Yogurts enriched in Caseinphosphopeptides
Carina Bergamini, Verónica Wolf, Cristina Perotti
Instituto de Lactología Industrial, Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).
Santiago del Estero 2829, Santa Fe S3000ADN. Argentina, cber@fiq.unl.edu.ar

INTRODUCTION
Casein phosphopeptides (CPP) are phosphorylated peptides produced by enzymatic hydrolysis of αs1, αs2 and β casein by several proteases, such as trypsin. The CPP containing the cluster sequence -SerP-SerP-SerGlu-Glu-, have demonstrated anticariogenic activity because their ability to remarkably stabilize calcium phosphate in solution and increase the level of calcium phosphate in dental plaque [1, 2].

Previously, Lorenzen and Meisel [3] have assayed the manufacture of a yogurt with increase in the CPP content by hydrolysis of milk base with trypsin. In the present work, we used a similar strategy to make a yogurt enriched in CPP, but we evaluated if an increase of the substrate (casein) in the milk base could lead to the production of higher levels of CPP. In addition we studied the protective effect of CPP against dental erosion by means of demineralization test using hydroxyapatite as a model system [4].

OBJECTIVES
The objective of this work was to obtain yogurts enriched with CPP by hydrolysis with trypsin of milks fortified with different levels of casein from skim milk powder and calcium caseinate. A comparative study of acidity, peptide profiles and anticariogenic activity between hydrolysed and control yogurts was performed during fermentation and/or storage.

MATERIAL AND METHODS

- **Manufacture of yogurts**
  Different types of yogurts, according to the scheme and experimental design shown in the Figure 1 and 2, were made applying the traditional method adapted to laboratory scale. An equipment consisting of four stainless steel tanks (1 L capacity) operated simultaneously were employed (Figure 3). Each yogurt was manufactured by duplicate in different days using different milk. 12 L of milk were used for each day of manufacture (1 L for each yogurt).

- **pH and Acidity:** pH during fermentation and Titratable acidity (TA) were determined by normalized methods from International Dairy Federation (IDF).
- **Peptide profile:** Peptide profiles of yogurts were analyzed by RP-HPLC, in which CPP were identified by comparison with peptide profiles of CPP obtained by selective precipitation with ethanol/calcium chloride at pH 5.5 from tryptic hydrolysates of sodium caseinate (5%). In these conditions, only CPP that have demonstrated anticariogenic activity are precipitated [5].
- **Anticariogenic activity:** The protective effect of CPP was determined according to Kanekanian et al. [4] with some modifications.

RESULTS AND DISCUSSION

1. Preparation of yogurt extracts.
2. Demineralization test

3. Procedure: The tubes were mixed thoroughly and allowed to stand for 20 min with intermittent stirring to allow maximum peptide binding to the HA. Then, the suspension was centrifuged at 1000 g for 10 min. The HA pellet was resuspended in 3 mL distilled water to remove excess of unbound peptide, which was discarded after centrifugation at 1000 g for 10 min. Then, 5 mL portions of the acid buffer (0.25 M acetate pH 4.2) were added to each tube as rapidly as possible, the contents were briefly stirred and after standing for 10 min the tubes were centrifuged (1000 g for 10 min). Supernatants were immediately decanted off into clean tubes for subsequent Ca and P analyses by colorometric methods.

The amount of Ca and P loss from HA was used to evaluate the protective effect of CPP against acid dissolution in comparison with the controls. Black and control samples containing (no CPP protection) or extract yogurt only (no HA, no Ca or P substitution and hence equivalent to 100% protection) were also prepared. The results were expressed as % of protection.

RESULTS

1. pH during fermentation and titratable acidity of yogurts
2. Peptide profiles
3. Demineralization test

CONCLUSION
The hydrolisis of milk with trypsin allowed the manufacture of yogurt enriched in CPP. Likewise, the higher content of casein in the milk base led to an increase in the CPP levels in hydrolysed yogurts.

It was demonstrated a protective role against mineral loss from hydroxyapatite by hydrolysed yogurts containing CPP. However, this anticariogenic activity was not proportionally increased in yogurts made with a milk base fortified with caseinate in which we found higher levels of CPP.