayr *et al*. 2017) (Tables 16.5, 16.6).

During agave juice fermentation higher alcohols are the most important aromatic compounds produced, representing between 50 to 90% of the total of volatile compounds produced during this stage. These include, mainly: amyl alcohol, 1-propanol, isobutanol and furfuryl alcohol (Gschaedler *et al*. 2015). Factors that influence their production are the yeast strain (Arellano *et al*. 2008; Díaz-Montaño *et al*. 2008; Segura *et al*. 2015), high fermentation temperature and low nitrogen concentration (Arellano *et al*. 2008; Arrizon *et al*. 2006). Esters are the most diverse compounds found, and their production depends mainly on the yeast strain. Non-*Saccharomyces* species as *K. marxianus*, *P. kluyveri* and *T. delbrueckii* have been reported to produce more esters than *S. cerevisiae* (Amaya-Delgado *et al*. 2013, Segura-García *et al*. 2015, Núñez-Guerrero *et al*. 2016). However, anaerobic conditions (Moran-Marroquín *et al*. 2011) as well as high nitrogen concentrations, can influence the production of these compounds in *A tequilana* and *A durangensis* juices (Arrizon *et al*. 2002; Rutiaga-Quiñones *et al*. 2013). At the end of tequila fermentation, Prado-Jaramillo *et al*. (2015) detected 71 compounds belonging to alcohols, acids, terpenes, furans, aldehydes, ketones and esters (Table 16.5).

Other volatile compounds produced during agave must fermentation are: acetals, aldehydes, acids, ketones, phenols, hydrocarbons, sulfur etc., although they have been identified, have not been quantified, and the parameters that influence their production are still unknown (Prado-Jaramillo *et al*. 2015) (Table 16.5).

It is important to highlight that agave species, place of origin, age, year season, spontaneous fermentation, and fermentation conditions can change the profile of volatile compounds produced (Pinal *et al*. 2009). The different yeast and bacteria species that participate in the spontaneous fermentation of agave-spirits can also impact the production of non-volatile compounds such as: ethyl acetate, ethyl lactate, acetic acid and lactic acid, that modify the titratable acidity of the must (Kirchmayr *et al*. 2017; Escalante-Minakata *et al*. 2008; Narváez-Zapata *et al*. 2010; Páez-Lerma *et al*. 2013).

There are few reports regarding the production and recovery of volatile compounds during the distillation of agave spirits (Prado-Ramírez *et al*. 2005). In these beverages more than 200 compounds have reported mainly: alcohols, acids, terpenes, furans, aldehydes, ketones and esters, whose profile varies depending on the species of agave used, its region of origin, the fermentation process and distillation conditions (Table 16.5) (Álvarez-Ainza *et al*. 2013; Prado-Jaramillo *et al.* 2015).

Agave distillates can be aged in wooden (oak, white oak, holm oak) barrels for months or years, depending of the type of distillate to be obtain (Fig 16.4k). In aged agave spirits more 327 compounds have been identified (Prado-Jaramillo *et al*. 2015). During Tequila aging the main compounds detected are: higher alcohols, methanol, esters, furans, gallic acid, vanillin, syringaldehyde, synapinaldehyde, coniferaldehyde, syringic acid, ferulic acid, esculetin, scopoletin, cis/trans whisky lactones, guaiacol, 4-ethyl guaiacol and, vainillin (González-Robles and Cook 2016).

16.4 Recent developments and future prospects

The great number of agave species and their many uses are a natural and cultural symbol of Mexico. For more than 10,000 years these plants have been used for different purposes (nutritional, medicinal, fibers extraction, production of alcoholic beverages, construction, etc.). This emphasises why the diversity of agaves must be protected to maintain their ecology, ethnobotany and evolution, ensuring their availability to support the economic and cultural needs of the Mexican people.

The production processes of *Agave* and *Dasylirion* spirits have to be understood and precisely described. The genetic diversity of the plants used as raw material as well as the production and composition of the must have to be determined. The microbiota present in the must that participates in the complex fermentation process has to be phenotypically and genotypically characterized, and their potential as starter cultures. Finally, the precise roles of microbes during spontaneous fermentations in the production of ethanol and aromatic compounds remains to be evaluated.

16.4 Yeasts in production of miscellaneous spirits

This section covers yeast and fermentation aspects of selected miscellaneous distilled spirits. It is outwith the scope of this Chapter to provide a comprehensive coverage of these topics and the reader is directed to several publications and websites for more detailed information (eg. for fruit-based spirits: http://www.pediacognac.com/en/; for sugar/molasses-based spirits: Piggot (2003); and for whey-based spirits: O'Shea (2003).

16.4.1 Yeasts in production of fruit-based spirits

Various spirits are produced following the distillation of fermented fruit sugars. For example, brandies are distilled wines and eau-de-vies are distilled fruit beverages. The fruits in question are those from which fermentable sugars, principally glucose and fructose, can be easily extracted and include grapes, apples, plums, apricots and several others.

For brandies, including the best-known example Cognac, the starting material for the distillation is wine. Yeast and fermentation aspects of wine production have been well covered in previous Chapters in this book and the reader is also directly to the recent elegant paper on wine yeasts by Pretorius (2016).

Several naturally-occurring and selected yeasts are involved in grape fermentations for spirit production, principally including: *S. cerevisiae*, *Candida famata* and *K. apiculata*. For Cognac production, the Bureau National Interprofessionnel du Cognac (BNIC, http://www.bnic.fr/cognac/) in France recommends 8 strains of yeast (Dr L. Lurton, BNIC, personal communication) which will produce different concentrations of secondary fermentation metabolites that act as flavour congeners in Cognac spirits (eg. ethyl acetate, isoamyl acetate, higher alcohols, 2-phenyethanol, ethyl laurate etc.).

