Chapter 15

Production of tequila from agave: historical influences and contemporary processes

M. Cedeño Cruz and J. Alvarez-Jacobs
Tequila Herradura, S.A. de C.V. Ex-Hda San Jose del Refugio, Amatitán, Jalisco, México

Introduction: Agave beverage alcohol products

Tequila is classically associated with Mexico, particularly with Jalisco, a state located in the west of the country. This beverage is obtained by distillation of fermented juice from only the agave plant *(Agave tequilana* Weber var. *Azul)* if 100% agave tequila is required. Fermentation is carried out by inoculated strains of *Saccharomyces cerevisiae* or in some cases by a spontaneous process. Up to 49% (w/v) sugars may come from a source other than agave, usually sugar cane or corn syrup, if 100% agave tequila is not required. This tequila could not be labeled as 100% agave tequila.

Different kinds of tequila are produced in 50 registered companies in Jalisco. The tequila products made by these companies differ mainly in proportions of agave used, production processes, microorganisms used in the fermentation, distillation equipment used and the maturation and aging times. The product known as 'silver' or white tequila must be distilled to a concentration not exceeding 55% alcohol in volume (v/v) and not less than 38% (v/v) from a fermented wort containing not less than 51% sugars from the agave plant. 'Gold' tequila is the white product to which caramel color (generally) has been added. Rested tequila (Reposado) and aged tequila (Añejo) are white tequila matured in wood containers, preferably oak casks, for at least 2 and 12 months, respectively, according to present regulations. In practice, the aging period is normally longer and depends on the characteristics each company wants to give to the final product. The products known as 100% agave, which are becoming more popular, could be white, rested or aged and are distilled from fermented wort with only agave as a source of sugars. The labels of these products must indicate that they were obtained using only agave and under Mexican government supervision.

Tequila is differentiated from the beverage known as 'mezcal' by the type of agave used in its elaboration. Mezcal is made from *Agave potatorum,* which grows in the state of Oaxaca. Most mezcal producers use a rudimentary fermentation and distillation process (Sánchez, 1991). There is no technical reason for, or any improvement in the organoleptic characteristics from, the worm inside the bottles of some mezcal brands. The worm is primarily a commercial ploy. Worms are grown in agave plants and introduced manually in the bottling line.

'Pulque' is another beverage obtained by fermentation of the juice obtained from several species of agave, *A. atrovirens* and *A. salmiana* among others, by a complex succession of yeast and bacteria that produce ethanol, a diversity of chemical compounds, and some polymers that give a sticky consistency to the final product (Rzedowski, 1978; Sánchez Marroquin and Hope, 1953). Pulque is sometimes mixed with fruits or vegetables, but has poor stability as it is neither distilled nor pasteurized.

Agave plants still serve as food in some states of México; and other fermented regional beverages are produced (e.g., 'Sotol' in the state of Chihuahua and 'Bacanora' in the state of Sonora), but only tequila and more recently mezcal have reached international recognition. Another difference between tequila and mezcal and all other regional drinks is that both are subject to an official standard that for tequila is NOM006-SCFI-1993 (Secofi, 1993), and production is supervised by the Mexican government.

Origin and history of tequila

The word 'tequila' is believed to originate from the tribe of *ticuilas* who long ago inhabited the hillside of a volcano bearing the same name located near the city of Tequila. Another possible origin is the Nahuatl word *tequitl,* which means *work* or *employment*, and the word *tlan,* which means *place*. Therefore 'tequila' would mean *place in which labor or work is done*.

The most ancient information revealing the existence of agave and its different uses is from the era before the Spaniards in several codices preserved to the present time (a codex, from the Latin *codex* meaning board or writing tablet, is a manuscript volume, especially of a classic work or scripture). The most important is the *Tonalmatlnahuatl* codex, which notes that certain tribes had learned to cook agave plants and used them as food and to compensate for the lack of water in desert lands. Also, these tribes discovered that cooked agave when soaked in water would ferment, producing a very appreciated beverage. In fact, this primitive and rudimentary method was used for centuries to produce beverages from agave, considered a sacred plant possessing divine properties. In other codices, such as *Nutall, Laud, Borgia* and *Florentine,* there are many references to uses of the agave plant for soap manufacture, a source of fiber, footwear, medicine and sewing needles as well as thread, paper and rope. In fact, Indians could distinguish the different species of agave by color, size, stem, leaf width and the different uses given each plant (Muria, 1990). The great religious importance of agave was apparent in those codices, as only warriors and priests used fermented drinks in ritual ceremonies.

In prehispanic México the general name for all species of agave (or mezcal as it is also known) was *Metl,* which is a representation of the goddess *Mayahuel*. The alcoholic drink produced was called *Iztac octli* (white wine). The first Spaniards to arrive in México referred to the plant as 'maguey', a name used for an identical plant they had seen in the Caribbean Islands, where they first encountered new world plants and animal life (Bottorff, 1971). It was not until arrival of the Spaniards, who brought knowledge of distillation techniques, that tequila took its present form (Goncalves, 1956).

There are only two main regions for tequila production in México. The oldest, the Tequila-Amatitán region that comprises the Amatitán village, developed at the end of the seventeenth century. The second region, the Jalisco Highlands, appeared in the last decade of the 19th century (Luna, 1991). The first tequila production process with a commercial purpose was established in the city of Tequila around the end of the 18th century. The main consumers were in the mining zones located in the state of Jalisco. The Spaniards tried to suppress consump-tion of tequila in order to reduce competition for brandy and other wines imported from Spain with a decree signed by Carlos III forbidding its sale and production under the pretext that its consumption was the cause of several illnesses.

The results were negative. The governor of the region later issued a decree imposing a tax on tequila in order to enrich the royal coffers, thus permitting its sale in all of New Spain. By the end of the 19th century, expansion of the tequila industry, helped by the railway, was evident. However it was not until the first casks were exported to the US that tequila was known beyond México's borders.

