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Production of a Mexican Alcoholic Beverage: Sotol

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Abstract: Sotol is a distilled alcoholic beverage (similar to Tequila) obtained by fermentation of sotol *Dasylirion* sp. a *Neolinacea* that grows in the semi-desert areas of Mexico. The liquor is obtained by cooking of the central part of sotol plants (known as "pineapples"), which are previously fermented and distilled. This traditional process does not have a quality control program. However, this alcoholic beverage is very popular and economically important. In this research the physiochemical parameters of raw materials and liquor manufacturing process were evaluated; in addition, microorganisms associated to different stages of alcoholic fermentation were isolated and identified. Results indicated an average concentration of 31.9% of reducing sugars in the extracts of cooked pineapples and, 10.15% of reducing sugars, in the fermentation tank, the fermentation temperature was 18-25 ^{0}C, pH was 4.4, soluble sugars content at the initial step was 10 Brix degrees (°BX) and 5 °BX at the final step. After the first distillation, the alcoholic grade was 35 °Gay Lussac (°G.L.). and after a second distillation it was, 46-50 °G.L. Associated to sotol plant were found different bacteria, yeasts and fungi species. However, diversity of microorganisms was diminished after the cooking process, but in the fermentation step, a fungus, 6 bacteria and 2 yeasts were isolated. Microorganisms were identified by traditional and automatic biochemical tests. Future research will be made to select the native *Saccharomyces cerevisieae* strain(s) according to their enological characteristics of the Sotol liquor.

Key words: Sotol, *Dasylirion*, fermentation, traditional process, alcoholic beverage

INTRODUCTION

Sotol is an alcoholic beverage obtained by distillation of fermented musts of heads or pineapples of the plant with the same name sotol or sereque, (*Dasylrion* sp.) of the *Neolinacea* family (Magellan and Hernandez, 2005; Vinomex, 2005). This plant grows abundantly in regions of the Chihuahuan desert. Sotol plant survives under frozen winters so much as to burning summers. This plant is dioecy, perennial, polycarpic and semicylindrical of big size with narrow leaves that leave the center toward the periphery. The oldest leaves do not fall when dying, but rather they are part of the shaft and they help it to be protected. Both sexes have a terminal inflorescence with stamens in male or pistils in female plants (Bogler, 1994). The plant core is not very high and it is known as head or pineapple, they generally have a mass from 20-40 kg in mature plants but pineapples of the oldest plant have a mass of 100 kg or more (Bogler, 1994; Lopez-Barbosa and Carry-Vargas, 2002).

In Mexico, 16 sotol species have been identified, but only some of them have an economic importance, *Dasylirion duranguense*, *Dasylirion cedrosanum* and *Dasylirion wheeleri* are the species more used to produce alcoholic beverages (Contreras and Ortega, 2005). These species cover big extensions, which guarantee their use for many years using sustainable technologies. Sotol plants can live more than 100 years, however it is considered the optimum age to being cut for liquor production is 10 years (Contreras and Ortega, 2005). This plant had a high economic importance through during the history of the Mexican towns located in the semiarid regions. It have been used (due its high carbohydrates content) as food and feed (Rivera, 2005) as ornamental plant in parks and churches, but mostly it is used for production of an alcoholic beverage named Sotol (Notimex, 2002; Coello, 2003; Contreras and Ortega, 2006).

Liquor production is regulated by the Mexican norms NOM-005-RECNAT-1997, NOM-007-RECNAT-1997 (SCFI, 2003) and NOM-159-SCFI-2004 (IMPI, 2002). Also as a protection for the Mexican producing states

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(Chihuahua, Durango and Coahuila) in 2002, the Origin denomination for sotol was established. However, industrial use of this plant must be regulated a sustainable approach which is established in the Mexican Official Norm NOM-159-SCFI-2004 (SCFI, 2003; Buendia and De la Garza, 2005).

Sotol contains simple and complex carbohydrates such as glucose, fructose and a series of fructo-oligosaccharides and fructosyl polymers, which play an important role during the fermentation process. However, also a great amount of other polysaccharides are present and these represent the bagasse residue.