Ester formation by Cognac yeasts is a desirable attribute as these compounds impart characteristic fruity and floral aromas to the spirit. The choice of yeast strain is therefore critical in defining spirit quality.

A typical composition of a freshly-distilled wine spirit like Cognac would comprise: ~72% v/v ethanol; ~28% v/v water and <1% volatile substances. Of the latter, 80 olfactive zones have been characterized by gas chromatography-olfactometry and many of these have been identified (e.g. in the following chemical classes: alcohol, esters, aldehydes, norisoprenoïds, terpenes, etc.) in concentrations ranging from nanogram/l to several hundred milligrams/l. Although Cognac aroma originates from the grapes, distillation and ageing, the role of yeast and progress of fermentation is of paramount importance for Cognac sensory characteristics. The BNIC have developed a Cognac aroma wheel that depicts the cycle of the seasons (Fig 16.5).

Fig 16.5 here

Cognac wine fermentations are complete within 4-8 days, depending on temperature which also influences volatile flavour and aroma compounds in the spirit. For example, increasing temperature (eg. 18 to 22°C) results in elevated levels of higher alcohols, but decreasing levels of isoamyl acetate. In order to have some control over congener profiles and also off-flavours, the yeasts employed for Cognac fermentations (at an inoculation rate of around $1x10^6$ cells/mL) must be able to initiate alcoholic fermentation of grape must rapidly to prevent growth of contaminant microbes. This is especially important since SO_2 is not allowed in Cognac production processes. In addition, the level of utilisable nitrogen in the grape must will dictate the kinetics and extent of yeast growth and nitrogen deficiency can be addressed by supplementation (within defined limits) with ammonium salts. Rapid yeast growth also exerts some degree of control over bacterial malolactic fermentation. Generally speaking, distillers producing wine-based spirits such as Cognac must select the most suitable strains of *S. cerevisiae* to dominate the fermentation and to liberate the correct balance of congeners into the spirit. In other words, the sensory profile of a wine spirit, as it is with other alcoholic beverages, is highly yeast strain-dependent.

16.4.2 Yeasts in production of sugarcane-based spirits

Various distilled spirits can be produced from sugar cane. Those emanating from the fermentation of the raw sugar cane juice are spirits such as Rhum Agricole (from Réunion and Martinique) and Cachaça (from Brazil), and those emanating from sugar cane molasses are spirits collectively called rum. There are several styles of rum, classed as white rum (having no colour, generally unaged in oak wood barrels); amber rum (with a golden colour, typically aged in oak barrels for between 1-3 years) and dark rum (typically aged 3 or more years). Molasses (or sugar cane juice) fermentations can either be spontaneous, relying on natural microorganisms (wild yeasts and bacteria) indigenous to the raw materials and distillery environments, or can be specially selected *S. cerevisiae* strains to be used as starter cultures. Fermentations can either be batch, continuous or semi-continuous processes, but in all cases, the main fermentable sugar available to yeast is sucrose. Therefore, yeasts with a high invertase activity are employed to facilitate sucrose hydrolysis to glucose and fructose. In addition to *S. cerevisiae,* other yeasts play important roles in rum fermentations such as *Schizosaccharomyces pombe* (Pech *et al*. 1994; Fahrasmane *et al*. 1988). This fission yeast can impart desirable flavour and aroma characteristics, particularly in dark, heavy rum styles. Compared with *S. cerevisiae*, *Schizo. pombe* conducts slower fermentations, produces less higher alcohols and fatty acids, but more esters. This yeast is favoured by low pH and higher sugar concentrations in molasses. *Schizo. pombe* also has interesting enological properties such as malo-ethanolic fermentation (Benito *et al*. 2012).

16.4.3 Yeasts in production of whey-based spirits

There are a variety of beverages, both alcoholic and non-alcoholic, derived from cheese whey, a by-product of the cheese-making process (Jeličić *et al*. 2008). In addition to whey “beer” and whey “wine”, distilled spirits are also made from cheese whey (O’Shea 2003). In all cases, the fermentation of lactose in the whey is conducted by lactose-fermenting yeasts, notably *K. marxianus* (Walker and O’Neill 1990). The spirits include whey-based vodka and such beverages are produced on a large scale in several countries (e.g. Ireland, New Zealand, Turkey). Because whey only contains around 5% w/v lactose, it has to be concentrated (following ultrafiltration to remove whey protein) using reverse osmosis to increase the final alcohol concentration in the beer prior to distillation. The alcohol levels produced in whey fermentations are also restricted by the relatively low ethanol tolerance of *Kluyveromyces* spp. employed and this is an area for further yeast research.

16.5 Conclusions and future prospects

The diversity of distilled spirits is linked to the diverse starting raw materials and the main available sugars for yeast fermentation, which are: maltose (for whiskies), glucose and fructose (for brandies), sucrose (for rums), lactose (for cheese whey-based spirits) and fructose (for Tequila and M.ezcal). The choice of yeast strain for such spirits must therefore reflect specific sugar fermentability For example, a fructophilic yeast would not be appropriate for malt wort fermentations for whisky.

Many developments have taken place aimed at improving the efficiency of sugar conversion to alcohol, and in selecting new strains of *S. cerevisiae* to impart desirable sensory characteristics to spirit beverages. Walker *et al.* (2012) have identified desirable characteristics for Scotch whisky distilling yeast strains.