The Agave plant

According to Granados (1985), the genus *Agave,* which means 'noble' in Greek, was defined by Linneaus in 1753 when he described the plant *A. americana* as the first agave species known to science. Agave plants, which are often confused with cacti, belong to the family Agavaceae and are succulent plants with spirally-arranged leaves forming a rosette. Some have definite trunks, but more often they are nearly stemless. The leaves are bluish green in color, over 1 m long in mature plants, and end in a sharpened brown thorn. As Backman (1944) pointed out, the widespread distribution of some 300 species, combined with the fact that the plants require approximately 8-12 years to mature and hybridize very easily, make the taxonomic and phylogenetic study of the genus *Agave* extremely complicated and very difficult. The family Agavaceae includes 20 genera and nearly 300 species. Of these, around 200 are found in México.

Several agave species are important from an economic point of view. Fiber derived from *A. foucroydes,* grown in the state of Yucatán, is known worldwide for its use in producing ropes and carpet. More recently, the pulp remaining after removal of the fiber has found use in animal feed. *A. salmiana* and *A. atrovirens* are valued for pulque production and *A. potatorum* for mezcal production. Finally, *A. tequilana* Weber var. *Azul,* named around 1900 by the German botanist Weber (Diguet, 1902), is used to produce tequila.

Cultivation and harvest

The blue agave, as it is known, is the only species out of hundreds of Agavaceae with the appropriate characteristics for tequila production. These include a high inulin concentration, low fiber content and the chemical compounds present in the plant that contribute to the final taste and flavor of tequila to give the beverage its particular character. Some have attempted to produce tequila in other states with other agave species, but without success. The blue agave is cultivated in the state of Jalisco in the two regions with the right climate and soil composition for its growth, namely the Highlands and the Tequila-Amatitán region. The temperature conditions for good agave yields are a minimum of 3^0C, an optimum of 26^0C and maximum of 47°C. Soil should be fertile but not very deep, 30 to 40 cm. Good drainage is required to avoid effects of flooding, which are very harmful for development of the agave. The plants must be planted at 800 to 1700 m above sea level where the annual rainfall is about 800 to 900 mm. The correct planting time is immediately before the rainy season, from June to September, so that the plants do not suffer from water stress during the first year of growth.

Propagation is accomplished by the vegetative route in agave. Sexual reproduction via seeds is not usual. Asexual bulbils develop in the inflorescence at the base of the flowers, producing small plants that after some time detach themselves from the floral peduncle and fall to the soil where they root. Another mode of asexual propagation is by suckers, which are a characteristic type of lateral bud or branch developing at the base of the main stem. Plants developing near each mother plant are separated at the age of 3-4 years. These baby plants are called 'first-class seed' because they are better and healthier (Sánchez, 1991). In practice, people use the word 'seed' to refer to such young plants, but from a botanical point of view these are rhizome shoots or suckers (Valenzuela, 1992). Plant cell culture is used experimentally by some agave producers, but is not being used commercially due to unavailability of trained technicians and laboratory facilities to small agave producers. Some developments are being carried out to improve agave plants, or to obtain plants resistant to pests; and these new plants will be ready in the next two years (CIATE, 1993).

Land for agave cultivation must be cleared and deep-ploughed, sometimes twice. Agave is planted approximately 2-4 m apart in straight lines called ruts. Sowing is done by hand in holes 15 cm in depth. Plant density is around 2000-4000 plants per hectare, depending on the plantation system used; and yields can be between 30,000 and 200,000 kg/ha, assuming that the weight of a harvested plant varies from 15 to 50 kg. This variation is caused by differences in soil conditions, quality of plants sown, rainfall, pests and fertilization. Sometimes agave is sown intercalated with nitrogen-fixing crops such as peanuts, beans, chickpeas or soybeans. After the agave has been in the soil for a year, visual inspection is carried out to replace sick or dead plants with new ones. This operation is called re-seeding. The percentage of dead plants depends on soil and plant characteristics, but is generally from 8 to 15% (Pérez, 1990).

Agaves regularly host borer insects that live in the stems, leaves and fruits during the larval stage. These include butterflies of the family Megathymidae and moths of the family Prodoxidae. Also, the fungi *Diplodia theo-bromae* and *Colletotrichum agavae* may cause serious damage to agave leaves (Halffter, 1975; Agricultural Research Service, 1972).

Ehrler (1967) discovered that unlike stomata in most plants, agave stomata close during the day and open by night. This prevents loss of water through transpiration during hot daylight hours, although it results in a hotter leaf surface than most plants could tolerate. The thick cuticle overlying the epidermis, which is quite evident in tequila agave, apparently prevents damage to the leaf from high temperature. This waxy cuticle produces turbidity in tequila because it dissolves in the distillation step and produces a haze in the final product when it is diluted or cooled. One way to avoid this is to treat tequila with activated charcoal and to filter it through pure cellulose filter pads. This, unfortunately, results in the loss of some aromas.

Agave fertilization is based on soil composition, plant age, and the type of chemical compound used. The normal procedure is to use urea as a nitrogen source in amounts of 30-70 g per plant added directly into the soil. In some areas, phosphorus and potassium fertilization is also required. As some agave regions are also involved in cattle, swine, and chicken breeding, manure is sometimes employed for fertilization (GEA, 1992).

The average maturity time for agave is 10 years. Every year following planting, fields are cultivated to loosen the soil and weed and pest control is carried out. Each plant matures individually. Harvest begins at eight years. The leaves are cut from the base and left in the field to recycle nutrients. The harvested plants free of leaves look like large pineapples and weigh from 20 to 90 kg. They are transported to the distillery. Only the better plants, meaning those of good size and high inulin content (measured as reducing sugars) are harvested; but on the 12th year, all plants remaining (the weakest plants) are cut. These are called 'drag' and are generally discarded. Agave composition varies seasonally, but an average would be (wet basis) 27% (w/w) reducing sugars, a juice content of 0.572 ml/g and a pH of 5.2.