There are not reports about the sotol production (including fermentation and distillation); however, there are documented evidences of the existence of distilleries in the XVII century in the "Nueva Viscaya County", mainly in "Santa María de las Parras", Coahuila, in "Presidio", Chihuahua and "Cedros" Zacatecas (Rivera, 2005).

After the Spanish colonization at Mexico, new processes were developed taking into accunt the experience obtained from wine processing and the distillation. These principles were combined with the pre-Hispanic techniques to elaborate fermented beverages (i.e. Pulque and Tesgüino) generating new alcoholic beverages like Mezcal, Tequila and Sotol.

However, the commercial sotol production has remained without changes during the last 150 years for this reason it is considered as artesian product. One of the main problems for the modernization of the sotol industry is the scarce of scientific information about plant composition, transformation process and microorganisms associated to fermentation (Mancilla-Margalli and López, 2002; Villacencio and Gray, 2005; Buendia and De la Garza, 2005; De la Garza and Aguilar, 2006). The objectives of this study, were to evaluate the traditional production of sotol, measuring the changes in the reducing sugars content during the process and to isolate and identify the microorganisms associated to the different steps for liquor processing to improve the quality, productivity and operation conditions of the process.

MATERIALS AND METHODS

Localization of the traditional production of Sotol: To study the empirical processing, of stool production a small industry located in the agricultural community of Cedros, Zacatecas, Mexico, located at 150 km from Saltillo City (Coahuila State) was selected to evaluate the process steps and conditions. It is important to note that the place is located in a rural region far from the urban zone.

Sugar content of raw material: Plants of *Dasylirion cedrosanum* with an average mass of 35 kg were collected from two different areas (Southeast of Coahuila and North of Zacatecas), plants were collected in valleys and from to mountain altitudes and in different years seasons winter and summer. Diameter and mass of pineapples were recorded. After that, sotol plants were cut, after that leaves and pineapples were removed and transported under refrigeration to the Food Science laboratory, School of Chemistry (UAdeC). Analysis performed were total and reducing sugar contents (Aranda, 2005).

Characterization of the handmade production processing: A flow diagram (Fig. 1) of the process was elaborated to identify the main steps (cooked pineapples, "majado of pineapples", samples of the fermentation cubs and distillation products). Physiochemical measurements were taken at each step: temperature, pH, total and reducing sugar content, soluble sugars as °BX, alcohol content (°G.L.) in distillation step samples. Alcohol content was determined using a hydrometer.

Isolation and identification of microorganisms: Vegetal tissue samples were collected at the follow processing

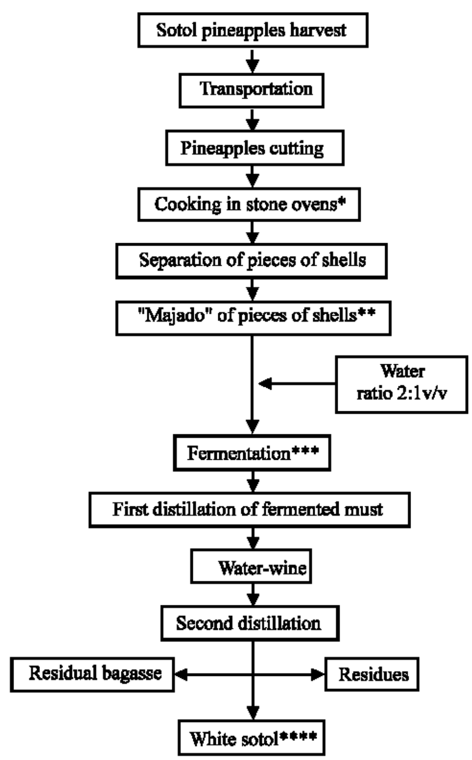

Fig. 1: Handmade production process of the sotol liquor. *3 days of cooking; ** 24°C for 2 days; ***20°C for 4 days

