BIOACTIVE PEPTIDES OBTAINED FROM WHEY PROTEIN CONCENTRATE BY ASPARTIL PROTEASE IMMOBILIZED IN ALGINATE BEADS

Xochitl Tovar[1], Claudia Muro-Urista[2], Alejandro Téllez-Jurado[1], Yuridia Mercado-Flores[1], Arturo Abreu-Corona[1], Carlos Gómez-Aldapa[3] y Ainhoa Arana-Cuenca[1]
[1]Universidad Politécnica de Pachuca, Laboratorio de microbiología molecular. Carr. Pachuca – Cd. Sahagún Km. 20, Zempoala, Hidalgo. México. C.P. 43830. [2]Instituto Tecnológico de Toluca.
[3]Universidad Autonoma del Estado de Hidalgo. Email: food_chemistry_xtj@hotmail.com.

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Introduction. Aspartil protease (AP) from *Sporisorium reilianum* shows extensive proteolytic activity towards whey proteins. The need for the immobilization of AP has been due to its great liberate antioxidant and antihypertensive peptides capacity and AP recovery and reuse (1).In this sense, the immobilization enzymes can be carried out in Ca-alginate beads, it represents one of the most widely used carriers for the immobilization of enzymes, also, it has several advantages over the enzymes in free solution.
The aim of this study was to optimize the time for obtaining peptides with biological activity.

Methods. The effect of whey protein concentrate (WCP) (μg protein) and hydrolysis time (min) on the antioxidant (mM AAEAA/100g WCP) and hypertensive activity (%ACE inhibition) (2) were evaluated using a central composite rotatable design (Table 1) in order to optimize the immobilization conditions. The beads obtained from the optimal region were observed under a scanning electron microscope also were determined relative activity after 20 cycles of use (3).

Table 1: Experimental design: process variables and their levels.

Factors	Low level	Middle level	High level
μg protein	179	299	897
Hydrolysis time (min)	10	35	60

Results. The optimization was performed using the methodology of overlapping surfaces, setting a criterion find immobilization conditions for the best values of antioxidant and antihypertensive activities. The optimization results indicated that the best theoretical conditions for WCP hydrolysis are: protein concentration: 498.33 μg protein; hydrolysis time: 16.25 min, resulting in an antioxidant activity of 1047 mM AAEAA/100g WCP and hypertensive activity of 82.3 %ACE inhibition. Experimentally, under these conditions, the antioxidant activity was: 1077.77±32.3 mM AAEAA/100g WCP and the hypertensive activity was 89±6.25 %ACE inhibition indicating a good fit of the proposed model. Also this results were comparated with quimotrypsin immovilized under the same conditions, however, lower biological activity was obtained (antioxidant activity: 520 mM AAEAA/100 g WCP and hypertensive activity: 50 %ACE inhibition). The obtained spheres showed under optimal conditions a porous lattice structure (Fig. 1).

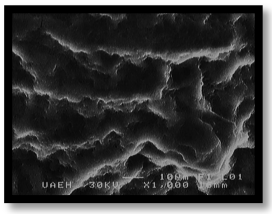

Fig. 1 Scanning electron micrographs of calcium alginate beads the area obtained at the optimum point (Cross Section).

The study of enzyme immobilized reuse under the optimized conditions no showed significant difference after 3 uses, AP retained 90% of its activity after 8 cycles, this feature of reusability is advantageous for industrial application.

Conclusions. The conditions used for immobilization of AP maintain the enzyme activity by 90% after 8 uses.
The optimization improved the antioxidant and antihypertensive activity peptides in comparison to the commercial enzyme immobilized.

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