Although scientific advances in knowledge of yeast genetics and molecular biology as applied to brewing and winemaking have been made in recent times this has proved of very limited practical value to the distiller. In particular, strain engineering employing recombinant DNA techniques has not found favour for production of spirit beverages, mainly due to public perception issues. In other words, the constraints in employing GM yeast strains in this field are primarily sociological, rather than technological. *Self-cloned* yeast strains, involving yeast-yeast genetic modification may be more attractive to consumers than yeast transformation with non-yeast genes and may also be more favourable from a regulatory standpoint (Argyros and Stonehouse 2017). Where such genetically manipulated (GM) strains are being successfully exploited is in the fuel ethanol sector (see Walker and Walker, 2018). Nevertheless, some molecular biological techniques applied to beverage spirit yeast strains have proved useful. For example, proteomic analysis of whisky yeast strains during fermentation has provided insight into stress responses of yeast during fermentation of industrial grain mashes (Hansen *et al.* 2006). Molecular genetics and bioinformatics applied to industrial strains of *S. cerevisiae* represent powerful tools in monitoring and control of fermentations for beer, wine and spirit production (Bond and Blomberg 2006). Future research into yeast physiology and genetics will lead to a deeper understanding of *S. cerevisiae* strains exploited for spirit fermentations. In turn, novel yeast strains with interesting new attributes with improved fermentation performance and flavour quality are on the horizon.

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Figure Captions

Fig 16.1 The key role of *Saccharomyces cerevisiae* in production of fermented beverages. Adapted from Walker and Stewart (2016)

Fig 16. 2 Categories of the main global whiskies. Adapted from Walker and Hill (2016)

Fig 16.3 Summary of the main processes involved in Scotch malt whisky production. Adapted from Walker and Hill (2016)

Fig 16.4 Mezcal elaboration process.

(a) *Agave angustifolia*
(b) Raw agave heads or *piñas*
(c) Pit oven filed with stones
(d) Brick oven
(e) Cooked agave heads or piñas
(f) Rudimentary mill or *tahona*
(g) Fermentation of cooked agave must with fiber in wooden vats
(h) Fermentation of agave must without fiber in a brick tank
(i) Distillation of fermented agave must in a rudimentary still
(j) Arab type still
(k) Mezcal aging in oak barrels (optional)

Figure 16.5 Cognac aroma depicting the cycles of the seasons From: http://www.bnic.fr/cognac/ © BNIC / Gérard MARTRON

Table 16.1 Yeasts used in the production of selected distilled alcoholic beverages. Adapted from Walker & Stewart (2016)

Beverage	Yeast Involved	Comments
Whisk(e)y	*Saccharomyces cerevisiae*	Scotch whisky producers currently use selected distilling strains of *S. cerevisiae* in three main formats, cream yeast, pressed (cake) and dried yeast. Malt whisky distilleries traditionally use pressed yeast, but larger grain distillers have now adopted cream yeast. Dried yeasts are not as prevalent as pressed and cream formats in whisky fermentations.
Rum	*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*	*Saccharomyces cerevisiae* strains in rum fermentations are developed as starter cultures and provide faster fermentation with more higher alcohols and fatty acids, but less esters resulting in lighter style rums. *Schizosaccharomyces pombe* in rum fermentations provides slower fermentations leading to less higher alcohols and fatty acids, but more esters resulting in heavy, strong aroma rums. Growth of *Schiz. pombe* is favoured by low pH, higher sugar concentrations.
Tequila, Mezcal, Bacanora	Natural yeasts in artisanal Agave fermentations	Various yeasts have been isolated from such processes: *S. cerevisiae*, *Kluyveromyces marxianus*, *Pichia* spp., *Brettanomyces* spp., *Rhodotorula* spp., etc.
Brandies, Gin, Vodka, etc.	*Saccharomyces cerevisiae*	For brandies, cognac, etc. the base wine is produced by pure starter cultures of *S. cerevisiae*. For gin, vodka, etc. selected distilling strains of *S. cerevisiae* will be used.
Cheese whey-derived beverages	*Kluyveromyces marxianus*	Lactose-fermenting yeast to produce ethanol destined for gin, vodka and cream liqueurs, etc.

Table16.2 Attributes of distiller’s yeasts compared with wine yeasts

Attribute	Distillers yeast	Wine yeasts
Genetics	Some whisky yeasts are natural hybrids between *S. cerevisiae* and *S. cerevisiae* var. *diastaticus*, but generally have complex ploidy (e.g. polyploid). Some Scotch whisky is made using mixtures of distiller’s and brewer’s yeast cultures. *S. cerevisiae* strains found in agave juice fermentations show high genetic diversity.	Wine yeasts are generally homothallic diploids, but some strains may be polyploid or aneuploid.
Metabolism	Rapid and complete fermentation of cereal sugars. Most distillers yeasts will ferment glucose, maltose, matotriose and some limited fermentation of maltodextrins (glucoamylase activity). *Agave* juice yeasts ferment fructose and glucose. Production of desirable flavour congeners (esters, organic acids, aldehydes, higher alcohols etc.). In Scotch whisky and *Agave* fermentations, there is also a contribution to spirit flavour made by lactic acid bacteria (see text).	Vigorous fermentation desired, with correct volatile acidity, aromatic character (esters, succinic acid) and viscosity (glycerol). Low acetaldehyde and correct balance of sulphur compound production.
Stress physiology	Stress-tolerance to temperature, ethanol, osmotic pressure and competitive microbes (lactic acid bacteria) desired.	In addition to tolerance to variable temperature, osmotic pressure and pH, wine yeasts should be SO_2 tolerant.

