



TITLE

METHOD FOR EXTRACTION AND/ OR ISOLATION OF BROMELAIN FROM PINEAPPLE

METHOD FOR EXTRACTION OF VEGETABLE ENZYMES FROM VEGETABLE BYPRODUCTS OF FOOD INDUSTRIES

HIGHLIGHTS

Innovative and sustainable technology applied to obtain vegetable enzymes of high biological value obtained through organic residues from the fruit and vegetable processing industries, for future application in the pharmaceutical, nutraceutical and food industries, looking for a circular economy approach.

This technology is an alternative to those currently applied in the enzyme-producing industry, as it eliminates the use of pollutants and potentially toxic chemicals, whose residues are normally present in this type of product (enzymes). Besides that, it allows extracts with high purity, high activity and high extraction yield (ca. 80%).

CONTEXT

Bromelain (BR) is a generic name given to proteolytic enzymes found in vegetable tissues such as peel, stem, fruit and leaves of the Bromeliaceae family, including pineapple stem (*Ananas comosus*). It is usually distinguished as either fruit BR (EC 3.4.22.33) or stem BR (EC 3.4.22.32) depending on its source, with all commercially available BR being derived from the stem. Stem and fruit BR have a molecular weight of 33 kDa and 28 kDa with an isoelectric point of 9.5 and 4.6, respectively (Harrach et al., 1995). The extract of BR has been shown to exhibit its activity over a pH range of 4.5-9.8.

This protease has wide range of applications in food and pharmaceutical industries. Recently, a wide range of therapeutic benefits have been attributed to BR such as the reversible inhibition of platelet aggregation, relief from bronchitis, improved recovery after surgical traumas, and the enhanced absorption of drugs, particularly of antibiotics (Amid et al., 2011). One of the important pharmaceutical applications of BR is the enzymatic debridement of necrotic tissues from ulcers and burn wounds, as well as, medical treatment of cancer patients. In the food industry, has been used as a meat tenderizing enzyme, but also is used in brewing and functional protein at the pre-digestion, digestive aids (Soares et al., 2012).

The biological precipitation can result in concentration and purification of BR, with maintenance of activity. Bringing as advantages easy scale-up, using equipment's relatively simple.

BENEFITS

This technology allows the removal of dangers related with application in products and processes for products used by humans and animals. The technology only uses compounds extracted from nature (biological precipitants), any kind of chemical reagents are added to the process, thus, every secondary effect related with its application is nulled.

On the other hand, it also allows to obtain products with a high degree of purity, in a single step and with a very reduced cost when compared to the existing one, which is very important to



eliminate interferences when creating new/ different products, for example in the food industry in emulsions or gel formulations and /or pastes; in the pharmaceutical industry which enables tablet size reduction (by the elimination of compounds prior to chemical precipitation).

Technology Specifications

Application of biological precipitants in low concentrations

Technology applied at room temperature

No buffering required

No need to add drinking water

No need for ultrafiltration

No need for dialysis

No high temperature baths required for separation of precipitant

Technology Capabilities

Operates in any industrial space without need for temperature and / or humidity control

It allows isolating, extracting and recovering compounds (proteins) with a high degree of purity

It allows to obtain other important ingredients for other processes

Reduces the number of equipment required (when compared to the current ones)

Reduces the number of steps (compared to the current ones)

Reduces process time (when compared to current ones)

It reduces the cost of obtaining the product (when compared to the current ones)

High degree of protein recovery and purification

Absence of chemical contaminants in final products

Absence of effluent treatment processes generated by the technology

It can be applied to several existing processes in different industries

PROOF OF CONCEPT

The use of natural compounds as precipitant agents, where the enzyme can be recovered by inversion of the conditions, is an advantage on the traditional methods.

- It was found that after isolation of br it was maintained the biological activity and increases the thermal stability of the enzyme around 8 °C;
- After biological precipitation, the enzyme can be re-dissolved and recovered;
- Yield of extraction by activity was ca. 85-90% at precipitate;
- The natural/biological precipitant acted as enzyme stabilizer; nontoxic and the food grade.

After application of biological precipitation was possible to dry the desirable enzymatic extract, producing fine yellow powder:



TECHNOLOGY OFFER

- It was maintained the initial features of the extract (enzymatic activity, stability, purity)

POTENCIAL COMMERCIAL USE /APPLICATIONS

Technology with the possibility of application in different complex plant matrices, for proteins extraction, either intra or extracellularly.

Allow extract compounds of high value, without unviabilization of any fractions, being the main fractions used for other propuses.

The proteins can be applied to a large number of products, processes and industries.

INVENTORS AND RESEARCH UNIT

Débora Campos, Maria Manuela Pintado, Guillermo Picó, Nadia Valetti, Lorenzo Castro, José Teixeira

Centre for Biotechnology and Fine Chemistry, Escola Superior de Biotecnologia - Universidade Católica Portuguesa.

COOPERATION OPTIONS

Sell the patent application

Licensing the exploitation rights

PATENT STATUS

Pedido de Patente Europeia: Nº Ep 17174447.7

CONTACTS (ESB)	E-MAIL	PHONE
Debora Campos	dcampos@porto.ucp.pt	+351 22 55 800 01
Manuela Pintado	mpintado@porto.ucp.pt	+351 22 55 800 00