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Purification Processes and Market Potential of Bromelain in Brazil

Diego de Freitas Coêlho[1*], Edgar Silveira[2] and Elias Basile Tambourgi[1]

1. Chemical Engineering School, Campinas State University, Campinas 13083-852, Brazil

2. Institute of Genetics and Biochemistry, UFU (Universidade Federal de Uberlândia), Patos de Minas 38700-128, Brazil

Abstract: Bromelain is the denomination given to the group of endoproteases obtained from members of *Bromeliacea* family. These enzymes have a wide range of proven applications and have been an object of study for worldwide researchers for decades. Over the years, several different downstream processes were studied in order to determine which technique would be worthwhile to be implemented in Brazil and provide the national market with such product. The objective of the present study is to relate the main studies in Brazil that has proven that bromelain purification can be cost-effective, in addition to the well-known benefits owned by such enzymes, and highlight the applications that create their market potential in the Brazilian market.

Key words: Bromelain, market potential, downstream processing, liquid-liquid extraction, aqueous two-phase systems.

1. Introduction

The first description related to isolation and discovery of proteases from tissue of plants belonging to *Bromeliaceae* family was written by the Venezuelan chemist Vicente Marcano in 1891, which work obtained these enzymes from pineapple (Fig. 1) [2] pulp (*Ananas comosus*. Linnaeus Merr [1]). One year later, Chittenden et al. [1] realised an extensive study on this subject, featuring its extract, proteolytic activity and suggesting "bromelain" as denomination to the enzyme in study. Afterwards, this denomination was extended to the set of all proteases obtained from plants belonging to *Bromeliaceae* family, which pineapple belongs.

In 1957, Taussig et al. [3] discovered that pineapple's stem held more bromelain than its pulp. Thus, enzymes from stem of *A. comosus* (*Ananas comosus*) are the most studied [4]. Therefore, bromelain from stem is widely used in food industry, mostly for its thermal stability, which enable enzyme sustain its biological activity at temperatures between 40 ^{o}C and 60 ^{o}C, in which most of enzymes are denatured [5].

The aqueous extract obtained from pineapple processing is a complex mixture of thiol-proteases (including bromelain from stem—EC 3.4.22.32, pulp—EC 3.4.22.33 and the ananain—EC 3.4.22.31) and components without any proteolytic activity [6]. Among these components with no activity are phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates [7]. Moreover, several other proteases from specimens belonging to *Bromeliaceae* family have been isolated and characterized: pinguinain, obtained from *Bromelia penguin* [8, 9]; palmerain, from *B. palmeri* (*Bromelia palmeri*); balansain I, from *B. balansae* [10]; karatasin, from *B. plumieri*; hieronymain I, from *B. Hieronymi* [11]; macrodontain I, from *Pseudananas macrodontes* [12]; penduliflorain I, from *Hohenbergia penduliflora* [13] and various other proteases from *B. antiacantha* [14].

Furthermore, the *curauá* (*Ananas erectifolius* LB Smith [15] (Fig. 2)), typical of Brazilian Amazon region, whose fiber has a wide application in the manufacture of pieces for automotive industries, is another species belonging to the family *Bromeliaceae* and, as expected, shows significant levels of bromelain [16].

[*]**Corresponding author:** Diego de Freitas Coêlho, M.Sc., research field: downstream process. E-mail: dfcoelho@feq.unicamp.br.

Fig. 1 ***Ananas comosus*.**

Fig. 2 ***Ananas erectifolius*.**

2. Bromelain's Purification and Extraction Processes in Brazil

In the development of a purification process, it is necessary to fully exploit the physicochemical properties in which biomolecules differ from each other, in order to develop a suitable separation process [17]. The challenge is to extract the target-molecule from a very specific mixture which all constituents, in its core, have the same main features. Therefore, there is no defined method that researchers should follow to develop a protocol that leads to a purified protein, being necessary whenever a step of laboratorial studies.

Bromelain's purification would not be an exception. Throughout the last two decades, several Brazilian researchers [18, 19] from the state of São Paulo have being studying and developing purification processes that could make possible to extract bromelain from almost all pineapple's parts. Most of researches focused on the study of its purification though ATPS (aqueous two-phase systems) (Fig. 3), which consists in the selective partition of target-molecule to one phase. The partition occurs as result of incompatibilities between properties of a polymer solution and a salt solution, with high ionic force, or between two polymers.

These chemicals constitute a suitable and appropriate medium to the extraction of biological compounds, once both phases have between 70% and 90% of water content, providing an environment that preserves its molecular stability and thus allowing its purification with, virtually, no loss of biological activity [20].

The first study that was entirely dedicated to bromelain at UNICAMP (State University of Campinas) was developed by César [21], which goal was to determine the best conditions to extract bromelain from pineapple extract, evaluating the influence of pH and concentration of components (polyethylene glycol and potassium phosphate) on phasesin process efficiency. Just one year later, Bertevello [22] proposed the use of the same system, but in a micro column of pulsed caps in order to evaluate enzyme recovery in continuous processes. His results showed that 60% of protein content was extracted and 59% of biological activity was held after purification, when compared with values obtained from crude extract.