steps: Tissue of sotol plants at the place of collection and cooked pineapples. Pineapples were perforated with sterile drills and a sample of tissue was taken with sterile hyssops, which was inoculated on Petri dishes. These plates contained different culture media according to the group of microorganisms. Nutritive agar was used for bacteria, potato dextrose agar for yeasts and mycological agar for fungi (Norris and Richmond, 1980; Cáceres *et al.*, 2005; Telles *et al.*, 2005). Samples of tissue from cut and "majadas" pineapples were used for microorganisms isolation also in this case the three previously mentioned culture media were employed and samples from the fermentation cubs were taken at the beginning and fourth day of bioprocess and inoculated on Petri dishes with the culture media described above. Isolated Microorganisms were transferred to new Petri dishes, purified and stored as pure strains in slants (Lozano *et al.*, 2005). Morphological characteristics of colonies, such as color, form and texture were evaluated; in addition, pictures of the colonies were taken to determine later other cell characteristics like particular forms and spore presence (Saldivar, 1992).

In addition, for bacteria and yeasts identification, biochemical tests were analyzed using the automatic system Dade Behring MicroScan model auto SCAN 4 CE which was used to determine the bacterial and yeast specie. Monospore cultures of fungi were used to evaluate colonial morphology and were identified by morphological keys. Finally an alcohol production test was performed using only yeast (Saldivar, 1992).

Technological changes proposed: Different tests were carried out to evaluate the effect of technological changes in different production steps like cooking of pineapples, sotol juice extraction and fermentation process to improvement of sotol production.

RESULTS AND DISCUSSION

In the traditional process of liquor production, plants are cut based on diameter instead of mass or plant age. Although, there are not experimental data to determine which is the most appropriate pineapple diameter or mass to liquor production, producers recommend pineapples with a diameter of 30-40 cm and a mass of 30-45 kg. Results showed that pineapple mass was increased when the diameter was higher (Table 1).

Results on sugar content in pineapples harvested at different year seasons showed that the highest sugar content was determined in sotol pineapples harvested during winter months and the lowest sugar content was determined in sotol pineapples harvested during the raining season (June to September). On the other hand, It was observed that sugar concentration is higher in the external part of the pineapple and it diminishes in the center part. While plants that grew at the mountain base have high sugar content in relation to that plants that grew at the top or the middle of the mountain (Table 2).

Table 1: Values of diameter and weight of sotol pineapples

Diameter (cm)	Weight (kg)
18-25	13-16
26-35	20-26
36-45	32-40
46-55	47-56
56-65	66-77

Table 2: Effect of place and season on the sugar content of sotol pineapples

Location	Total sugar content (g L^{-1})	Reducing sugar content (g L^{-1})
Mountain bases		
(Winter)	37.92	14.23
(Summer)	31.23	12.43
Mountain middle		
(Winter)	28.89	7.50
(Summer)	25.14	6.34
Mountain top		
(Winter)	28.92	8.70
(Summer)	24.54	6.25

Sotol liquor is obtained cutting the all plant, after removing leaves, only pineapples are left. Pineapple are cooked to achieve the hydrolysis of its carbohydrates, later they are cut in pieces and left at room temperature for 1-2 days in a process call "majado" (it is a kind of yeast solid state culture of the sotol pineapples pieces). Soon after all pineapple pieces are mixed with 2 fold of water volume and a spontaneous alcoholic fermentation start. In this fermentation only native microorganisms are involved, after that the produced ferment is distilled two times in copper stills and later is packed or diluted with drinking water depending on the required concentration of alcohol. All process is summarized in Fig. 1.

Sotol plants are collected in more and more distant places from the processing areas because no reforestation is carried out. Cut pineapples are terrestrial transported until manufacture factory, at this place pineapples are cooked in stone ovens which are fed with trunks of palms, pineapples are cooked during two-three days with high relative humidity but to atmospheric pressure; after cooking, they are stored until a minimum of 600 pineapples is reached. Cooked pineapples present a dark color in their surfaces due to fire action and a brown color inside as product of the Maillard reactions of their sugars.

