



## MULTIFUNCTIONAL PEPTIDES RELEASED DURING MILK FERMENATION WITH *Lactobacillus casei* SHIROTA

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*Keywords: bioactive peptides, mineral binding, Lactobacillus casei Shirota*

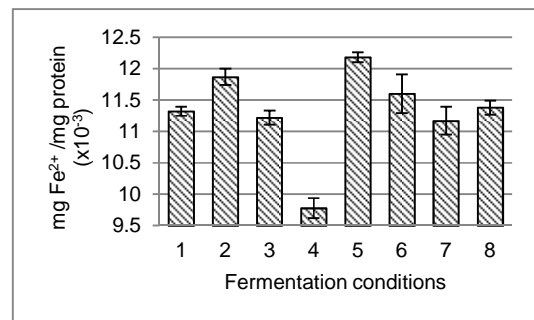
**Introduction.** Growth of lactic acid bacteria (LAB) in milk depends on the presence of peptides and free amino acids. Natural concentration of these components is low in milk. Cell envelope proteinase (CEP), main component of the proteolytic system of LAB, hydrolyses milk proteins to release several peptides which are necessary for the growth. Some of these peptides present several bioactivities<sup>(1)</sup>. The aim of this study was to determine the conditions of pH and temperature for the synthesis of calcium and iron binding peptides in the fermentation of milk with *Lactobacillus casei* Shirota. A factorial design based on optimal conditions for the growth of *L. casei* and CEP activity<sup>(2)</sup> was used.

**Methods.** A factorial design 2x2x2 was applied (time, temperature and pH according to table 1). *Lactobacillus casei* Shirota was inoculated in skim milk (10% solids) under appropriate conditions. Fermented milk was centrifuged (1000 rpm/30 min) in order to obtain a cell free extract (CFE). Protein content of CFE samples were determined through Lowry<sup>(3)</sup> method and standardized to 2mg/mL. Iron binding activity was determined as reported by Farvin et al<sup>(4)</sup> while calcium binding activity as reported by Figueroa-Hernández et al<sup>(5)</sup>.

**Results.** Iron binding activities of CFE are shown in figure 1. Fermentation conditions that showed higher activities were 2 and 5, in which  $11.87 \times 10^{-3}$  and  $12.18 \times 10^{-3}$  mg Fe<sup>2+</sup>/mg protein, respectively were obtained. With respect to calcium binding activity, there was no significant difference among the 8 treatments evaluated until now.

**Table 1.** Fermentations conditions evaluated

		time (h)			
		12		20	
		Temperature (°C)		Temperature (°C)	
		39.5	42.0	39.5	42.0
pH	6.25	1	2	3	4
	6.50	5	6	7	8



**Fig.1** Iron binding activity of CFE

**Conclusions.** Fermentation conditions had an effect on iron binding activity, obtaining higher activities at 12h/40°C/pH 6.5 and 12h/40°C/pH 6.5 with values of  $11.87 \times 10^{-3}$  and  $12.18 \times 10^{-3}$  mg Fe<sup>2+</sup>/mg protein respectively. Fermentation conditions evaluated had no effect on calcium binding activity.

**Acknowledgements** to CONACyT for granting a scholarship to pursue studies. (Scholarship number 50370).

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