Table 16.3 Important yeast-derived flavour congeners in distilled spirits

Compound Class	Example	Flavour/Aroma
Higher alcohols	*n*-propanol	Alcoholic
	Isobutanol	Pharmacy
	Iso-amyl alcohol (3-methylbutan-1-ol)	Fusel, alcoholic, fruity, banana
	Phenylethanol	Roses, perfume
Esters	Ethyl acetate	Solvent, acetone
	Ethyl butyrate	Pineapple, banana, mango
	Ethyl caproate	Apple, aniseed
	Ethyl caprylate	Apple
	Ethyl hexanoate	Pineapple, unripe banana
	Ethyl lactate	Butter/cream
	Ethyl octanoate	Sour apple, apricot
	Iso-amyl acetate	Banana, fruity
Aldehydes	Acetaldehyde	Green apple
Vicinal diketones	Diacetyl	Butter, butterscotch
Phenolics	4-Vinyl guaiacol	Clove-like
S-Compounds	Hydrogen sulphide	Rotten eggs

Table 16.4 Bacteria and yeasts isolated from the must fermentation process of Mexican distilled *Agave* and *Dasylirion* spirits

Beverage	***Agave* species**	**States of production**	**Microbiota**[1]	**References**
Tequila	*A. tequilana* var. azul	Jalisco, regions of the states of Guanajuato, Michoacán, Nayarit and Tamaulipas	LAB, non-*Saccharomyces* (*Candida* spp., *C. intermedia*, *C. magnoliae*, *Dekkera anomala*, *D. bruxellensis*, *Hanseniaspora* spp., *H. uvarum, H. vinae, Issatchenkia orientalis, Kazachstania humilis, Kluyveromyces lactis*, *K. marxianus, Meyerozyma guilliermondii, Pichia membranifaciens, Torulaspora delbrueckii, Zygosaccharomyces bailii*) and *Saccharomyces* (*S. cerevisiae*) yeasts In industrialized process selected *S. cerevisiae* strains	Lachance (1995) Cedeño (2003) Gschaedler *et al.* (2004)
Mezcal	*A. fourcroydes*	Yucatán	Non-*Saccharomyces (C. parapsilosis, Clavispora lusitaniae*, *Debaryomyces hansenii, I. orientalis, K. Marxianus*, *Meyerozyma caribbica*, *M. guilliermondii*, *Millerozyma farinosa, Ogataea angusta, P. membranifaciens, T. delbrueckii, Wickerhamomyces anomalus*) Basidiomycetous yeasts (*Rhodotorula* spp., *Rhodotorula mucilaginosa*	Lappe *et al.* (2004)
Mezcal	*A. angustifolia*	Oaxaca	LAB, AAB; non-*Saccharomyces* (*Candida* spp., *C. apicola*, *C. boidinii*, *C. parapsilosis*, *C.zemplinina*, *Citeromyces matritensis*, *Cl. lusitaniae, D. hansenii*, *.D. anomala*, *Diutina rugosa, Hanseniaspora* spp., *H. guilliermondii*, *H. osmophila*, *I. orientalis, K. lactis*, *K. marxianus*, *M. guilliermondii*, *Pichia fermentans, P. mandshurica*, *P. membranifaciens*, *Schizosaccharomyces*	Andrade Meneses and Ruiz Terán (2004) Kirchmayr *et al*. (2017)

			pombe, *T. delbrueckii*, *W. anomalus*, *Z. bailii*, *Z. bisporus*, *Z. rouxii*) and *Saccharomyces* (*S. cerevisiae*) yeasts Basidiomycetous yeasts *(Cryptococcus uniguttulatus*, *Naganishia albida*, *Pseudozyma prolifica*, *Rhodosporidiobolus fluvialis*, *Rhodotorula glutinis*, *Rh. mucilaginosa, Sporidiobolus salmonicolor*	
Mezcal	*A. salmiana* subsp. *crassispina*	San Luis Potosí	LAB, *Zymomonas mobilis*; non-*Saccharomyces (C. ethanolica*, *Cl. lusitaniae, I. orientalis*, *Kazachstania exigua*, *K. marxianus. P. fermentans*, *Pichia. kluyverii, T. delbrueckii*, *Z. bailii*) and *Saccharomyces* (*S. cerevisiae*) yeasts	Escalante-Minakata *et al.* (2008) Verdugo Valdez *et al.* (2011)
Mezcal	*A. angustifolia* *A. lechuguilla* *A. montium-sancticaroli*	Tamaulipas	LAB, Non-*Saccharomyces (C. parapsilosis, Cl. lusitaniae, K. marxianus, M. guilliermondii, P. kluyveri*, *T. delbrueckii, Yamadazyma mexicana, Z. bailii*) and *Saccharomyces* (*S. cerevisiae*) yeasts Basidiomycetous yeasts (*Rh. mucilaginosa*)	Arratia (2009) Narváez Zapata *et al.* (2010)
Mezcal	*A. durangensis*	Durango	Non-*Saccharomyces (H. uvarum*, *K. marxianus*, *T. delbrueckii*, *P. fermentans, Saturnispora diversa*) and *Saccharomyces* (*S. cerevisiae*) yeasts	Páez-Lerma *et al.* (2013)
Mezcal	*A. cupreata* *A. inaequidens*	Michoacán	Non-*Saccharomyces* (*C. magnoliae, H. uvarum, I. orientalis,*) and *Saccharomyces* (*S. cerevisiae*) yeasts	Kirchmayr *et al.* (2014)
Bacanora	*A. angustifolia*	Sonora	Non-*Saccharomyces* (*Candida blankii*, *C. silvatica*, *D. bruxellensis, Mi. farinosa*, *M. guilliermondii*, *Ogataea polymorpha*, *T. delbrueckii*) and *Saccharomyces* (*S. cerevisiae*) yeasts. Basidiomycetous yeasts (*Rhodotorula* sp.)	Vallejo Córdoba *et al.* (2005) Zamora-Quiñonez (2006) Álvarez Ainza *et al* (2015)
Raicilla	*A. angustifolia* *A. inaequidens* *A. maximiliana*	Jalisco	Non-*Saccharomyces (Cl. lusitaniae*, *K. marxianus*) and *Saccharomyces* (*S. cerevisiae*) yeasts	Arrizon *et al.* (2007)