In the following years, researchers diversified the types of processes and components used on ATPS to purify bromelain. Campese [23] evaluated the use of POLICAJU, a ramified heteropolysaccharide exuded naturally from cashew tree (*Anacardium occidentale* L.) or through incisions in the tree's trunk or branches. Besides, it has provided better results than work cited above (around 70% of total protein and enzyme activity), the POLICAJU is a natural nontoxic polymer of low cost and found largely in the northeast of Brazilian.

There is also a trend to research operational conditions that allows bromelain precipitation from crude extract using ethanol as precipitant agent [24]. The salting-out effect induced by solvent addition causes precipitation of any protein and, if added fractionally, allows that pools of similar proteins precipitate selectively [25]. However, there are some issues related to temperature increase while ethanol is being added that requires more complex control methods [26], and therefore makes this option unattractive.

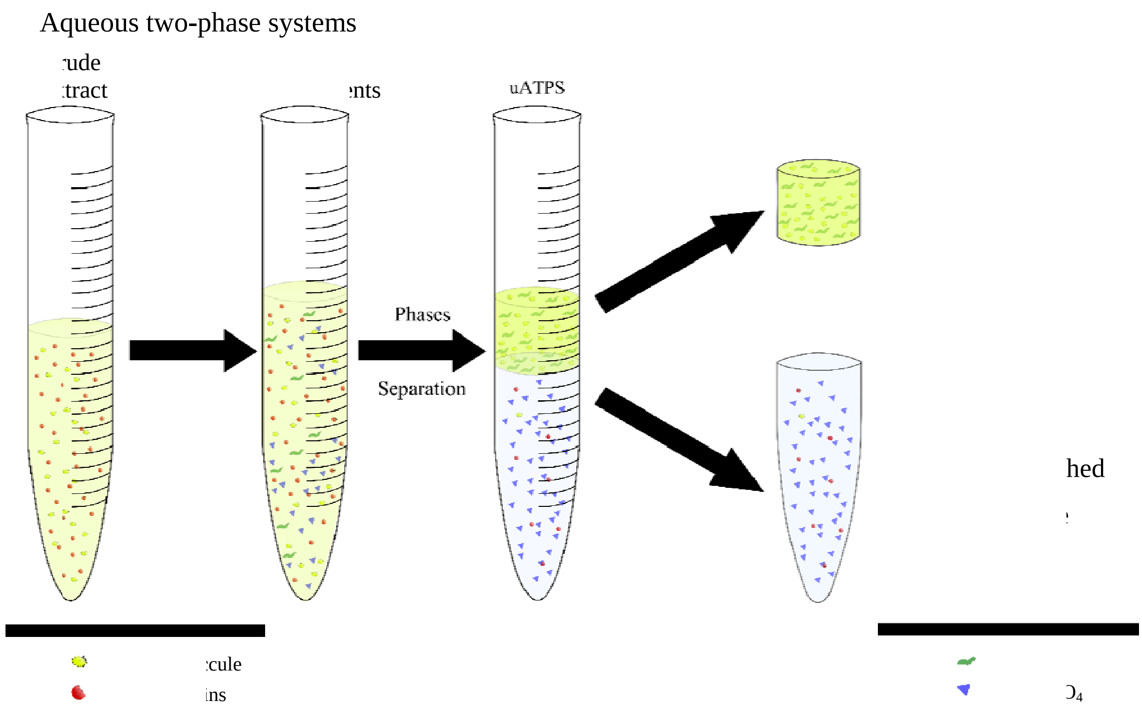

Fig. 3 ATPS illustration.

Fig. 4 Pineapple's residues.

As pineapple is widely used, either by final consumers or by the industry as raw material, much of this research chose agroindustrial waste (from pineapple processing, Fig. 4 [27]), as crude extract to get the target-molecule with same quality as obtained from its pulp.

However, the promising pharmacological use of bromelain, associated with its use in clinical laboratory procedures and Brazilian dependence of exterior market have driven a boost in its researches. Newer techniques were introduced, such as its extraction by reverse micelles in batch systems [28] or expanded bed adsorption [29]. Additional characterisation studies were realised at different temperatures and pH [30] to evaluate activity and thermal stability of bromelain obtained from bark and stem of pineapple [30]. Also, there was an economic feasibility study of extraction processes for that enzyme [16]. Moreover, each new approach reached better results, with respect to either total protein recovery or even enzymatic activity.

Among the most recent works, we can mention that one performed by Coêlho [17], which integrated a precipitation step to a fractional aqueous two-phase system, aiming synergetic effect in a liquid-liquid extraction. The precipitation step has been successfully integrated and has removed mostly of unwanted proteins, increasing purity of the product. The system obtained, that denominated unconventional ATPS, showed excellent results, reaching yields above 80% and purification factor of up to 11.8, that is almost two times more than the purification factor obtained in recent researches [31].