Tequila production

Tequilas differ greatly depending on agave quality and origin (Highlands or Tequila-Amatitán regions). The production process also strongly influences quality of the final product. Some distilleries still employ rudimentary production methods, just as they did several decades ago (Pla and Tapia, 1990). Most companies, however, employ technological advances that improve process efficiency and consistency; and some have implemented a ISO-9000 standard. Whatever processes are used, tequila manufacture comprises four main steps: cooking, milling, fermentation and distillation.

Processing harvesting agave

Agave is transported from the fields to the factories as soon as possible to avoid weight losses, because today most distilleries pay by weight and not by inulin content. The heads are unloaded from the truck in the receiving area of the factory and must be protected from the sun and rain in order to avoid withering and fungal growth. Although the agave has already been inspected during growth and at harvest, it is examined again to reject visually unacceptable plants or those damaged by pests. At this point, a representative sample of agave is taken for laboratory analysis. Modified AOAC (1990) procedures are used to determine reducing sugar content (after acid hydrolysis of inulin) along with pH, moisture, dry weight, juice and ash content.

Agave heads usually weigh between 20 and 60 kg, although some can reach 100 kg. They are cut to sizes that facilitate uniform cooking and handling. Different agave cutting systems exist, but the use of axes and a specialized tool called 'coa' are the most popular. The heads are cut in halves or quarters, depending on the weight, and the pieces are arranged manually in an oven or autoclave. Band saws can also be used to cut the agave heads. Band saws are faster and less labor-intensive than the manual procedure, but the belts break frequently because of the resinous consistency of agave. Some factories tear uncooked agave first with a knife and place the resulting pieces mixed with water into autoclaves to be cooked.

Other raw materials for tequila production

When producing 100% agave tequila, the only source of carbohydrate is the inulin hydrolyzed from agave in the cooking step. For other kinds of tequila the law permits the use of other sugars in amounts of up to 49% by weight in wort formulation. There are no legal specifications regarding the type of adjunct sugar sources to be used in tequila manufacture; and theoretically any kind of fermentable sugar can be used for the formulation of wort. In practice and from an economical point of view, only cane sugar, *Piloncillo*, cane molasses and acid- or enzyme-hydrolyzed corn syrup are employed. Cane sugar is received in 50 kg bags and stored in a dry, cool place for subsequent utilization. *Piloncillo* consists of brown cones of crystallized complete cane juice, sometimes individually wrapped in corn or cane leaves and packed in sacks. Cane molasses is also used but it is difficult to handle and there is risk of spoilage over a long storage period. Acid- or enzyme-hydrolyzed corn syrup may be used to formulate the wort. All sugars used in wort formulation are routinely analyzed by measuring solids content and reducing and fermentable sugar content.

The cooking step: hydrolysis of inulin

Cooking the agave serves three purposes. First, the low pH (4.5) together with the high temperature hydrolyze inulin and other components of the plant. The correct composition of these compounds is still unknown. In addition, cooked agave has a soft consistency that facilitates the milling operation.

In the pre-Hispanic era, agave cooking was carried out in holes filled with stones heated using wood for fuel. The stones retained the heat for the time needed to cook the agave. Nowadays some distillers have replaced the stuffed stone holes with brick ovens and heating is conducted by steam injection after the cut, raw agave has been introduced into the oven. Oven cooking is slow, and steam injection lasts around 36-48 hrs to obtain temperatures of 100 ^{0}C. After that period, the steam is shut off and the agave is left in the oven for a further two days to complete the cooking process. During this step, a sweet liquid called 'cooking honey' is collected and used later as a source of free sugars, mainly fructose. Also during this step some of the sugars are caramelized; and some of the compounds that contribute significantly to the aroma and flavor in wort formulation are due to its high content of fermentable sugars (>10% w/v). Finally, the oven door is opened to allow the cooked agave to cool. The agave is then ready for milling.

In most distilleries brick ovens have been replaced by steel autoclaves. Autoclaves have superior efficiency and allow good pressure and temperature control, enabling a homogeneous and economic cooking. In a typical autoclave cooking operation, steam is injected for 1 hr so that the condensed steam washes the agave. This condensed liquid is called 'bitter honey' and is discarded because it contains waxes from the agave cuticle and has a low sugar content (<1 % w/w). Steam is injected for an additional 6 hrs to obtain a pressure of 1.2 kg/cm^2 and a temperature of 121 °C. At the end of that time the agave remains in the autoclave for another 6 hrs without additional steam, cooking slowly in the remaining heat. This step produces a syrup with a high sugar concentration (>10% by weight) that is later used to formulate the initial wort. To calculate the yield and efficiency of this step, the amount of cooking honey and its reducing sugar content as well as the cooked agave are measured.

The main difference between autoclaved and oven-baked agave is that careful control of cooking time, temperature and steam pressure must be maintained in autoclaves to prevent overcooking or burning the agave. Overcooking gives a smoky taste to the tequila, increases the concentration of furfural in the final product and reduces ethanol yield due to the caramelization of some of the fermentable agave sugars. This is why some factories with both cooking systems reserve the ovens for their better-quality products. Although it is easier to obtain well-cooked agave in an oven than in an autoclave, there is no difference in terms of flavor and fermentability between agave cooked in autoclaves or in ovens if both are correctly controlled.

Extraction of agave juice: milling

Milling has gone through three historical stages. In ancient days cooked agave was crushed with wood or steel mallets to extract the juice. Later, a rudimentary mill consisting of a large circular stone 1.3 m in diameter and 50 cm thick was used. Driven by animals, the stone turned in a circular pit containing cooked agave and extracted the juice. The resulting juice was collected by hand in wood basins and carried to fermentation tanks. By the 1950s modern systems were implemented in which cooked agave was passed through a cutter to be shredded (except in factories that did this operation before cooking); and with a combination of milling and water extraction, sugars were extracted. The mills used for agave are similar to those used in the sugarcane industry but are smaller in size (normally 50 cm wide). This system is still employed in most distilleries.