Sotol	*Dasylirion* spp.	Chihuahua Durango Coahuila	Non-*Saccharomyces (Dekkera* sp.*,* *Kloeckera* sp.*, Hanseniaspora* sp., *Kluyveromyces* spp.*, K. marxianus, Zygosaccharomyces* sp.*,* and *Saccharomyces* (*S. cerevisiae*) yeasts	De la Garza-Toledo *et al.* (2008)

[1] Microbiota in spontaneous fermentation. LAB= Lactic acid bacteria, AAB= Acetic acid bacteria

Table 16.5 Volatile compounds production along the elaboration process of agave distilled beverages

Process stage	Beverage	Agave used	Volatile compounds	References
Raw material	Tequila	*A. tequilana* var. azul	Terpenes, alcohols, furans, acids, aldehydes, esters, hydrocarbures	Prado-Jaramillo *et al.* (2015)
	Mezcal, Tequila	*A. angustifolia*, *A. salmiana* *A. tequilana* var. azul	Acids, esters, terpenes	Peña-Alvárez *et al.* (2006)
	Mezcal	*A. salmiana* subsp. *crassispina, A. salmiana* var. *salmiana, A. angustifolia, A. cupreata, A. karwinskii*	Acids, lipids	Martínez-Aguilar *et al* (2009)
Cooking/ hydrolysis	Tequila	*A. tequilana* var. azul	Furans, pyrans, aldehydes, nitrogen and sulfur compounds terpenes, alcohols, furans, acids, aldehydes, esters, hydrocarbures	Mancilla-Margalli and López (2002) Prado-Jaramillo *et al*. (2015)
	Mezcal	*A. salmianas*	Furans	García-Soto *et al*. (2011)
Fermentation	Tequila	*A. tequilana* var. azul	Terpenes, alcohols, higher alcohols, furans, acids, esters, aldehydes, hydrocarbures	Arrizon *et al.* (2006) Arellano *et al*. (2008) Díaz-Montaño *et al.* (2008) Pinal *et al.* (2009) Moran-Marroquín *et al.* (2011) Valle-Rodríguez *et al.* (2012) Amaya-Delgado *et al* (2013) González-Robles *et al.* (2015) Prado-Jaramillo *et al.* (2015) Segura-García *et al.* (2015)
	Mezcal	*A. angustifolia*	Esters, higher alcohols, aldehydes	Kirchmayr *et al.* (2017)
	Mezcal	*A. salmiana* subsp. *crassispina*	Esters, higher alcohols, aldehydes	Verdugo-Valdez *et al.* (2011) De León-Rodríguez *et al.* (2008)

	Mezcal	*A. angustifolia, A. potatorum*	Alcohols, esters, ketones, acids, furans	Vera-Guzmán *et al*. (2009, 2012)
	Tequila Raicilla	*A. tequilana* var. azul *A. maximilana* *A. inaequidens*	Alcohols, esters, aldehydes	Arrizon et al. (2007)
	Mezcal	*A. durangensis*	Alcohols, higher alchohols, esters, acids, acetals, ketones, aldehydes, esters, terpenes	Soto-García *et al.* (2009) Rutiaga-Quiñones *et al*. (2012) De los Ríos-Deras *et al.* (2015) Nuñez-Guerrero *et al.* (2016)
	Mezcal	*Agave* spp.	Acetals, acids, alcohols, esters, ketones, aldehydes, phenols, terpenes	Molina-Guerrero *et al* (2007)
	Mezcal	*A. angustifolia, A. lechugilla* *A. americana*	Acids	Narváez-Zapata *et al.* (2010)
	Bacanora	*A. angustifolia*	Alcohols, aldehydes, esters,	Álvarez-Ainza *et al.* (2013)
	Mezcal	Definied wort medium	Alcohols, aldehydes, esters	Arrizon *et al.* (2006)
Distillation	Mezcal	*A. salmiana*	Esters, higher alcohols, furans, acids, aldehydes	De León-Rodríguez *et al*. (2006)
	Mezcal	*A. angustifolia, A. potatorum*	Alcohols, esters, ketones, acids, furans, terpens	Vera-Guzmán *et al*. (2009, 2018)
	Tequila Raicilla	*A. tequilana* var. azul *A. maximiliana, A. inaequidens*	Terpenes	Arrizon *et al.* (2007)
	Raicilla Sisal Tequila Mezcal Bacanora Sotol	*A. maximiliana,* *A. sisalana* *A. tequilana* var. azul *A. salmiana, A. potatorum, A. angustifolia, A. durangensis* *A. angustifolia* *Dasylirion* spp.	Esters, higher alchohols, furans, acids, aldehydes	De León-Rodríguez *et al.* (2008)
	Tequila Mezcal, Bacanora Sotol	*A. tequilana* var. azul *A. angustifolia* *Dasylirion* spp.	Alcohols, esters, aldehydes	Lachenmeier *et al*. (2006)
	Sotol	*Dasylirion* spp.	Alcohols, esters, acids, furfural, ketones	De la Garza *et al.* (2010)

