IDENTIFICATION 0F PEPTIDES PRODUCED IN MILK FERMENTED BY PROBIOTIC BACTERIA.

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INTRODUCTION. Considerable research has been done to study the health benefits of fermented milks. A possible mechanism of the beneficial effects is the one that involves biogenic compounds, such as bioactive peptides. These are produced by the action of microorganisms and make the products directly beneficial without the need for live bacteria (1). Many studies have demonstrated that existence of bioactive peptides in dairy fermented products is due to the proteolytic system of lactic acid bacteria. The aim of this study was to determine the existence of probable bioactive peptides in milk fermented by the probiotics *L. acidophilus* and *S. thermophilus*, as well as to study the changes in their concentration during the first 13 days of refrigerated storage.

METHODOLOGY. Fermented milk was centrifuged and peptides were determined in the supernatant. Peptides were monitored during the first 13 days of refrigerated storage by modified PAGE, according to Shaggër and Von Jagow (2); their size and concentration were determined by HPLC on a size exclusion chromatography column (Biosep-SEC 2000) using a diode array detector. Lectures were made at 210, 257 and 280 nm.

RESULTS AND DISCUSSION. Around 12 peptides were detected by both PAGE and HPLC, but their concentration varied during refrigerated storage from 227 to 357 µg/m. Several molecular weights were observed, some of which showed coincidences with others reported as bioactive in fermented milks (table 1). Peptide concentration was different depending on their size: in the first stages of storage peptides larger than 5 kDa were predominant, but as time increased, the concentration of lower MW peptides increased (Figure 1). This suggests that peptide production was probably a staged process, in which low molecular weight peptides (lower than 2 kDa) were formed by the hydrolysis of midrange peptides (2-6 kDa) which were once originated by proteins or peptides over 6 kDa.

The peptide whose concentration varied most was the one of 850 Da, which corresponds to one reported in the literature as antihypertensive. Further more, several bioactive peptides with antihypertensive and opioid activity have been reported to have high aromatic aminoacid contents in their primary structure (1). When the chromatograms were analyzed at different wavelengths, this peptide showed more intensity at aromatic region (257 and 280 nm), demonstrating that this petide has a high aromatic aminoacid content (Fig. 2) in its primary structure, and thus suggesting that it might have antihypertensive activity.

CONCLUSION. Some of the peptides identified in this study could be bioactive and might partially explain the probiotic properties attributed to *S. thermophillus* and *L. acidophilus*. Furthermore, the most abundant peptide might be an antihypertensive one.

MW of peptides detected by SDS-PAGE (kDa)	MW of peptides detected by HPLC (kDa)	MW of Reported peptides (kDa)	Probable Function
3.97	3.86	3.97	MA
3.03	3.09	3.11	IM, ATM
2.58	2.68	2.70	MA, ACEI, IM.
1.56	1.61	1.62	ACE-I, MA,
1.31	1.42	1.39	ACE-I, MA
1.15	1.14	1.18, 1.15	MA, ACE-I
0.836	0.870	0.885	ACE-I, opioide

Table 1. Molecular weight peptides relation between electrophoresis and HPLC analyses and comparison with reported others.

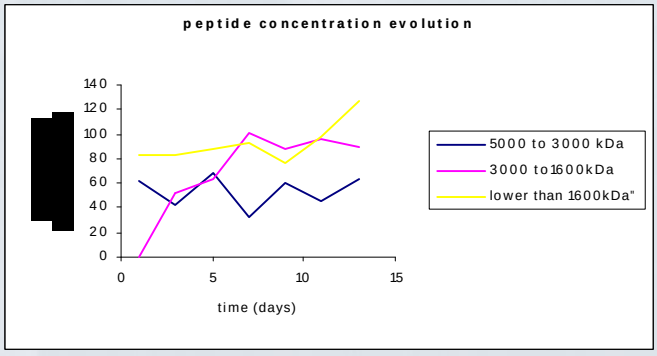

Fig. 1. Evolution of peptide concentration during 13 days on refrigerated storage.

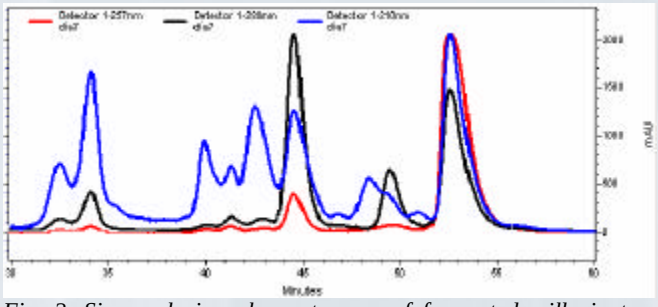

Fig. 2. Size exclusion chromatogram of fermented milk, in tree different wavelengths 210, 257 and 280nm

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