Juice obtained in milling is mixed with the syrup obtained in the cooking step and with a solution of sugars, normally from sugarcane (if the tequila to be produced is not 100% agave), and finally pumped into a fermenter. Although the amount of sugar employed as an adjunct is regulated by law and must be less than 49% by weight at the beginning of the fermentation, each factory has its own formulation.

The milling step generates a by-product called bagasse, which represents about 40% of the total weight of the milled agave on a wet weight basis. Bagasse composition (dry weight basis) is 43% cellulose, 19% hemicellulose, 15% lignin, 3% total nitrogen, 1% pectin, 10% residual sugars and 9% other compounds. The bagasse, mixed with clay, is used to make bricks; but it is also the subject of research to find alternative uses. Examples are use of bagasse as an animal feed or as a substrate on which to grow edible fungi. Attempts are also underway to recover its components (cellulose, hemicellulose and pectin) using high-efficiency thermochemical reactors (Alonso *et al.*, 1993), to obtain furfurals, make particle board, or enzymes (cellulase and pectinase). Many of these projects are at the laboratory stage, and there is not enough information to evaluate feasibility.

In milling, as in all steps of the tequila process, a sugar balance is computed to determine the yield. If the yield decreases, the extraction pressure in the mill and the water/agave ratio are increased to improve efficiency.

Fermentation

Wort formulation

To produce 100% agave tequila, only agave may be used and the initial sugar concentration ranges from 4 to 10% w/v, depending on the amount of water added in milling. When other sugars are employed, they are previously dissolved and mixed with agave juice to obtain an initial sugar concentration of 8-16%, depending on sugar tolerance of the yeast strain. Wort formulation in most of the distilleries is based solely on previous experience. A few distilleries base wort formulation on composition of raw materials and nutritional requirements for yeast growth and fermentation. In these distilleries response surface methodology is the preferred method to optimize nutrient concentrations, using fermentation efficiencies and the taste of the resulting tequila as responses (Montgomery, 1984). To complement nutritional deficiencies of agave juice and sugars employed in the growth and fermentation steps, urea, ammonium sulfate, ammonium phosphate or magnesium sulfate could be added. Because the pH of the agave juice is around 4.5, there is no need for adjustment and the same wort composition is used for both inoculum growth and fermentation.

Yeasts

Some companies do not inoculate a specific strain of S. *cerevisiae* and instead allow natural fermentation to proceed. Others inoculate the wort with fresh packages of baker's yeast or a commercial dried yeast to obtain initial populations of 20-50 x 10^6 cells/ml. The dried yeasts were originally prepared for wine, beer, whisky or bread production; and sometimes the quality of the tequila obtained using these yeasts is not satisfactory, with large variations in flavor and aroma. To achieve high yields and maintain a constant quality in their tequila, some companies have been using yeast strains isolated from a natural fermentation of cooked agave juice. Nutrients are added; along with special conditions such as a high sugar concentration or temperature. These isolated and selected yeast strains have been deposited in national microbial culture collections, the most important being the Biotechnology and Bioengineering Department Culture Collection of CINVESTAV-IPN, located In México City.

Inoculum growth

When an inoculum is used, it is grown in the laboratory from a pure culture of a strain of *S. cerevisiae* maintained on agar slants in lyophilized form or frozen in liquid nitrogen. All laboratory propagation is carried out under aseptic conditions using a culture medium with the same ingredients used in the normal process but enriched to promote cellular growth. The inoculum is scaled-up with continuous aeration to produce enough volume to inoculate fermentation tanks at 10% of the final volume. Populations of 200-300 x 10^6 cells/ml are normally achieved. Strict cleanliness is maintained in this step as bacterial contamination is highly undesirable. When contamination is detected, antibiotics or ammonium bifluoride are used as antimicrobial agents. Once an inoculum is grown, it is maintained by mixing 10% of the volume of an active culture with fresh agave juice and nutrients. Although inoculation with commercial yeast greatly improves yield and turnover time, some companies prefer a more complex (in terms of the microbial diversity) fermentation. While yields might be lower and turnover time higher, the range of microorganism produces more compounds contributing to a more flavored tequila. It is also important to recognize that a change in taste and flavor could negatively affect the market for a particular brand of tequila.

Fermentation of agave wort

Once a wort is formulated with the required nutrients and temperature is around 30 °C, it may be inoculated with 5 to 10% (volume) of a previously grown *S. cerevisiae* culture with a population of 100-200 million cells/ml. Otherwise, microorganisms present in the wort carry out the fermentation. If an inoculum is not added, the fermentation could last as long as seven days. With an inoculum the fermentation time ranges from 20 hrs in the faster process to three days in the slower one.

Production of ethyl alcohol by yeast is associated with formation of many fermentation compounds that contribute to the final flavor of the tequila. These are organoleptic compounds or their precursors produced either in subsequent maturation of the wort before it goes to the distillation step, in the distillation process, or in the barrels if tequila is aged. The factors influencing formation of the organoleptic compounds in alcoholic beverages have been reviewed by many authors (Engan, 1981; Berry, 1984; McDonald *et al*., 1984; Ramsay and Berry, 1984; Geiger and Piendl, 1976). Experience in the tequila industry is that the amount of organoleptic compounds produced is lower in fast fermentations than in slow fermentations. As a consequence, the flavor and general quality of tequila obtained from worts fermented slowly is best. The rate of fermentation depends mainly on the yeast strain used, medium composition and operating conditions. The wort sugar content decreases from an initial value of 4-11% to 0.4% (w/v) reducing sugars if an efficient yeast strain is employed. Otherwise, the residual sugar content could be higher, increasing the production costs.

