



MEZCAL FERMENTATION

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Keywords: Mezcal fermentation, Non-Saccharomyces, Saccharomyces yeast,

In this talk we discuss the identification of the yeast population during a mezcal fermentation and we relate it to some flavour compounds.

Mezcal is a regional Mexican spirit produced by the distillation of fermented agave must which can be obtained from different species of the agave plants, such as *Agave salmiana*, *A. agustifolia* Haw, *A. potatorum* Zucc, *A. weberi*, and *A. esperrima*, among others. According to official regulations, there are two types of mezcal. The first one is produced from the fermentation of sugars obtained only from agave (100% agave sugars). The second type is produced from the fermentation of a mixture of sugars, of which only 80% come from agave, and up to 20 % are obtained from other sources. These two types of mezcal can each be subdivided in three subcategories: aged mezcal, which is maintained in oak casks for at least 12 months, rested mezcal, which is placed in oak casks from 2 to 6 months, and young mezcal, which is bottled soon after distillation is finished.

Mezcal production is a four to five step process (five in the case of aged mezcal). The first step is the cooking of the agave stem, which is carried out in stone or rustic ovens by rural producers and in autoclaves in semi or industrial producers. During cooking, the plant tissues become soft, and polyfructans are hydrolysed yielding mainly fructose. This occurs in a wide range of temperatures, that range from 80 to 120 °C, and time intervals, that last for 18 to 96 h, depending on where the stems are cooking. The second step is the milling of the agave stems. This is carried out using mechanical stone mills or, as in the case of rural producers, wooden mallets are used to get the juice out of the plant tissue. The third step is the fermentation, which is carried out at ambient temperature. The fermentation is placed in wood or stainless steel tanks where the agave must can either be left to ferment with the natural microbiota or inoculated with baker's or commercial yeast from the alcoholic drink industry. The stem fibres can be either be removed or left in the must. The fermentable sugar content in the agave must vary from 9 to 15 °Bx. The fermentation of the

agave must is developed by *Non-Saccharomyces* and *Saccharomyces* yeast, but lactic acid bacteria and *Zymomonas mobilis* have also been isolated from the fermentation. The main product in this spirit is ethanol produced by the fermentation of the sugars by added or natural microorganisms. There are, however, alcohols with three or more carbons, ethyl esters, and organic acids which contribute to the organoleptic characteristics produced by the microbiota. The final concentration of ethanol after the fermentation depends on the initial fermentable sugars content. The mezcal compounds are distilled in different kind of recipients. Rural producers may use clay pots, whereas industrial or semi-industrial producers use cooper recipients where the heating of the must is conducted. The use of distillation columns varies, as well as the recipients where mezcal is collected. The final aged mezcal product contains compounds from the agave plant, from the fermentation process and from the oak.

We have identified the yeast population during a mezcal fermentation and we relate it with the fusel oil production. The mezcal fermentation from rural producers was followed. Samples were taken at 0, 24, 48, 72, 96 and 120h in Sola de Vega in the state of Oaxaca, México. The samples were plated out in YM, WL- and lysine agar and pure yeast colonies were isolated at different stages of the mezcal fermentation. Fifteen different morphologies were found and they were characterized using metabolic tests and API 20C AUX. The strains were further analyzed using the RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. The sequence of the D1/D2 region of the 26S rADN was also used for the identification of the isolates. The main group of yeasts found belongs to *Candida* genera. This is followed by a group of *Zygosaccharomyces* and *Rhodotorula*. *S. cerevisiae* was also found though in low quantities at the beginning of the fermentation, and it remained until the end of the fermentation. Ethanol and fusel oil alcohols were analyzed by GC chromatography in the must during the fermentation and in the distilled product.