

## MONITORING THE FERMENTATION OF SUGARCANE JUICE BY YEAST FOR THE PRODUCTION OF CACHAÇA BY MALDI-TOF MS

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Cachaça is the Brazilian sugar cane spirit produced from fermented sugar cane juice with an ethanol content between 38% (v/v) and 48% (v/v) at 20 °C. The monitoring of yeast population during controlled fermentation using mixed inocula is often made with plate count followed by the use of a molecular technique. MALDI-TOF MS is frequently used in clinical diagnosis of yeasts and bacteria, however, in recent years, some studies have used this technique to identify yeasts used in different fermentation processes. Although this technique is not fully consolidated for monitoring yeasts in fermentative processes, its cost, time consumption and reliability make it an interesting tool in experiments with selected yeasts. Therefore, the aims of this study were to evaluate the efficiency of MALDI-TOF MS as a tool to monitor the inoculated yeast (*Meyerozyma caribbica* and *Saccharomyces cerevisiae*) fermenting sugar cane juice under different conditions to produce cachaça. The efficiency for monitoring the studied inocula by MALDI-TOF MS and protein extraction from colonies or directly from cell suspension in culture media was evaluated in three different experiments. A first assay was performed to evaluate the MALDI TOF MS capacity for distinction between mixed yeast inoculum and pure yeast inocula, the efficiency of protein extraction from yeast colonies or from cell suspension and to check possible interferences of sugar cane juice on the yeast mass spectra. In the second assay, the technique was used to monitor the mixed inoculum composed by different populations of *M. caribbica* fermenting sugar cane juice with different °Brix. Finally, in the third assay, MALDI-TOF MS was used to monitor the mixed inoculum and pure inoculum of *S. cerevisiae* fermenting 15 L of 16 °Brix sugar cane juice for three successive batches. The feasibility of MALDI-TOF MS use under studied conditions was demonstrated by the comparison of the results obtained from yeast cultivation in YPD broth, YPD agar and sugar cane juice, showing that there was no interference of sugar cane juice in protein profile. The data seem to suggest that the protein extraction from fermenting must can generate significantly different spectral signals for different selected inocula that may be used for monitoring the cachaça fermentative process under studied conditions.

Keywords: Yeast co-inoculation. MALDI-TOF MS. Cachaça.

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