Fermentation vessels vary considerably in volume, depending on the distillery. Their capacity ranges from 12,000 liters for small tanks to 150,000 liters for the largest ones; and they are constructed of stainless steel in order to resist the acidity of the wort. Ethanol production can be detected almost from the onset; and a pH drop from 4.5 to 3.9 is characteristic of the fermentation. The alcohol content at the end of fermentation lies between 4 and 9% v/v, depending on the initial sugar concentration. Alcohol losses may be significant because many fermentation tanks are open, allowing evaporation of alcohol with carbon dioxide. Some of the largest distilleries have a cooling system that keeps fermentation temperature within a tolerable range for yeast, but small producers do not have these systems. The fermentation temperature can exceed 40 °C, causing the fermentation to stop with an accompanying loss of ethanol and flavors that consequently decreases yields and affects the quality of the tequila. Fermentations carried out with pure agave juice tend to foam, sometimes requiring the addition of silicones. In worts with added sugars, foaming is usually not a problem.

Non-aseptic conditions are employed in fermentation, and in consequence bacterial activity may increase. The size of the bacterial flora depends on a number of factors including the extent to which bacteria grow during yeast propagation (if used), the abundance of bacteria on the raw materials and hygiene standards in the distillery. There is no doubt that the activity of these bacteria contributes to the organoleptic characteristics of the final product. Occasionally, the size of the bacterial population in fermenting wort may become too large (>20 x 10^6 cells/ml), in which case the bacteria use the sugars, decreasing ethanol yields and sometimes excreting undesirable compounds. The same compounds used in the propagation step may be used here to decrease common bacterial contaminants found in tequila worts. Lactobacillus, Streptococcus, Leuconostoc, and Pediococcus are the most common contaminants, but Acetobacter may be found in fermented worts that are left inactive for a long time prior to distillation.

In contrast to other distilled beverages, the organoleptic characteristics of tequila come from the raw material (cooked agave) as well as from the fermentation process. In most of the processes used in the tequila industry, fermentation is spontaneous with the participation of microorganisms from the environment, mostly yeasts and a few bacteria. This peculiarity brings about a wide variety of compositions and organoleptic properties. However, the special characteristics of the wort make it a selective medium for the growth of certain kind of yeasts such as *Saccharomyces* and to a lesser extent, acetic and lactic bacteria. In experiments isolating microbial flora from musts of various origins, different microorganisms were isolated. In most cases, this difference in flora is responsible for the wide variety of organoleptic characteristics of tequila brands (Pinal, 1999). Where a single purified strain is used, the final flavor and aroma are more neutral since the bouquet created by the contribution of several stains is richer than that obtained from only one type of yeast. Moreover, when yeast produced for bakery applications is used, the final product is also more neutral. It is also recognized that when non-100% agave tequila is made, a poorer bouquet is obtained because since a more defined medium yields a more defined product.

Organoleptic compounds generated during fermentation

Fusel oil

As in many other alcoholic fermentation processes, higher alcohols are the most abundant compounds produced along with ethanol. We have found (in decreasing order of abundance) isoamyl alcohol, isobutanol, active isoamyl alcohol and phenylethanol. It has been established that production of isoamyl and isobutyl alcohols begins after the sugar level is lowered substantially and continues for several hours after the alcoholic fermentation ends. In contrast, ethanol production begins in the first hours of the fermentation and ends with logarithmic yeast growth (Pinal *et al.*, 1997).

The most important factor influencing the amount of isoamyl alcohol and isobutanol is the yeast strain. It was found that a native strain isolated from tequila must produces a higher amount of such compounds when compared with a strain usually employed in bakeries. These results agree with those reported for Scotch whisky (Ramsay and Berry, 1984) and beer (García *et al.*, 1994).

The carbon:nitrogen ratio also has a significant influence on higher alcohol production. In tequila musts, which contain mainly fructose (≈95%) as a carbon source and an inorganic nitrogen source (ammonium sulfate), it was found for both native and bakery yeast strains that low carbon:nitrogen ratios result in low amounts of isoamyl alcohol: 19 mg/L in bakery strains and 30 mg/L for native strains *vs* 27 and 64 mg/L, respectively, for high carbon:nitrogen ratios. A similar relationship exists for isobutyl alcohol production (Figures 1 and 2).

Temperature is a third factor affecting isobutyl and isoamyl alcohol production with higher temperatures (e.g. 38 *vs* 32°C) yielding higher concentrations of those alcohols. On statistical analysis it was found that in addition to the direct effects of yeast strain, carbon:nitrogen ratio and temperature, the interaction of these three factors also had an impact on higher alcohol concentration in tequila (Pinal *et al.*, 1997). These results are consistent with the fact that with high carbon:nitrogen ratios there is a tendency to use amino acids as a nitrogen source, which implies the production of fusel oil as a by-product (by the Erlich pathway). On the other hand, variables such as the type of nitrogen source (urea or ammonium sulfate) or the amount of inoculum used for fermentation had little or no effect on the production of higher alcohols. Pareto diagrams involving all the variables tested are depicted in Figures 3 and 4.

Methanol

Another characteristic compound present in tequila is methanol. It is the general idea that

