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 S3000AOM. Argentina, cvberg@fiq.unl.edu.ar

INTRODUCTION

Casein phosphopeptides (CPP) are phosphorylated peptides produced by enzymatic hydrolysis of αs1, αs2 and β casein by several proteasas, such as trypsin. The CPP containing the cluster sequence -SerP-SerP-SerP-Glu-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 led 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 3, were made applying the traditional method adapted to laboratory scale. An equipment consisting of four stainless steel vats (5 L capacity) operated simultaneously were employed (Figure 2). Each yogurt was manufactured by duplicate in different days using different milk. 12 L of milk were used for each day of manufacture (3 L for each yogurt).

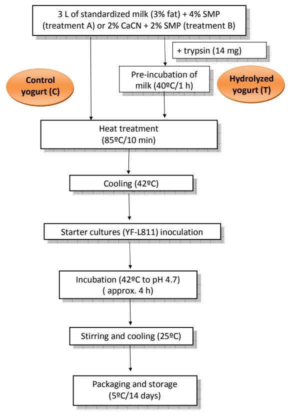


Figure 1. Scheme of yogurt-making

Figure 2. Equipment used for yogurt-making.

Treatment	Yogurts	Type and quantity of added milk ingredients	Addition of sucrose (8%)
A	C	4% SMP	No
	C_s		Yes
	T		No
	T_s		Yes
B	C	2% CN + 2% SMP	No
	C_s		Yes
	T		No
	T_s		Yes

Figure 3. Experimental design of yogurt-making. SMP: skim milk powder; CN: calcium caseinate. C: control yogurts. T: yogurts made with milk pre-hydrolyzed with trypsin. S: sucrose.

pH and Acidity:

pH during fermentation and Titratable acidity (TA) were determined by normalized methods from International Dairy Federation (IDF).

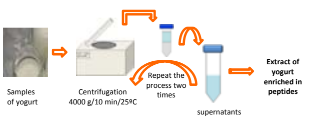
Peptide profile:

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 3.5 from tryptic hydrolysates of sodium caseinate (5%). In these conditions, only CPP that have demonstrated anticariogenic activity are precipitated [1].

Anticariogenic activity:

The protective effect of yogurts was determined according to Kanekanian et al. [4] with some modifications.

1. Preparation of yogurt extracts.



2. Demineralization test

The demineralization test was made by triplicate for each sample according to the following scheme.

Samples	HA	Buffer pH 7.5 (Tris-HCL)	Volume of extract
C yogurt	50 mg	3 mL	2 mL
T yogurt	50 mg	3 mL	2 mL
Blank	50 mg	5 mL	-
Control of C yogurt	-	3 mL	2 mL
Control of T yogurt	-	3 mL	2 mL

3. Procedure: The tubes were mixed thoroughly and allowed to stand for 20 min with intermittent stirring to permit maximum peptide binding to the HA. Then, the suspension was centrifuged at 3000 g for 10 min. The HA pellet was re-suspended in 3 mL distilled water to remove excess of unbound peptide, which was discarded after centrifugation at 3000 g for 10 min. Then, 5 mL portions of the acidic buffer (0.25 M sodium acetate pH 4.2) were added to each tube as rapidly as possible, the contents briefly stirred and after standing for 10 min the tubes were centrifuged (3000 g for 10 min). Supernatants were immediately decanted off into clean tubes for subsequent Ca and P analyses by colorimetric methods.

The amount of Ca and P loss from HA was used to evaluate the protective effect of T yogurts against acid dissolution in comparison with the controls. Blank and control samples containing HA only (0% protection) or extract yogurt only (no HA, so no Ca or P solubilisation and hence equivalent to 100% protection) were also prepared. The results were expressed as % of protection.

RESULTS AND DISCUSSION

pH during fermentation and acidity of yogurts

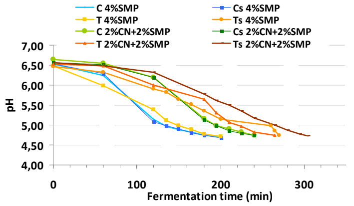


Figure 4. Evolution of pH during yogurt-making. C: control yogurts, T: yogurts made with milk pre-hydrolyzed with trypsin. S: sucrose.

- The addition of trypsin produced an increase in the fermentation time for T and Ts yogurts in relation to C and Cs yogurts in the treatment B (25 and 65 min, respectively), but only for Ts in the treatment A (65 min).
- All yogurts of the treatment B showed a delay in the fermentation time in comparison with yogurts of the treatment A. These results could be due to the buffer properties of CN which was added in these yogurts.
- For hydrolysed yogurts, the addition of sucrose produced an increase in the fermentation time. In effect, the process was longer for Ts than T yogurts for both treatments: 70 and 40 min for treatment A and B, respectively.

