STABILITY TO TECHNOLOGICAL PROCESSES OF ANTIHYPERTENSIVE PEPTIDES

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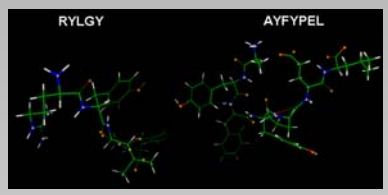
1. Introduction

- In a previous study, two novel antihypertensive peptides, which corresponded to fragments f(90-94) (RYLGY) and f(143-149) (AYFYPEL) of α_{s1}-CN, have been identified in a peptic bovine casein hydrolysate (Contreras et al., 2009).
- A relevant question for the industrial application of bioactive peptides is if these compounds would survive to processing conditions. However, there are little data about this issue.



The main objective was to evaluate the resistance of the peptides RYLGY and AYFYPEL to drying, homogenization and pasteurization, and their stability in yoghurts stored at 4 °C. The stability of the ACE-inhibitory activity and the antihypertensive activity was also evaluated.

2. Material and Methods

Preparation of the casein hydrolysate

Food grade conditions (previously optimized)

- Casein
- Water
- Enzyme
- With/without an additive (for improving taste)

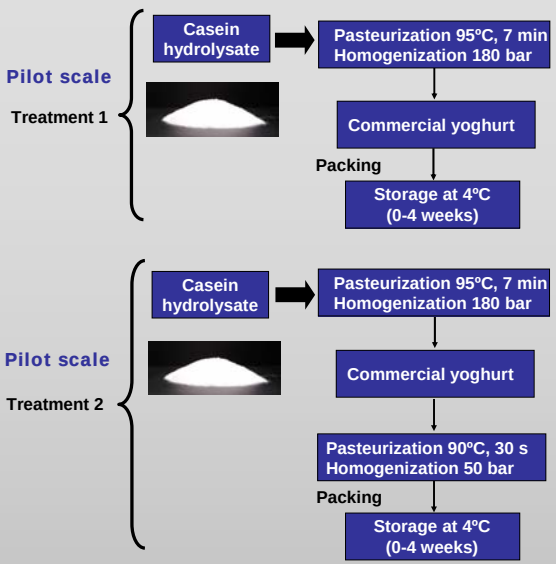
Spray- /Freeze-drying

Laboratory — Large scale

Hydrolysis tank 140 L


Fig. 1. Industrial spray-drier

Incorporation of the casein hydrolysate into yoghurt



Large scale (produced by a Food Company)

Production → Storage at 4ºC (0-45 days)


Yoghurts prepared on a large scale

- Yoghurt starter cultures
- Yoghurt starter cultures + *Lb. casei*

In addition, the peptides were quantified by HPLC-MS, using the method of Contreras et al. (2010). *Anal Bioanal Chem* 397, 2825-2832. The ACE-inhibitory activity was determined as previously described Quirós et al. (2009). *Peptides* 30, 1848–1853, and the antihypertensive activity was evaluated accordingly to Contreras et al. (2009). *Int Dairy J* 19, 566-573.

3. Results and discussion

3.1. Effect of drying

Table 1. Content of peptides RYLGY and AYFYPEL (mg/g of dried product) in the casein hydrolysate prepared on a laboratory scale. The ACE-inhibitory activity was expressed as IC_{50} value (µg of dried product/mL).

Additive	Drying method	Content of RYLGY (mg/g)	Content of AYFYPEL (mg/g)	IC_{50} (µg of dried product/mL)
Before hydrolysis	Spray-drying	0.67[a] ± 0.04	2.44[a] ± 0.11	126.12[a] ± 11.71
	Freeze-drying	0.72[a] ± 0.01	2.56[a] ± 0.02	122.88[a] ± 21.93
After hydrolysis	Spray-drying	0.74[a] ± 0.004	2.49[a] ± 0.07	131.56[a] ± 8.08
	Freeze-drying	0.68[a] ± 0.07	2.24[a] ± 0.20	131.04[a] ± 18.89

Large scale → RYLGY = 0.79 mg/g and AYFYPEL = 2.17 mg/g
IC_{50} = 99.11 µg of dried product/mL

The moment of the incorporation of a food additive did not affect the peptide content in the final ingredient (Table 1). In addition, the content of both peptides and the ACE-inhibitory activity were comparable after spray-drying and freeze-drying and between both scales, with (Table 1) or without (data not shown) the incorporation of the additive during the processing of the hydrolysate.

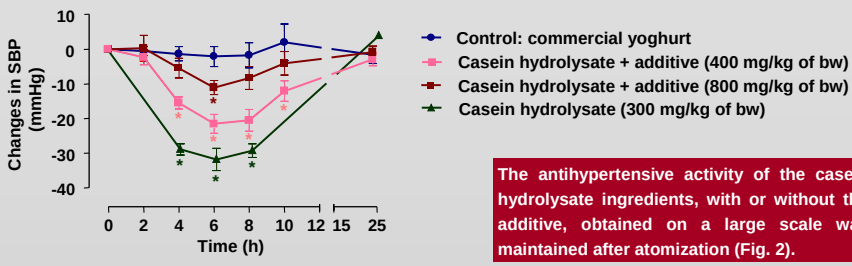


Fig. 2. Antihypertensive activity in SHR after oral administration of different powdered ingredients.

The antihypertensive activity of the casein hydrolysate ingredients, with or without the additive, obtained on a large scale was maintained after atomization (Fig. 2).

3.2. Effect of pasteurization and homogenization. Stability during shelf-life

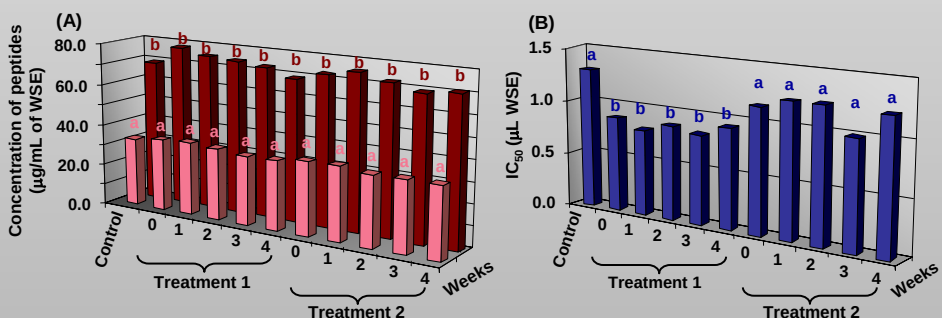


Fig. 3. (A) Effects of pasteurization and homogenization on the concentration (µg/mL of WSE) of peptides RYLGY (□) and AYFYPEL (■) and their stability in yoghurts at refrigerated storage. (B) The ACE inhibitory activity was expressed as IC_{50} value (µL of WSE).

The active peptides were stable during the processes of homogenization and pasteurization. Moreover, no significant reduction of both peptides was detected during the shelf-life of the yoghurts prepared on pilot (Fig. 3) and large scale (data not shown).

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