AGAVE SAP (*aguamiel*) CHARACTERIZATION AT DIFFERENT PRODUCTION TIMES FROM THREE AGAVE SPECIES FROM HUITZILAC, MORELOS

Nadia Maturano-Ramírez; Clarita Olvera and Agustín López-Munguía; Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología UNAM, Cuernavaca 62210; @ibt.unam.

Key words: agave sap (aguamiel), fructans, fructooligosaccharides

Introduction. Carbohydrates in agave plants are composed by complex type of fructans called agavins [1], which are synthesized by fructosyltransferases (FTFs). Agave sap (*aguamiel*) accumulation in agave plants after the creation of a central core involves the transfer of nutrients from the plant to the sap, as well as the hydrolysis of fructans to monosaccharide and fructo-oligosaccharides (FOS) with a degree of polymerization (DP) of 3-6 [2]. Agave sap collection is usually performed every 12 hr for a period of 4 to 6 months [2]. This sap is a rich media for naturally occurring fermentation processes mainly performed by lactic acid bacteria (LAB), proteobacteria and yeasts. *Aguamiel* and its fermentation product, *pulque*, are traditionally consumed as a beverage with interesting nutritional properties. LAB are the main population present during the initial fermentation steps in *pulque* production [3]. The aim of this research was to explore the evolution of agave sap carbohydrate composition and concentration during the first 12 hr of accumulation at the stem in order to evaluate composition changes trough time previous to collection.

Methods. *Aguamiel* pH was measured at site with Merck pH indicator strips. 0.04-0.05% NaN_3 was added to avoid contamination of agave sap samples. Glucose, fructose and sucrose were determined by HPLC. FOS content was determined by difference between initial free fructose and fructose after complete hydrolysis. FOS profile was identified by HPAEC-PAD. Presence of organic acid and ethanol was determined by HPLC UV/IR detection. Protein content was measured by Bradford method.

Results. Samples were taken at 2, 5 and 12 hr after scrapping the agave stem. This analysis was performed on three different species common in pulque production: *A. salmiana Otto ex Salm-Dyck, A. aff. americana L* and *A. americana L.* all of them of around 10 years old. Sampling took place after 1.5 months of aguamiel production.
Agave sap volume accumulated in the empty cavity after scrapping was from 500mL at 2hr up to 2L at 12 hr, yielding about 4L of sap/day. It was found that while fructose and glucose concentration increased with accumulation time sucrose decreased. On the other hand, FOS concentration did not appear to change. This behavior was reproduced in the three agave species sampled (Fig. 1 to 3). No agavins were found in *aguamiel*, while the main FOS in *A. salmiana Otto ex Salm-Dyck* sap was a DP3 and DP3-5 for *A. aff. americana L* and *A. americana L.* A pH of 8 was found in all three species sap after 2hr, but decreased to 6-5 after 12hr, due to acetic and lactic acid production caused by spontaneous fermentation.

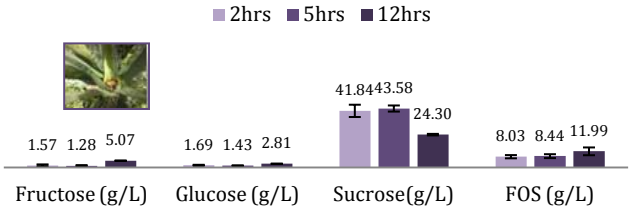

Fig. 1 Fructose, glucose, sucrose and FOS concentration through sampling time for *Agave salmiana Otto ex Salm-Dyck*

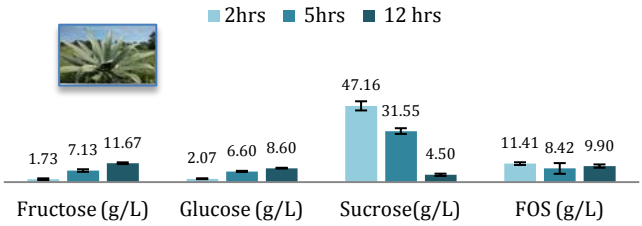

Fig. 2 Fructose, glucose, sucrose and FOS concentration through sampling time for *Agave aff. americana L*

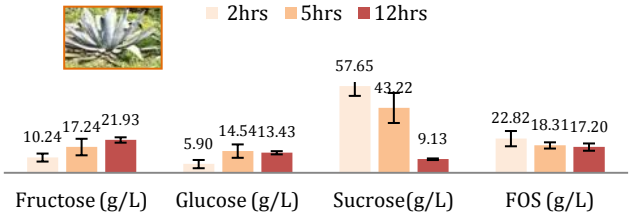

Fig. 3 Fructose, glucose, sucrose and FOS concentration through sampling time for *Agave americana L*

Propionic acid was detected in all samples after 12hr. Protein content also decreased in time from 0.16 to 0.27g/L at 2 hr to 0.09 to 0.13 g/L at 12hr. Interestingly, no ethanol was found in any samples indicating that ethanol producing strains, are absent at the sampled plant core

Conclusions. Microbial modification of *aguamiel* composition takes place in the plant during accumulation, complicating collection for further processing. Carbohydrate content and distribution varies between species, FOS content did not appear to change trough accumulation time suggesting a permanent transformation of agavin to FOS**.**

Acknowledgements. Dr. García-Mendoza for plant classification. Financial support by CONACyT scholarship No. 444901.

References.

1.Ávila Á., *et al.*,(2007) *Plant Science*. **173**(4): p. 478-486.
2.Ortiz R.I., *et al.*, (2008) *JAF C.* **56**(10): p. 3682-3687.
3.Escalante A., *et al.*, (2004). *IJFoodmicro* **235**(2): p. 273-279.