VIABILITY AND PROTEOLYTIC ACTIVITIES OF *Lactobacillus casei shirota* AND *Lactobacillus rhamnosus* GG IN FERMENTED MILK ENRICHED WITH AGAVE JUICE (*AGUAMIEL*) IN COLD STORAGE

Martínez_Ramírez, X; González_Olivares, L.G.[a]; Añorve_Morga, J[a;], Castañeda_Ovando, A.[a]; Contreras_López, E.[a]; Jaimez_Ordaz, J.[a]; Cruz_Guerrero, A.E.[b]

[a]Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Hidalgo, Carr. Pachuca-Tulancingo km. 4.5, Pachuca, Hgo., C.P. 42067, México

[b]Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, AP 55-355 México D.F.

correo: lgonzales@uaeh.edu.mx

Key words: Probiotics, prebiotics, agave juice

Introduction. Several investigations have focused on improving growth and activities of many different probiotics with inulin and Hi-maize starch (1); however, there is a lack of information regarding their effects on *Lactobacillus* spp. While the addition of prebiotics may have functional benefits for probiotics and subsequently for consumers, this approach may affect the textural characteristics of fermented milk. Therefore the objectives of this study were: i) to investigate the effect of addition of agave juice (*aguamiel*) on survival of two selected probiotic organisms, *Lactobacillus casei shirota* and *Lactobacillus rhamnosus* GG during cold storage; ii) to determine the proteolytic activity of the organisms in the presence of the prebiotics, during fermentation and storage at 4°C.

Methods. Two fermentation systems were prepared with 10% of skim milk powder, each of them were supplemented with 2% of carbon source from inulin and agave juice. These systems were inoculated with $1X10^6$ CFU/ml of each microorganism studied. The fermentation was made at 42°C for *L. rhamnosus* GG and 37°C for *L. casei.shirota.* The pH was monitored during fermentation which was stopped when a 4.5 value was reached. The proteolytic activity was measured using 2,4,6-trinitrobenzene sulfonic acid test (TNBS) (2) and SDS-PAGE electrophoresis. The enumeration of probiotics was performed as reported by Donkor et al. (2007) (3).

Results. The effect of prebiotic addition on the survival (table 1) and proteolytic activity (Fig. 1) of *L. rhamnosus* GG and *L. casei*. *shirota* was investigated over 14 days of cold storage at 4°C. The initial cell concentration of probiotics on day 1 was affected on different ways by the addition of prebiotics. Inulin and agave juice supplementation resulted in improved growth of probiotics during fermentation prior to the storage. Moreover, with agave juice the initial probiotics concentration increased by almost 1 log cycle in comparison with that observed for Inulin. The concentration of probiotics in all fermented milk assayed was above the lowest recommended level of $1X10^6$ CFU/ml at the end of the storage. The results showed a significant improvement ($P<0.05$) of proteolytic activity by probiotic organisms in presence of selected prebiotics (Fig. 1). On the other hand, the concentration of probiotics was significantly increased ($P<0.05$) at the end of the fermentation in the two systems compared with the initial concentration as well as the concentration at the end of the cold storage in both kind of prebiotics. The final peptide concentration at the end of storage was highest in the agave juice system. The electrophoresis analysis of fermentation by *L.casei shirota* showed the presence of peptides lower than 6 kDa. These results suggest that this kind of peptides were formed during fermentation and its concentration continued increasing until the end of the cold storage (4).

	L.casei		*L. rhamnosus*	
	Inulin log UFC	agave juice log UFC	Inulin log UFC	agave juice log UFC
day 0	9.39	9.44	9.48	9.49
day 7	8.81	9.31	9.26	9.45
day 14	8.36	8.23	8.08	8.37

Table 1 Survival comparison of probiotics in cold storage at 4°C

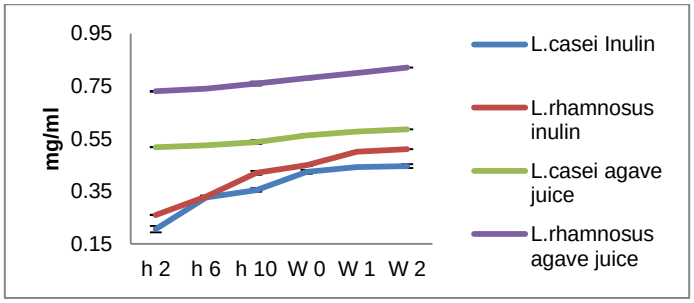

Fig. 1. Proteolític comparison of probiotics during fermentation (h) and cold storage (w)

Conclusions. During the study was observed that the system with agave juice showed more proteolityc activity than the system supplemented with inulin and increased survival of probiotics microorganisms. In the study was demostrated acumulation of low molecular weight peptides from the fermentation to cold storage.

References.

1. Borchers T. A. et al., (2009). *J. of Gastroenterology.* 44. (1): 26-46
2. Rojas_Ronquillo, R. et al (2012) *Int. Dairy J.*8:1-8
3. Donkor O.N, et al., (2007). *Int. Dairy. J.* 17: 1321-1331
4. González_Olivares L.G. et al (2011) *Rev. Mex. Ing. Quimica* 10(2): 179-188