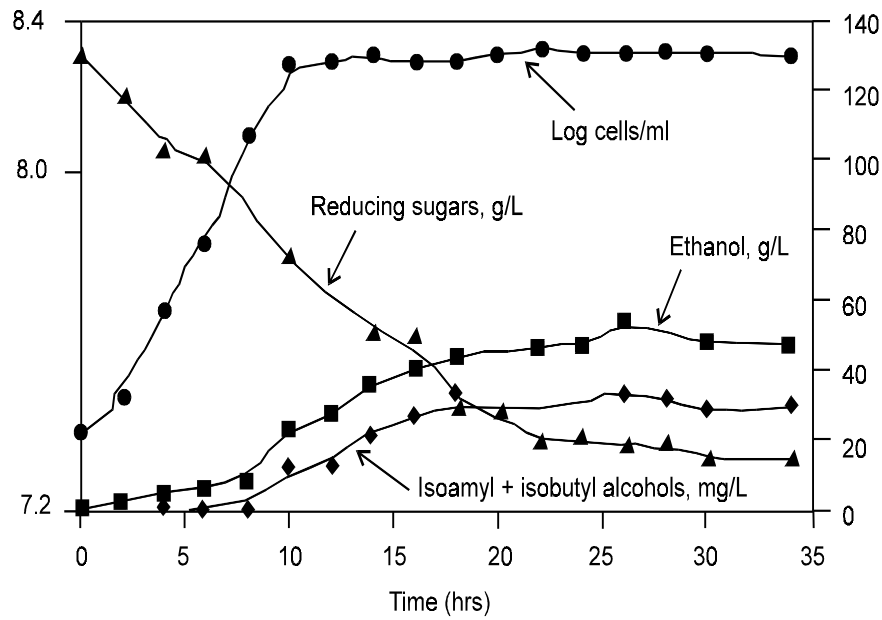

Figure 1. Higher alcohol production in tequila wort by a bakers yeast strain.

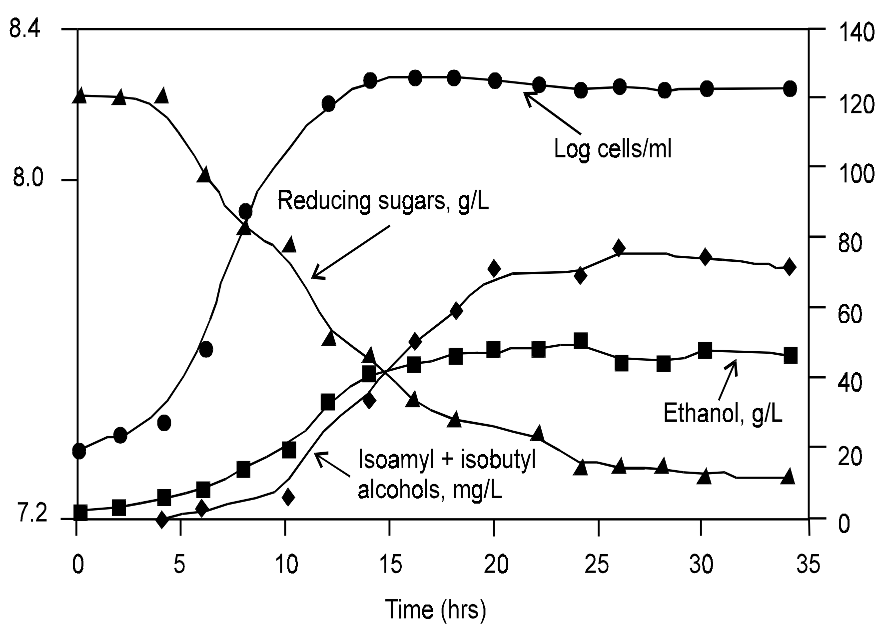

Figure 2. Higher alcohol production in tequila wort by a native yeast strain.

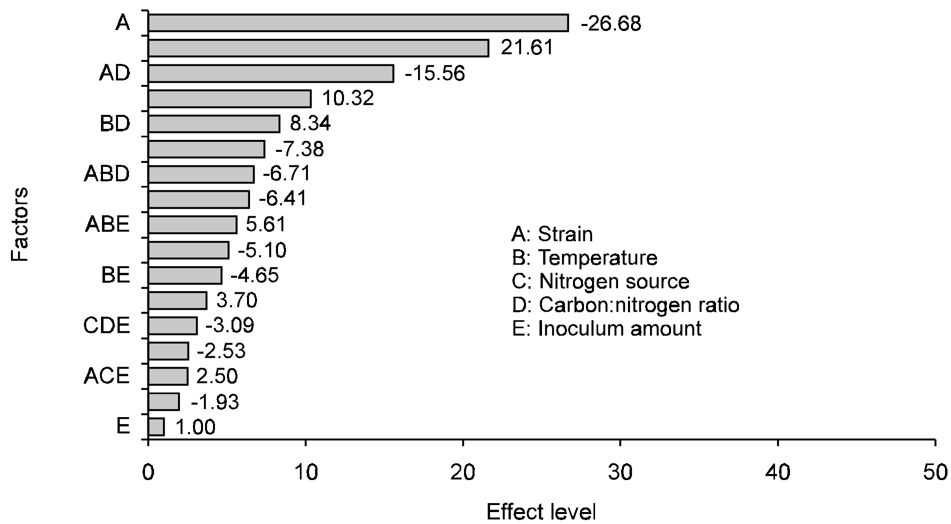

Figure 3. Pareto diagrams for isoamyl alcohol production in tequila.

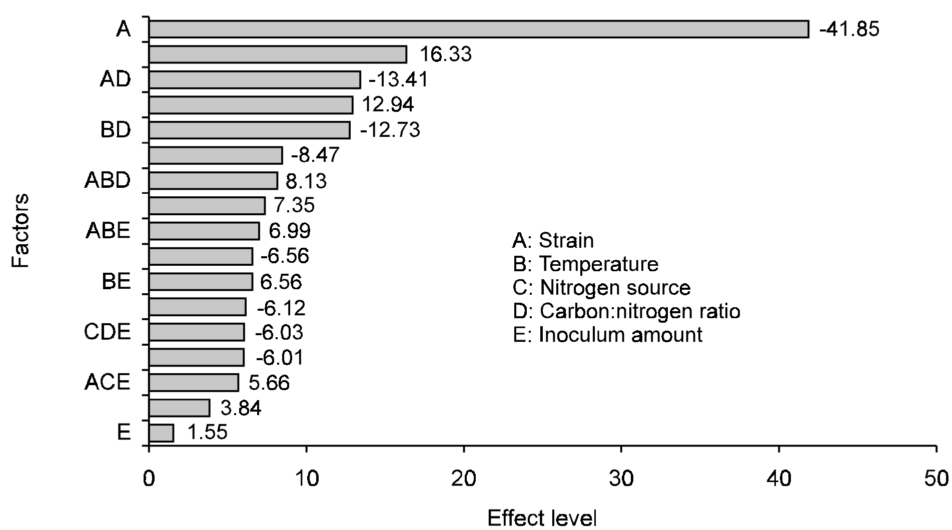

Figure 4. Pareto diagrams for isobutyl alcohol production in tequila.

methanol is generated through hydrolysis of methylated pectins present in the agave plant. Nevertheless, it is also believed that some yeast strains, natural or inoculated, have pectin methyl esterases. Some authors describe various amounts of methanol in musts with the same composition but fermented with different strains (Téllez, 1999).