3. Potential in the Brazilian Market

Proteolytic enzymes, or proteases, catalyze the cleavage of peptide bonds in proteins, fragmenting them into simpler compounds such as polypeptides of lower molecular weight or in amino acids [32]. Proteases represent a class of enzymes with important roles in physiological processes and have wide commercial application, being among most used industrial enzymes and are responsible for 60%-65% of international sale of enzymes [33, 34].

In Brazil, processes for extraction and purification of bromelain for use as a pharmaceutical have been studied for over a decade. However, the small number of researchers involved and the restriction to laboratory-scale feasibility of a process that can meet the national market forces Brazilian industry to import it.

While bromelain can be used to replace environmentally harmful processes or the development of new applications in several industries, this manuscript shall highlight those belonging to the food and pharmaceutical industries, whose involvement has stimulated world production of proteases and higher imports by Brazil.

Thus, local production of bromelain will benefit industries that can use it to replace environmentally harmful processes or even on development of new applications, in special those belonging to food and pharmacological areas, whose involvement has stimulated global production of proteases and higher imports by Brazil.

3.1 Pharmaceutical Use of Bromelain

Bromelain, due to side effects absence and its effectiveness after oral administration, earned growing acceptance as a herbal drug and may be found as tablets and capsules [7].

However, clinical studies using bromelain have been done extensively over the past 50 years. In 1962, Seligman demonstrated anti-inflammatory activity of bromelain and, since then, a large number of clinical studies support use of herbal extracts containing bromelain to anti-inflammatory treatment [35].

In addition to anti-inflammatory activity, these enzymes offer a broad spectrum of proven therapeutic applications, that includes antithrombotic and fibrinolytic activities [7]. It also has shown the ability to modify the cell surface structures by peptide and, consequently, to prevent edemas, platelet aggregation and metastasis [36].

Besides, bromelain enhances antibiotic effects, once it increases permeability of tissue [37], enzymatic debridement of necrotic ulcers and burn injuries [38], modulation of cytokines and immune system[39], mucolytic properties, assist digestion, adjunct to wound healing and increased cardiorespiratory fitness [29].

Recent studies have shown evidences that bromelain can be a promising candidate to use in development of enzyme therapies for oncology patients [6], although its anticancer effects appear to be a result of a systemic response that needs to be investigated.

3.2 Its Use in Food Industry

In bakery conventional processes production, the addition of proteases on flour with high protein content makes it more adequate to the continuous processes commonly used, because it requires mixing time rather low and dough with greater extensibility to processing [40], resulting in breads with increased volume and better symmetry, texture and granularity of crumb [32]. According to Cole (1973), the use of proteases may reduce by up to 30% of mixing time without impairing the quality on final product. Furthermore, addition of proteases in cookies processing allows the technologist a better control of problems related to mass retraction and product's excessive hardening caused by agglomeration of gluten-forming proteins.

In the brewing industry, the benefits brought by use of proteolytic enzymes were determined empirically, as they had no idea of how it acts on wort. Then, it was discovered that when added in the final process of brewing, these enzymes hydrolyse tannin-protein complexes formed during fermentation. These complexes become insoluble at lower temperatures, precipitating or clouding the beer (chill-proofing).

The problem then is to establish conditions in which enzyme can hydrolyse these complexes up to polypeptides (and not smaller peptides and amino acids), valuables for flavour and foam retention (quality indicators of beer) [40].

There is also the possibility of using bromelain to promote softening of meat by breaking the present fibrous material. In this context, researchers have devoted their efforts to tenderize meat by injection of proteolytic enzymes in animal carcasses. The method proved to be quite promising, offering only difficulty to distribute enzymes uniformly and requiring the injection prior the death of the animal and, therefore, being distributed throughout the vascular system before slaughter [40, 41].

3.3 Other Applications

Another application for proteases is in the leather industry, in which it could be used to remove skin. This process is much safer and milder than the traditional methods, involving the use of sodium sulphide. However, it requires relatively large amounts of enzyme between 0.1%-1.0% (m/m) and the process must be rigorously controlled in order to avoid decrease of leather quality.

4. Conclusions

Over the years, we could clearly see that the growing use of proteases in the world has stimulated the Brazilian researchers to develop a process for the extraction and purification of bromelain. In addition, bromelain has shown promising applications in several medical segments due to its poorly understood physiological function. So that, some national industries have been organizing and seeking the most appropriate method for bromelain production and/or analysing the current market and future applications of these enzymes.

A major attraction for the production of this group of proteases is the fact that it is possible to extract it from all parts of pineapple and agribusiness offers a great opportunity to add value to the large amount of waste generated (such as leaves, crown, stem and bark), which concentrates appreciable fractions of bromelain.

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