METAL BINDING PEPTIDES PRODUCTION BY *LACTOCOCCUS LACTIS* SUBSP. *CREMORIS* NCFB 712

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Introduction. Metal binding peptides or caseinphosphopeptides (CPP) have the ability to increase the absorption of calcium and other divalent minerals. Most of CPP contain an anionic cluster (SerP-SerP-SerP-Glu-Glu), which is related with metal binding activity (1, 2). These peptides are generated from casein but can also be produced from serum proteins such as β-lactoglobulin and α-lactalbumin (3). During fermentation of milk with lactic acid bacteria (BAL), bioactive sequences could be generated. The aim of this study was to evaluate the production of metal binding peptides during milk fermentation with *Lactococcus lactis* subsp.*cremoris* NCFB 712 under two different pH conditions.

Methods. Fermentations were performed with pH and without pH control. Peptide binding of iron and calcium were determinated in different samples in order to demonstrate their bioactivity. Iron binding capacity was determinated by the technique proposed by Hwang *et al*., 2001 (4). Calcium binding capacity of the samples was measured by the technique proposed by Brown & Rydqvist, 1981 (5). Molecular weight of peptides were determinated by HPLC.

Results. Samples of fermentation with pH control showed an increase in bounded calcium concentration from eight hours to 24 hours of fermentation. The same effect was observed without pH control fermentation, but in a lesser extent (Fig 1).

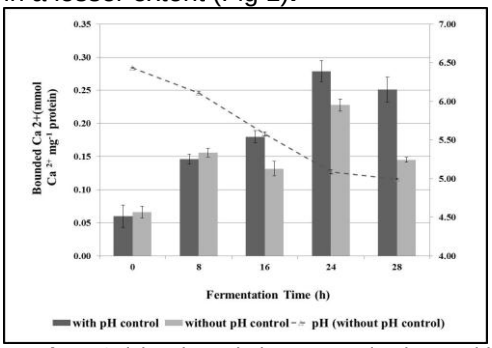

Fig. 1 Calcium bounded concentration by peptides generated during milk fermentation by *Lactococcus lactis* subsp. *cremoris* NCFB 712

It was found a correlation between proteolytic activity and calcium bounded of 0.967 and 0.830 for fermentation with pH control and without it respectively. This meant that calcium binding peptide production was closely related to proteolytic activity of bacteria. In relation to Iron binding capacity, samples fermentations showed a high initial value (30%) since some of the milk proteins, like β-lactoglobulin and α-lactalbumin have the ability to bound iron. However, only the fermentation with pH control showed an increase in bounded iron (60%). Molecular weight of peptides generated during milk fermentation by *Lactococcus lactis* subsp. *cremoris* NCFB 712 that coincided with metal binding peptides are showed in table 1.

Table 1. Molecular weight of peptides generated during milk fermentation that coincided with metal binding peptides. a: with pH control, b: without pH control.

Experimental MW (kDa)	Reported MW(kDa)	Reported sequence	Reported metal binding capacity
3.13 a	3.11	β-CN(1-25)-4P	4.64±0.77 mol Ca^{2+} mol^{-1} peptide
2.79 a	2.71	α_{s1}-CN(59-79)-5P	6.84±0.45 mol Ca^{2+} mol^{-1} peptide
2.05 a,b	2.07	α_{s1}-CN(43-58)-2P	Ca^{2+} & Fe^{2+}
1.06 a,b	1.01	β-CN(15-20)-4P	6.2 mM Ca^{2+} mL^{-1}
0.71 a,b	0.67	β-LG(78-83)	0.4 mM Ca^{2+} mL^{-1}

Conclusions. It was demonstrated that *Lactococcus lactis* subsp *cremoris* NCFB 712 has the ability to generate iron and calcium binding peptides. Fermentations with pH control showed higher calcium binding activity than fermentations without pH control. Proteolytic activity and calcium binding activity was closely correlated.

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