	Mezcal	*A. durangensis*	Esters, higher alchohols, acids aldehydes, tepens, , ketones	De los Rios-Deras *et al.* (2015)
	Tequila	*A. tequilana* var. azul	Alcohols, esters, aldehydes, furans, terpenes, acids, hydrocarbures	Prado-Ramírez *et al.* (2005) Ceballos-Magaña *et al.* (2013) Prado-Jaramillo *et al.* (2015)
Aging	Tequila	*A. tequilana* var. azul	Acetals, terpenes, aldehydes, esters, ketones, furans phenols, acids, hydrocarburs, alcohols	López-Ramírez *et al.* (2013) Ceballos-Magaña *et al.* (2013 Prado-Jaramillo *et al.* (2015) González-Robles and Cook (2016)
	Tequila, Mezcal	*A. tequilana* var. azul *A. angustifolia*	Furans	Muñoz-Muñoz *et al.* (2009)

Figure Captions

Fig 16.1 The key role of *Saccharomyces cerevisiae* in production of fermented beverages. Adapted from Walker and Stewart (2016)

Fig 16. 2 Categories of the main global whiskies. Adapted from Walker and Hill (2016)

Fig 16.3 Summary of the main processes involved in Scotch malt whisky production. Adapted from Walker and Hill (2016)

Fig 16.4 Mezcal elaboration process.

(a) *Agave angustifolia*
(b) Raw agave heads or *piñas*
(c) it oven filed with stones
(d) Brick oven
(e) Cooked agave heads or piñas
(f) Rudimentary mill or *tahona*
(g) Fermentation of cooked agave must with fiber in wooden vats
(h) Fermentation of agave must without fiber in a brick tank
(i) Distillation of fermented agave must in a rudimentary still
(j) Arab type still
(k) Mezcal aging in oak barrels (optional)

Figure 16.5 Cognac aroma depicting the cycles of the seasons From: http://www.bnic.fr/cognac/ © BNIC / Gérard MARTRON

Fig 16.1

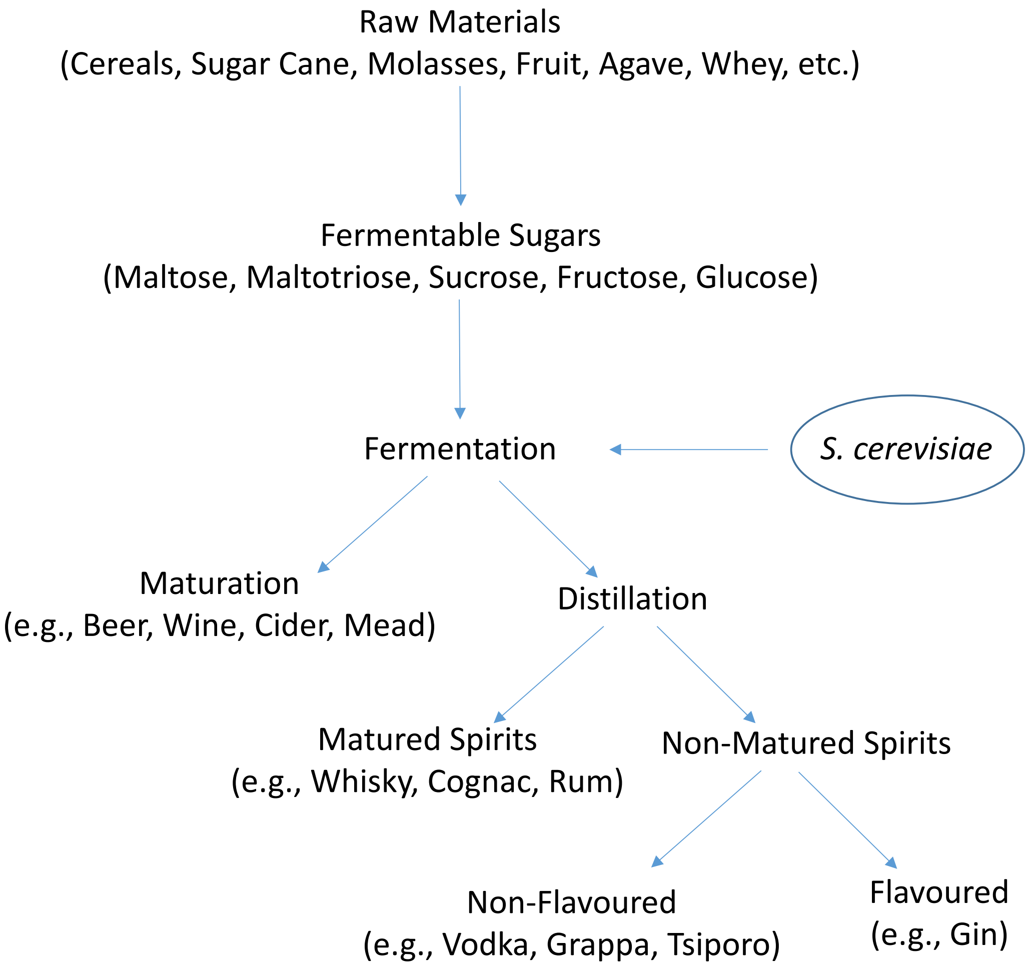

Raw Materials
(Cereals, Sugar Cane, Molasses, Fruit, Agave, Whey, etc.)
Fermentable Sugars
(Maltose, Maltotriose, Sucrose, Fructose, Glucose)
Fermentation
S. cerevisiae
Maturation
(e.g., Beer, Wine, Cider, Mead)
Distillation
Matured Spirits
(e.g., Whisky, Cognac, Rum)
Non-Matured Spirits
Non-Flavoured
(e.g., Vodka, Grappa, Tsiporo)
Flavoured
(e.g., Gin)