The cooked pineapples are cut in small pieces and they are piled in 3 m long and 0.6 m deep tanks. After that, pineapples are cut in smaller pieces, lightly wetting and packed and compacted. Then pineapples piles remain for one or two days, during this time a slight increase of the temperature take place because microbial growth. When,

Table 3: Parameters of the traditional sotol production

Characteristics	Value
Content average of humidity of raw pineapples	11.3%
Content of soluble sugars in the cooked pineapple	31°Bx
Time of having "majado"	2-3 days
Temperature average of the "majado"	24°C
Content of soluble sugars of the extracts of cooked pineapple	Center 18°Bx Periphery 31°Bx
Content of soluble sugars of the extracts of having "majado"	25 °BX
Temperature of fermentation	20°C
pH to the second day of the fermentation	4.4
Time of fermentation	4-5 days
Soluble sugars to the beginning of the fermentation (pineapples with water, in relationship 1:2)	10°Bx
Soluble sugars at the end of the fermentation	5°Bx
Soluble sugars in the residual of the distillation	5°Bx
Content of alcohol in the first distillation	35°GL
Content of alcohol after the second distillation	46-50°GL

finish this temperature increase, it is considered that this step end. This operation is known as "majado". The "majado" is a kind of solid state fermentation in which producers consider as a mean to achieve a polysaccharides saccharification or a cellulose hydrolysis of pineapples "shells". Although, after the analyses were performed, results showed that the "majado" is a form to increase native yeast inoculum and the start of an alcoholic fermentation.

Sotol fermentation is not carried out in a traditional way, where must is obtained from a juice extracted from fruits. In this handmade liquor, must is obtained mixing pieces of "majadas" pineapples, with water in a proportion of 1 volume of pieces of pineapples and 2 volumes of water. After "majado", pineapple pieces are placed in wooden fermentation cubes located at floor level where a natural or spontaneous fermentation take place.

After fermentation ends, cubes content (solids and liquid) is transferred to a copper pot for distillation, heat is generated by burning palm trunks. Distillation chamber is elaborated with wood while the condensation tramp is metallic and it is submerged in a water tank with a continuous water flow. When first distillation start the liquor has an alcohol concentration of 36°G.L. Alcohol concentrations are going down as distillation continues. This distillation is stopped when alcohol concentration reach 12 °G.L. The product of the first distillation is re-distillated again. The average alcohol concentration of the second distillation product is 50 °G.L., after that liquor which is bottled and sold as White Sotol.

Evaluated parameters in situ and during manufacture process allowed having the first scientific knowledge about this handmade liquor production system by example, temperature during "majado" is 24°C but during fermentation is 20°C. Values of °BX decrease during the all handmade processing from 31 % in cooked pineapples to 5% at fermentation end. On the other hand, alcohol concentration increased from 36°G.L. in the first distillation to 50°G.L. in the second distillation (Table 3).

There is a lack of knowledge about influence of microorganisms in sotol fermentation. Microorganism (bacteria, filamentous fungi and yeasts) were isolated during the different processing steps to determine their influence in the liquor characteristics. Samples were collected from the following points:

- *In situ* inside sotol plants.
- Raw pineapples.
- Cooked pineapples.
- "Majado" pineapples.
- Must.

The results showed that due to sotol plant anatomy, a high percentage of humidity is maintained which allow that different microorganism (bacteria, filamentous fungi and yeast) grow. Colony number of microorganisms decreased after cooking of pineapples in the stone ovens. However, it was observed that during "majado", the colonies number of yeasts increased, which were identified with the MicroScan. Two yeasts were identified as *Saccharomyces cerevisieae* (creamy colonies) and another as *Protatheca* sp. (yellow one identified), although it is possible that also other yeast like *Candida glabrata* or *Candida kefir* are involved during sotol fermentation. During "majado" at least 4 types of Gram negative bacteria are involved, they were identified by morphological and biochemical tests as *Bacillus licheniformis*, *Bacillus subtilis*, *Acetobacer* sp. and ***Bacillus deiformis.*** These bacteria were detected in the first steps of fermentation. However, at the end of fermentation only *Saccharomycee cerevisieae* was detected. In addition 11 different fungi were identified in the raw pineapples, but after the cooking, in the "majado" as well during fermentation only was detected the presence a fungus (*Mucor circinelloides*). Some *Mucor* sp. have been reported as pathogens while others as producers of complex compounds, like acetic acid or ethanol (Saldivar, 1992; Huang and Shan, 2007; Millati and Edebo, 2007).

