Optimization of Bromelain extraction from pineapple by-products through natural polymer complex formation

D. Campos [(1)], E. Coscueta [(2)], L. Pastrana-Castro [(3)], J. Teixeira [(4)], G. Picó [(2)], M. Pintado[(1)]

[(1)]*CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal*

+351917351461; dcampos@porto.ucp.pt

[(2)]*IPROBYQ – Institute of Biotechnological Processes – College of Biochemistry and Pharmaceutical Sciences, National University of Rosario, Suipacha 570, S2002LRK Rosario, Argentina;* [(3)]*INL – International Iberian Nanotechnology Laboratory, 4710-330, Braga, Portugal;* [(4)]*Centro de Engenharia Biológica, Universidade do Minho, Campus Gualtar, 4710-057, Braga, Portugal*

Introduction – The isolation and purification of by-products are very important processes in the biotechnology industry, representing 80–90% of total production costs. Furthermore, the development of simple and viable methods for protein purification has been an essential pre-requisite for many advances in biotechnology (Hari Krishna and Karanth 2002).

Bromelain (BR) is a crude, aqueous extract from the stems and immature fruits of pineapples (*Ananas comosus*) and can be found in the tissue of plants of the *Bromeliaceae* family and belongs to cysteine-proteinases family (Rowan and Buttle 1994). Several processes of extraction were developed, but all with some disadvantage (high cost, low purity, high use of chemical products). Two types of BR were described, from stem (EC. 3.4.22.32) and from fruit (EC. 3.4.22.33), previously called bromelin (Cooreman 1978).

These group of enzymes offer a wide spectrum of therapeutic efficacies: they demonstrate, *in vitro* and *in vivo*, antiedemateous, antiinflammatory, antithrombotic and fibrinolytic activities (Maurer 2001), increasing the importance of determining a viable extraction and purification method for this enzyme. A new method of purification and isolation of BR was developed, using a natural food safe polysaccharide (carrageenan).

Carrageenan (Carr) is derived from certain species of red seaweeds (*Rhodophyceae*) and shows a wide range of rheological and functional properties. Previous reports have demonstrated the use of this polysaccharide to isolate and immobilize enzymes (Fabian, Huynh et al. 2010).

Experimental/ Results and Discussion - Using extracts produced from two types of pineapple residues (stems and peels) complex formation of BR-Carr was studied and improved to obtain an isolate of BR by precipitation. Thus, statistical models designs were created. First, was developed a model to exclude non-influent independent variables and narrow ranges, thus in the initial factorial design (Central Composite Design – CCD) were studied four independent variables (protein concentration, polysaccharide concentration, pH and time of contact between protein and polysaccharide) described elsewhere (Phillips and Williams 2009) influents at protein-polysaccharide complexes formation and three dependents variables evaluated. The independent variables that presented no effect on the responses were eliminated and ranges were optimized for the following statistical designs. Box-Behnken Design (BBD) was the followed statistical model design used to optimize the BR, from each extract.

Conclusions - Through the experimental results was possible to concluded that, it was possible to separate and precipitate BR from an aqueous extract maintained its biological activity. High recovery yield – 80-90% - of active bromelain was obtained for both extracts (stems and peels) making possible to obtain 1,3-1,6 g of BR from 100 g of pineapple by-products using a low polysaccharide concentration (0.2-0,3% w/v).

References

1. Cooreman, W. (1978). "Bromelain." Pharmaceutical Enzymes-Properties and Assay Methods: 107-121.
2. Fabian, C., L. Huynh and Y. Ju (2010). "Precipitation of rice bran protein using carrageenan and alginate." LWT-food Science and Technology **43**(2): 375-379.
3. Hari Krishna, S. and N. Karanth (2002). "Lipases and lipase-catalyzed esterification reactions in nonaqueous media." Catalysis Reviews **44**(4): 499-591.
4. Maurer, H. (2001). "Bromelain: biochemistry, pharmacology and medical use." Cellular and Molecular Life Sciences CMLS **58**(9): 1234-1245.
5. Phillips, G. O. and P. A. Williams (2009). Handbook of hydrocolloids, Elsevier.
6. Rowan, A. D. and D. J. Buttle (1994). "[38] Pineapple cysteine endopeptidases." Methods in Enzymology **244**: 555-568.