Fig 16.2

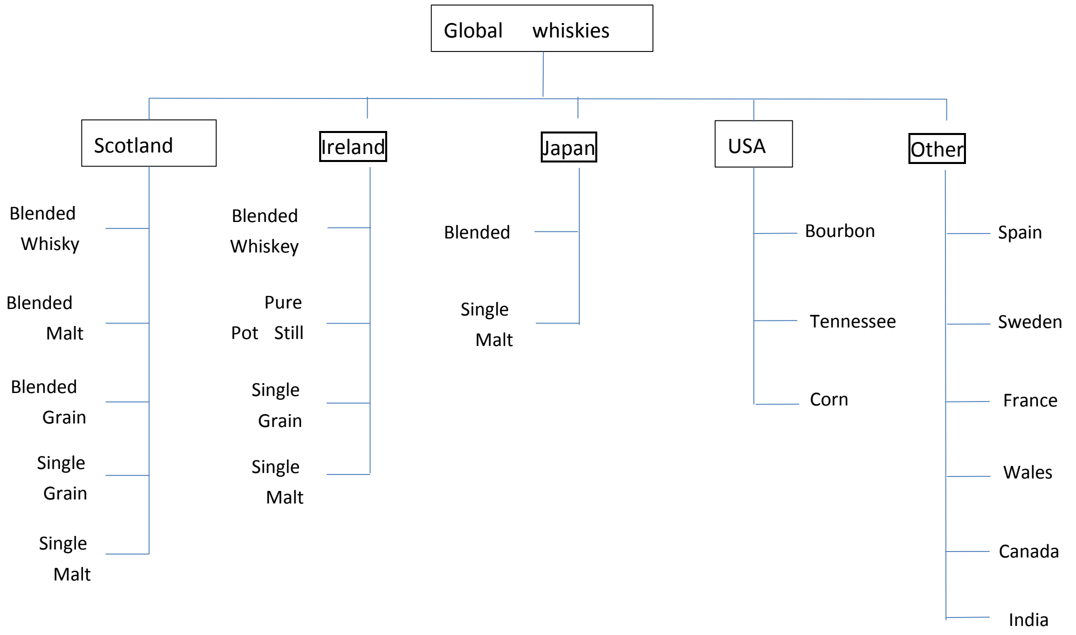
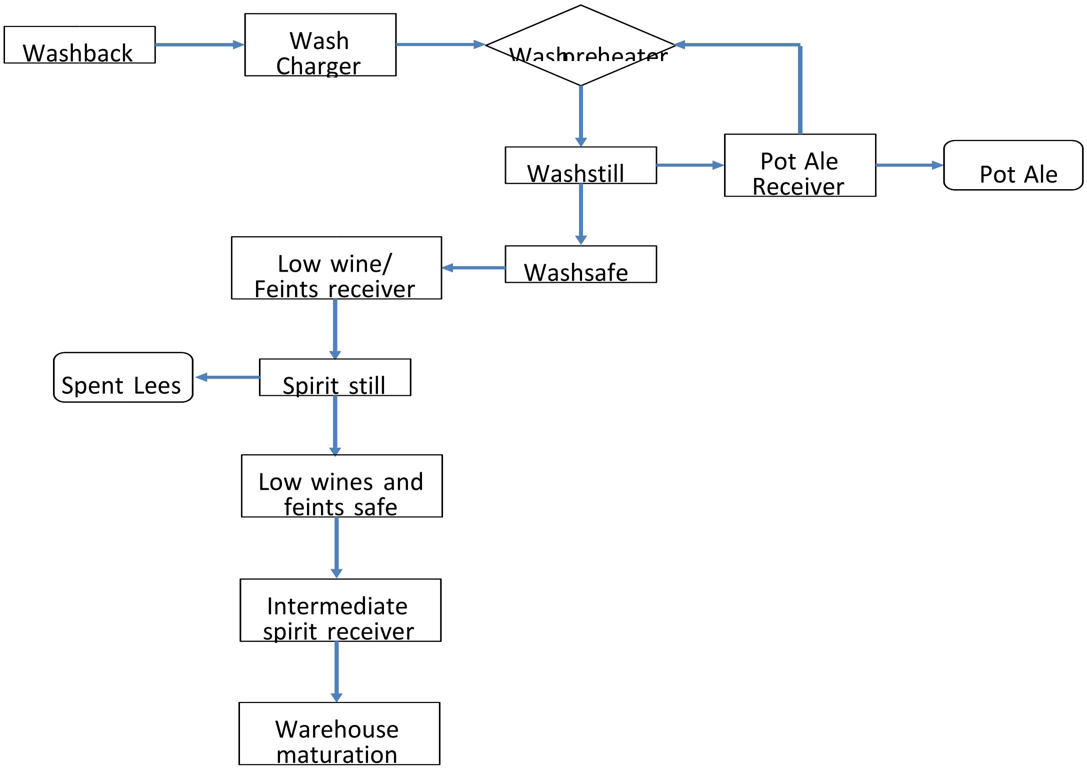
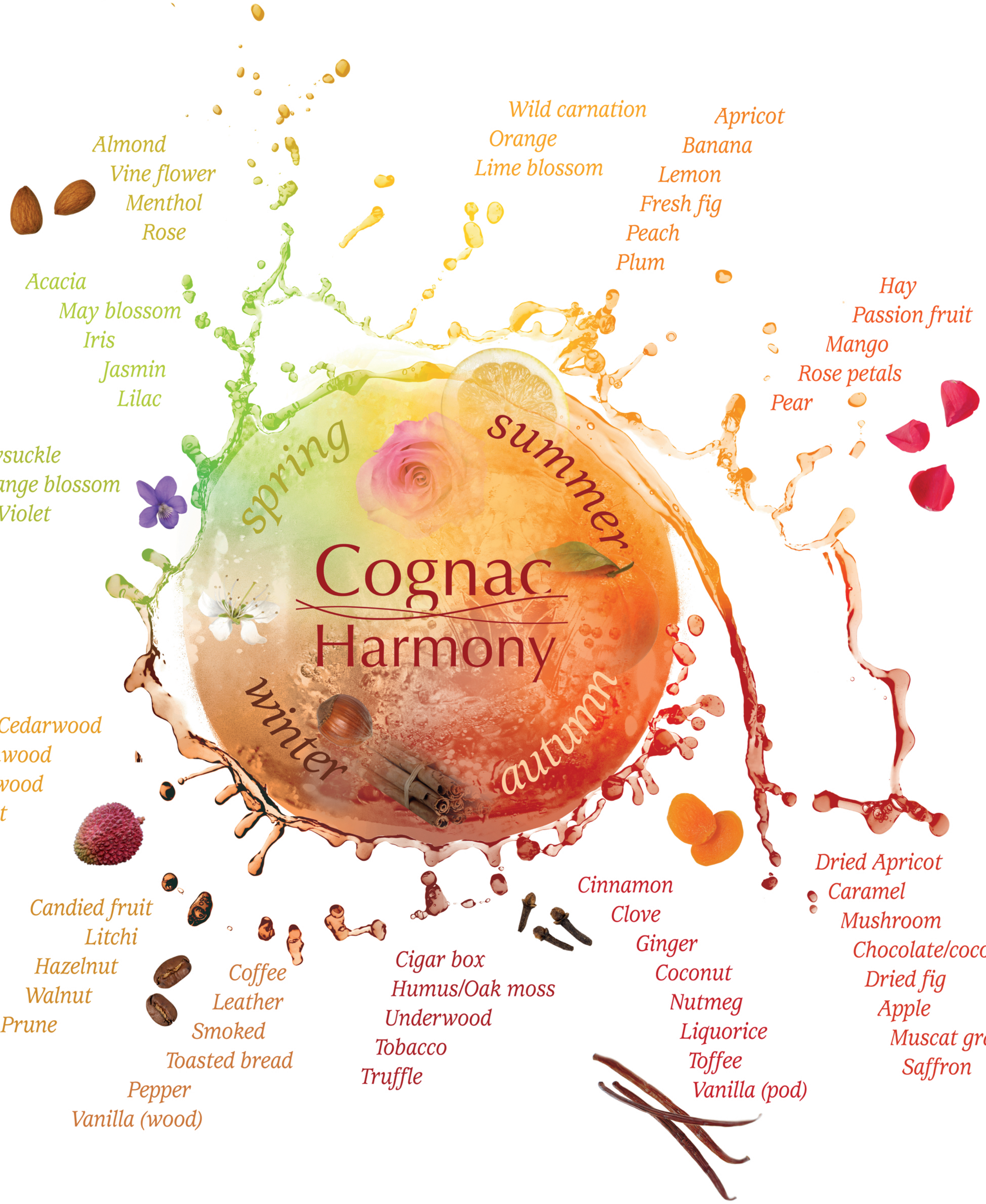