Aldehydes

Along with the production of ethyl acetate, the oxidation of ethanol also generates acetaldehyde, an intermediate in the production of acetic acid. It is well known in commercial practice that an oxidation process instead of fermentation begins after the sugar concentration declines, thus provoking the increase in the acetaldehyde level. However, there is no formal report of such phenomena in tequila, as it is described in beer (Hammond, 1991).

Organic acids

Small organic acids (up to six carbons) and larger molecules (fatty acids) are produced during fermentation. The smaller molecules can be products of intermediate metabolism of the normal microbial flora; and their production depends on the presence of oxygen. The larger fatty acids are synthesized for membrane structures during cell growth and can also appear at the end of the fermentation when lysis takes place. The presence of octanoic and decanoic acids in the final product has been described particularly for tequila.

Esters

Esters are very important compounds in their particular contribution to flavor and aroma, since they have the lowest organoleptic threshold values (Ramsay and Berry, 1984). In particular, ethyl acetate is the most abundant and important compound of this family. Ethyl acetate has been reported to be the second most abundant compound in tequila after isoamyl alcohol. The quantity of this compound present in the final product can vary widely, since it is synthesized from acetic acid (in form of acetyl-CoA) and ethanol. Acetic acid can also be produced by the oxidation of ethanol when the fermentation has ceased and an oxidative process starts on the surface of the fermentation tank by *Saccharomyces* and many other yeasts such as *Brettanomyces*. Therefore, long fermentation periods (a current practice in the tequila industry) yield high ethanol oxidation. In addition, in open fermentation tanks with worts at low pH containing alcohol, ethanol is also transformed to acetic acid (itself a precursor of ethyl acetate) by bacteria of the genera Acetobacter. Besides ethyl acetate, the presence of several other esters has been described including ethyl and isoamyl esters. Some of the most important esters found in silver tequila are listed in Table 1.

Table 1. Most abundant esters found in silver tequila.[1]

Ester	%
Ethyl acetate	17.77
Ethyl decanoate	2.78
Ethyl lactate	2.74
Ethyl octanoate	1.92
Ethyl dodecanoate	0.95
Ethyl butanoate	0.63
Isoamyl acetate	0.58
Ethyl propanoate	0.57
Ethyl hexanoate	0.48
Ethyl hexadecanoate	0.48

[1]Estarrón *et al.*, 1999.

Distilling

Distillation involves the separation and concentration of the alcohol from the fermented wort. In addition to ethanol and other desirable secondary products, wort contains solid agave particles consisting mainly of cellulose and pectin, and yeast cells in addition to proteins, mineral salts and some organic acids. Although a great number of types and degrees of distillation are possible, the most common systems used in the tequila industry are pot stills and rectification columns. The pot still is considered the earliest form of distilling equipment. It is of the simplest design, consisting of a kettle to hold the fermented wort, a steam coil, and a condenser or a plate heat exchanger. Pot stills are often made of copper, which ‘fixes’, according to Thorne *et al*. (1971), malodorous volatile sulfur compounds produced during fermentation. Batch distillation using pot stills is carried out in two steps. First the fermented wort is distilled to increase the alcohol concentration to 20-30% by volume, separating out the first fraction called heads, and the last fraction, called tails.

Composition of these fractions varies depending on many factors including the yeast strain employed, wort nutrient composition, fermentation time and distillation technique; but in general, heads are rich in low boiling point compounds such as acetaldehyde, ethyl acetate, methanol, 1-propanol, 2-propanol, 1-butanol, and 2-methyl propanol, which give a very pleasant flavor and taste to tequila. Heads are normally mixed with the wort being distilled. The tails contain high boiling point components such as isoamyl alcohol, amyl alcohol, 2-furaldehyde, acetic acid and ethylactate, giving a strong taste and flavor to the tequila; and when the concentration is above 0.5 mg/ml, the final product becomes unpleasant. This fraction is not used.

In the second step, the liquid obtained from the first stage is re-distilled in a similar pot still in order to obtain a final product that is 110° proof if it is sold in bulk (reducing transport costs) or 80° proof if it is to be bottled. Some companies obtain high proof tequila and dilute it with demineralized water or water purified by reverse osmosis.

In continuous distillation systems, the fermented wort enters the feed plate of the column and flows downward, crossing a series of trays. Steam is injected from the bottom in a coil and strips the wort of its volatile components. Vapors condense higher in the column, depending on component volatility, allowing liquids to be drawn off or recycled at the various plates as appropriate. Sometimes tequila obtained in this way is mixed with tequila from pot stills to balance the amount of organoleptic compounds because in general, tequila obtained through continuous columns has less aroma and taste than tequila obtained from pot stills.

The presence of methanol in tequila is still a subject of discussion because whether methanol is produced only by a chemical reaction or in combination with a microbial hydrolysis has not been satisfactorily demonstrated. The chemical reaction is demethylation of agave pectin by the high pH during cooking and the first distillation steps. The microbial reaction could be the hydrolysis of agave pectin by the enzyme pectin methyl esterase produced by some microorganisms during the fermentation step, but this has not yet been demonstrated. Preliminary results favor the first theory, but some research is still needed on this matter because it is important to maintain the methanol concentration within the limits established by the official standard, which is 300 mg methanol per 100 ml of anhydrous ethyl alcohol.

