POTENTIAL USE OF *KLUYVEROMYCES MARXIANUS* PURE CULTURE FOR TEQUILA FERMENTATION

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Introduction. The fermentation step in tequila industry is mainly carried out by inoculation of the wort with *S. cerevisiae*; few factories are still maintaining spontaneous fermentation. Currently in wine fermentation non-*Saccharomyces* are employed in order to increase the concentration of volatile compounds (1), in general fermentations are performed sequentially. This study evaluated the use of *K. marxianus* pure culture for the fermentation of Agave juice for the production of both ethanol and volatile compounds.

Methods. Fermentations of Agave juice were conducted in 3 L bioreactor, with two strains: *S. cerevisiae* (AR5) and *K. marxianus* (DU3) in conditions similar to the tequila industry (30℃, 100 g/l initial sugars, 0.5 g/L $(NH4)_2SO_4$). Monitoring of the fermentation was carried out according to Pinal et al (2009) (2) and ethanol and major volatile compounds concentrations measured by Headspace-Gas Chromatography (HS-GC) (3).

Results. The *K. marxianus* exhibits behavior similar to *S. cerevisiae*, presenting significant growth (100 x 10^6 cel/ml) and high production of ethanol (more than 40 g/l in the fermented must). The sugar was almost exhausted and the yields of conversion of sugar to ethanol are 0.44 g/g and 0.36 g/g respectively for AR5 and DU3, however DU3 presents an interesting profile of volatile compounds production particularly of ethyl acetate (Fig 1).

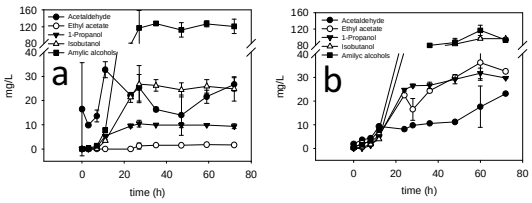

Fig. 1 Kinetics of volatiles compounds production for AR5 (a) and DU3 (b).

Previous studies with *Kloeckera apiculata* (4, 5) demonstrated that it was able to ferment and produce higher concentrations of volatile compounds compared to *S. cerevisiae* however an addition of yeast extract or asparagine was necessary. In this work Agave juice enriched with only ammonium sulphate is fermented (like in the industry), so the strain DU3 has no nutritional limitations.
Another recent study compared the behavior of *K. marxianus* and *S. cerevisiae* in fermentation of agave juice (6) and demonstrated that the yields of conversion of sugars to ethanol were higher for *K. marxianus*, but in the distillate, the concentration of ethyl acetate was lower whereas increased higher alcohols concentration. So in comparison with this work the benefit in the aromatic profile is not so clear.

Conclusions. The use of *K. marxianus* (strain DU3) to produce tequila is a viable option, because it is able to grow and ferment in pure culture, without the addition of other nutrients. Moreover the production of volatile compounds (esters and higher alcohols) increases comparing with the production observed with *S. cerevisiae* which represents an advantage for the flavor and aroma of tequila.

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