Global whiskies
Scotland
Blended Whisky
Blended Malt
Blended Grain
Single Grain
Single Malt
Ireland
Blended Whiskey
Pure Pot Still
Single Grain
Single Malt
Japan
Blended
Single Malt
USA
Bourbon
Tennessee
Corn
Other
Spain
Sweden
France
Wales
Canada
India

Fig 16.3


Washback
Wash Charger
Washpreheater
Washstill
Pot Ale Receiver
Pot Ale
Washsafe
Low wine/ Feints receiver
Spent Lees
Spirit still
Low wines and feints safe
Intermediate spirit receiver
Warehouse maturation

The Cognac Aroma Wheel

Principal aromatic notes



Almond
Vine flower
Menthol
Rose

Acacia
May blossom
Iris
Jasmin
Lilac

Butter
Honeysuckle
Orange blossom
Violet

Wild carnation
Orange
Lime blossom

Apricot
Banana
Lemon
Fresh fig
Peach
Plum

Hay
Passion fruit
Mango
Rose petals
Pear

Dried Apricot
Caramel
Mushroom
Chocolate/cocoa
Dried fig
Apple
Muscat grape
Saffron

Cinnamon
Clove
Ginger
Coconut
Nutmeg
Liquorice
Toffee
Vanilla (pod)

Cigar box
Humus/Oak moss
Underwood
Tobacco
Truffle

Coffee
Leather
Smoked
Toasted bread
Pepper
Vanilla (wood)

Candied fruit
Litchi
Hazelnut
Walnut
Prune

Cedarwood
Oakwood
Sandalwood
Orange zest

Cognac's palette fits the seasonal cycle perfectly, symbolizing its aromatic wealth.


a
b
c
d
e
f
g
h
i
j
k