Effluent disposal

The discharge from pot stills or distillation columns is known as stillage, slops or vinasse; and in a typical tequila distillery 7 to 10 liters of effluent are produced per liter of tequila at 100° proof. Tequila stillage has a biological oxygen demand (BOD) of 25 to 60 g/L. In addition to the dissolved salts (mainly potassium, calcium, and sulfate ions) and the low pH (<3.9) of the stillage, there are significant disposal or treatment problems. A general solution to the disposal problem does not exist because every factory has its own production process and is located either in a city or near agave fields. As a result of the difficulties of treating vinasses and due to their high concentrations of dissolved matter, a host of utilization schemes have been proposed. Some of the methods indicated below are under investigation and others are in use.

Recycling reduces the volume of waste to be treated. Stillage can be recirculated, mixing 5 to 10% of the total volume of the waste obtained with clean water to substitute for the dilution water used to prepare the initial wort. This can be carried out for a number of cycles, usually no more than five, because the concentration of dissolved salts increases and could affect the fermentation process. Also, great care must be taken with the final taste and flavor of the tequila because some components present in stillage could affect the organoleptic characteristics of the final product. Currently, only one tequila company uses this system.

Direct land application as irrigation water and fertilizer in agave fields is under careful evaluation to determine the optimum loading rates and the effects on the agave over the long time it takes to reach maturity. Evaporation or combustion of stilllage could provide fertilizer or potash, but the high cost of such a process is a serious limitation (Sheenan and Greenfield, 1980). The production of biomass and biochemicals including fodder yeast is a possibility, but the remaining liquor still has a high BOD (Quinn and Marchant, 1980). Stillage may be used as a food supplement for cattle, but it has an undesirable laxative effect on animals. Biological, aerobic, or anaerobic treatment offers a real means of disposal, but the cost is likely to be as high as the fermentation costs themselves (Speece, 1983; Maiorella, 1983). Ultimately, tequila vinasses should be viewed as a raw material rather than a waste, and a strategy should be devised that maximizes economic and social benefits and reduces recovery costs.

Maturation

Distillation is the final stage of tequila production if silver or white tequila is the desired product. For rested or aged tequila, maturation is carried out in 200 liter white oak casks or in larger wood tanks. The time legally required is two months for rested tequila and 12 months for aged tequila. Tequila is generally matured for longer periods, depending on the characteristics each company desires for its particular brand.

As tequila ages in barrels, it is subject to changes that will determine its final quality. Thickness and quality of the stave, depth of the char, temperature and humidity in the barrelling area, entry proof (40-110° proof), length of storage and number of cycles for the barrel (in México barrels may be re-used several times) all affect the final taste and aroma of the tequila. Fusel oil content decreases during maturation owing to the adsorbant nature of the char, smoothing the final product. Complex wood constituents are extracted by the tequila, providing color and the particular taste. Reactions among certain tequila compounds yield new components; and oxidation reactions change some of the original components in tequila and those extracted from the wood. As a result of all of these changes, the concentration of acids, esters and aldehydes is increased, while the concentration of fusel oils decreases as tequila reposes in barrels.

After aging and dilution with demineralized water (if necessary) the color of the tequila may be adjusted to the desired value by the addition of caramel. Alternatively, some companies blend different batches of tequila to obtain a standardized final product.

Government inspectors supervise the entire aging process. Prior to bottling, tequila is filtered through cellulose filter pads or polypropylene cartridges. Sometimes afterwards a pretreatment with charcoal is used to eliminate turbidity.

Future developments

Research and future developments in the production of tequila and agave cultivation can take many directions, but the implementation of any change must allow quality of the final product to be maintained and provide substantial improvement in the process. There are several key areas where important developments could take place. Development of new varieties of agave endowed with resistance to pests or extremely dry environments, higher inulin content, low wax content in the leaf cuticle and superior growth rates would be of benefit. Mechanization would improve aspects of cultivation and harvest of agave. In addtition, optimization of the cooking and fermentation steps would improve yields and reduce the amount of waste, while yeast strain selection could improve ability to ferment musts with high concentrations of sugars. Finally, low cost alternatives for waste (bagasse and stillage) treatment are needed.

Summary

Tequila is a beverage obtained from the distillation of fermented juice from the agave plant *(Agave tequilana.* Weber var. *Azul).* The use of agave to produce drinks in México dates from long ago when certain tribes used it for religious ceremonies. It was not until the arrival of the Spaniards, who brought distillation technique knowledge, that tequila acquired its present form. The agave plant, which is generally confused with cacti, is propagated through traditional methods or by means of plant cell culture. It is grown for 8 to 10 years before it can be harvested and sent to the distillery.

The tequila production process comprises four stages. In the first step, agave is cooked to hydrolyze the polymers present in the plant, mainly inulin, into fermentable sugars. In some factories this step is accomplished using stone ovens and in others it is carried out in autoclaves. The second stage is sugar extraction from cooked agave through milling; and the agave juice obtained through this step can be mixed with sugars from other sources, normally sugar cane, if 100% agave tequila is not desired. The third and most important stage is fermentation in which sugars are transformed into ethanol and other compounds such as esters and organic acids. These, along with other substances derived from the cooked agave, give the characteristic flavor and taste to tequila. It is of great importance to have a good yeast strain and nutritionally balanced wort for tequila production, as losses could be as high as 35% of the total production if inefficient yeast is used or nutrients do not appear in the right proportions. In the last stage, fermented wort is distilled, normally using pot stills or in some cases rectification columns, to obtain the final product. At the end of the distillation process white tequila is obtained. Maturation in white oak barrels is required for rested or aged tequila. The minimum maturation times are 2 and 12 months, respectively, for rested and aged tequila as required by government regulations. At every step of the production process, most companies employ several quality control analyses in order to ensure the quality of the product and the efficiency of the process. Some major producers of tequila are certified through an ISO-9000 standard. Tequila production is governed by the official norm NOM-006-SCFI-1993, which must be followed by all tequila producers to guarantee a good quality final product.

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