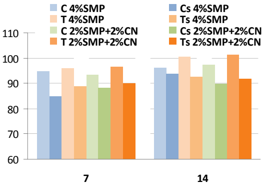


Figure 5. Acidity values during storage of yogurts. C: control yogurts, T: yogurts made with milk pre-hydrolyzed with trypsin. S: sucrose.

- Values of titratable acidity (TA) for all samples ranged from 85 to 105°D, which are within the normal range established in the Argentinean Legislation [5].
- Yogurts with sucrose showed lower levels of acidity than the similar yogurts without this sugar.
- Hydrolysed yogurts showed slightly higher acidity values than control yogurts.

Peptide profiles

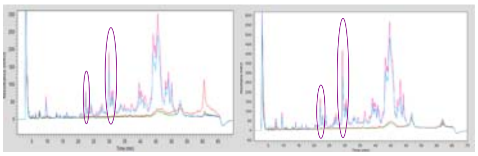


Figure 6. Peptide profiles of C and T yogurts from both treatments (A and B). C and Cs yogurts: red and green lines, T and Ts yogurts: pink and light blue lines. Ellipses enclose the peaks identified as CPP by comparison with peptide profiles of CPP obtained by selective precipitation from casein tryptic hydrolysate.

- The peptides profile of C yogurts showed the presence of a few peaks whereas the chromatograms of T yogurts were more complex, with a great number of peptides.
- The presence of CPP in the peptide profiles was only detected in those yogurts made with hydrolysed milk.
- Peptide profiles of T yogurts made with and without added sucrose were similar, but slightly lower peak area values were detected in those containing sucrose.

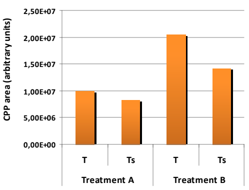


Figure 7. Area of peaks identified as CPP in hydrolysed yogurts.

- CPP levels, quantified as the sum of the area of all peaks identified as CPP in the peptide profiles, were higher in hydrolysed yogurts prepared with the inclusion of CN.
- These results could be due to the higher concentration of the casein substrate for the production of CPP by trypsin in yogurts from treatment B.

Demineralization test

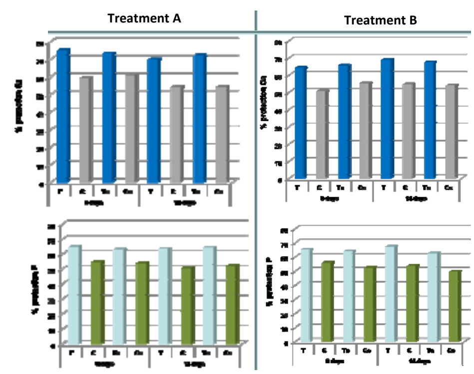


Figure 8. Degree of protection of the C and T yogurts from both treatments (A and B) against Ca and P dissolution from HA, expressed as %.

- Yogurts made with hydrolysed milk (T) showed a higher protective effect against acidic erosion than C yogurts. The maximum degree of protection under our experimental conditions was achieved in T yogurts made from 4% SMP (15% in relation to respective C yogurt).
- In spite of the higher levels of CPP in T yogurts made with treatment B, the anticariogenic activity was not greatly different to that seen in T yogurts from treatment A.
- The anticariogenic activity was similar in yogurts made with and without sacarose.
- Only slight variations in protective effects were observed during storage of the products.

CONCLUSION

- The hydrolysis 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.

[1] Reynolds E., Riley P., Adamson N. (1994) A selective precipitation purification procedure for multiple phosphoseryl-containing peptides and methods for their identification. Analytical Biochemistry 217 277-284.
[2] Reynolds E. (1998). Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: A review. Special Care in Dentistry 18 (1), 8-16.
[3] Lorenzen P.C., Meisel H. (2005). Influence of tripsin action in yogurt milk on the release of caseinophosphopeptide-rich fractions and physical properties of the fermented products. International Journal of Dairy Technology, 58 (2) 119-124.
[4] Kanekanian A., Williams R., Brownsell V., Andrews A. (2008) Caseinophosphopeptides and dental protection: Concentration and pH studies. Food Chemistry 107, 1015-1021.
[5]Código Alimentario Argentino. Cap. VIII, Art. 576.www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp.