Introduction. Fructans from different sources have received special attention in the last decade. Different polymerization degree (PD) fructans show singular technical properties (e.g. fat substitutes) and biological effects, (e.g. control of obesity and related metabolic disorders [1]). The effect of PD of inulin on its technical and biological effects, have been extensively studied, however scarce work is ongoing for Agave fructans (AF). AF polymerize and de-polymerize according to plant development stages, environmental and other conditions. A higher content of Fructooligosaccharides (FOS) with a DP<10 have been identified in two years agave plants [2], however they have not been fully quantified. In this work we developed an analytical protocol to separate and quantify water soluble carbohydrates from several agave juice (AJ) samples. This protocol was successfully employed to study the carbohydrate profile of one to five years agave plants.

Methods. Agave tequilana plants of one to five years were collected from a plantation in Tequila, Jalisco. Water soluble carbohydrates were extracted with 25 °C water and separated by ultrafiltration, using a 3 kDa membrane in two different fractions (PD > 10 and PD < 10). Efficiency of the fractions separation was confirmed by High Performance Thin Layer Chromatography (HPTLC) and MALDI-TOF. Both fractions obtained were hydrolyzed into monosaccharides with a cationic resin (IR120 Rohm and Haas), Saccharose (S), Glucose (G), and Fructose (F) content were quantified by HPLC using a BioRad Aminex 42-C column, before and after hydrolysis. Fructan content in both fractions were estimated by subtracting S, G, F initially present in AJ, from those generated after hydrolysis of the AJ permeate obtained with a 3kDa membrane.

Results and discussion. The ultrafiltration fractions obtained from AJ, Retentate (R) and Permeate (P), were analyzed by HPTLC (Figure 1). R remained as a deposit spot in HPTLC plates, suggesting that PD > 10 fructans are principally present. On the other hand, P was almost free of PD > 10. These results were confirmed by MALDITOF analysis. Both fractions were completely hydrolyzed into monosaccharides by a IR120 cationic resin and allowed us to quantify the concentration of the different fructan fractions (PD > 10 and PD < 10). In order to further validate the protocol, it was employed to separate and quantify the fructan fractions of different PD in agaves from 1 to 5 years. Figure 2, shows that the total fructans profile increased according to agave age, while free F and G decreased. On the other hand, FOS accumulates in the Agave plants reaching a maximum at three years (67%), and then, their content decreases (25%). Meanwhile, the opposite behavior was shown by DP>10 fructans. This behavior is largely observed in other plants which accumulates fructans, as carbohydrate storage [3].

Fig. 1. HPTLC of AJ fractions after ultrafiltration through a 3 kDa membrane. S:Saccharose, G:Glucose, F:Fructose, FOS:Fructooligosaccharides (Orafti P95). AJ:agave juice, P:permeate, R:retentate

Fig. 2. Water soluble carbohydrates content in Agave tequilana of different ages.

Conclusions.

This work demonstrated that the implemented protocol is very useful for the separation and quantification of diferente DP fructans in Agave tequilana Weber var. azul.

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References.