Pineapple cooking in stone ovens is one of the slowest steps in this handmade process, in this study was evaluate the influence of pineapple cooking using high pressure pots, varying the cooking time, but maintaining the temperature to 121°C and cooking with water or with an sulfuric acid solution (1 %). The sugar content was determined in each case (Table 4). Results showed that it is possible to reduce cooking time from 3 days to one hour using high pressure pots. Adding of an acid solution did not increase cooking efficiency.

Fermentation of pineapples pieces mixed with water reduces fermentation efficiency and increase volume of charged to fermentation cubes. In this study was evaluated the use of a roller mill for pineapple juice extraction. Results showed that juice obtained with the roller mill had a high sugar concentration (30%). This juice was diluted with water until to reach 12% of sugar content, pH was adjusted and $(NH_4)_2SO_4$ al 0.1%. Must was sterilized and inoculated with a native *Saccharomyces cerivisieae* strain isolated from sotol and preliminary tests for alcohol production were done.

The sotol is a plant difficult to domesticate and therefore of being cultivated. This study showed that sugar content in sotol pineapples varies depending on year season, as well as through plant and according to geographical growing conditions (Table 2). This is associated to a more fertile soil in the valleys than in the mountain tops. This result may contribute to establish a sustainable management for wild sotol populations and for planning of cropping sotol plantations.

Information of sotol chemical composition is scarce and about changes happening in their chemical compounds during the cooking process in stone ovens and during fermentation. Evaluated parameters *in situ* and during manufacture process allowed having the first scientific knowledge about this handmade liquor production system.

On the other hand, there is a lack of knowledge about influence of microorganisms in sotol fermentation, because it is carried out in a spontaneous form (Most of the producers say that their product is pure because they do not add anything and less that it is called yeast). One yeast identified as *Saccharomyces cerevisieae* and another as *Protatheca* sp., 4 species of gram negative bacteria *Bacillus licheniformis*, *Bacillus subtilis*, *Acetobacter* sp. and *Bacillus deiformis.* and a fungus (*Mucor circinelloides*) were identified associated to sotol liquor processing.

It is possible to improve sotol liquor production with some of technological modifications as pineapple cooking in pressure pots, juice extraction with roller mills and fermentation with industrial strains of *Saccharomyces cerevisieae*. Production of one liter of sotol liquor is cheaper than production of a liter of tequila because production of a liter of sotol liquor requires 7 kg of sotol pineapple (at a cost of 0.046 US dollar/kg) while production of a liter of tequila requires 5 kg of blue agave (at a cost of $1.56 US dollar/kg). By this reason, with a sustainable sotol production process this industry may be productive.

Table 4: Effect of cooking conditions on the total sugars content

Type of cooked solution	Time (min)	% of total sugars in the extracts
With water	15	20.0
With water	30	31.4
With water	50	15.0
With water-acid	15	21.7
With water-acid	30	18.1

CONCLUSION

The sotol production is made with rudimentary operations, only based on the transferred practical knowledge from generation to generation. There is a complete absence of theoretical knowledge on physical measurements for quality control, operation units and microbial principles of fermentation. Results showed that it is possible to improve sotol liquor production with some of technological modifications as pineapple cooking in pressure pots, juice extraction with roller mills and fermentation with industrial strains of *Saccharomyces cerevisieae.* Further studies are needed to define the effect of the plant sex on the alcohol productivity using molecular techniques, also, to define the kinetic parameters of the fermentation, to evaluate the effect ot mixed or axenic cultures on the volatile components profle; to optimize the distillation process and reuse the bagasse residue for syrups production.

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