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Purification of Bromelain Enzyme from Curauá (*Ananaserectifolius LB Smith*) White Variety, by Aqueous Two-Phase System PEG 4000/Potassium Phosphate

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Abstract: The Curauá (*Ananaserectifolius LB Smith*) is a species belonging to the family *Bromeliaceae*. Occurs in the states of Para, Amazonas, Amapá, Acre, Mato Grosso and Goiás, has two varieties, purple and white, and its fibers are used in automotive and textile industries due to its strength, softness and lightweight. Currently, only the fiber is used in industry, the rest is considered waste. However, this residue contains compounds with important properties to be discovered and studied. An enzyme complex found in this residue is bromelain, a group of proteolysis' enzymes with application in several areas such as in the food, pharmaceutical and cosmetics industries. Today, its use is aiming the pharmaceutical industry, the production of ointments, gels, creams and lotions because they offer a wide range of therapeutic efficacies: antiedemas, anti-inflammatory, antithrombotic and fibrinolytic activities. This work studied the enzyme purification and recovery presented in the leaves of Curauá by means of an ATPS (aqueous two-phase system) PEG 4000/potassium phosphate. The protein content was measured by Bradford reagent and the enzymatic activity was measured by the Biuret reagent. Batch assays were performed aiming the enzyme extraction and recuperation, using the partition coefficient as indicator. It was used pH 7.0, 8.0 and 9.0, varying the proportional composition between the polymeric and the saline phases (tie-lines). The white variety of Curauá was used. The best purification factor was 4.53 and about 113.04 μg/mL of total protein measured by Bradford.

Key words: Curauá, purification, bromelain.

1. Introduction

Enzymes are organic molecules present in cells of living organisms, with the specific function of catalyzing chemical reactions. The enzyme starts increasing velocity of a chemical reaction by acting as a catalyzer [1]. A catalyzer does not modify the balance of a reaction, however, it reduces the amount of energy required to make the reaction to happen.

Economically viable, the use of enzymes in various industrial sectors has been increasing over the years [2-3]. Among enzymes with industrial use, the bromelain stands out due to its diversity in applications: leather processing in textile industries; softening fibers and detergent production [4]; in food industry providing meat softening [5]; and in breweries it is used to purify beers hydrolyzing tannin-protein complexes produced during fermentation [5].

Bromelain is also used in the pharmacological research area due to its anti-inflammatory effect [6] and its potential to help treating health problems such as angina, indigestion and breathing problems [7]. In

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therapies against cancer, it is used to increase lysis of cancer cells [8].

Curauá (*Ananaserectifolius*) is a little known and studied species. Its cultivation started at Lago Grande de Curuaí, city of Santarém in Para state, and has been expanding to other regions of the same state. Researches have shown that Curauá fiber presents excellent quality, being compared to fiberglass due to its resistance, softness and low weight [9, 10]. In automotive industry, it has been used to produce sunshade for trucks [11].

Currently, the separation and purification process of bioproducts is a very important segment for industries, as it can represent from 80% to 90% of the production cost. Therefore, the development of an efficient and low cost process is highly relevant [12].

The liquid-liquid extraction has been arousing interest as an intermediate separation step that either replaces more expensive methods of separation or reduces the number of separation steps required by the process. This separation process is based on the distribution of the solute between the phases and partial miscibility of the liquids [13].

The liquid-liquid extraction process consists of transferring a substance from a liquid mixture to another immiscible (or partially miscible) liquid phase by putting them in contact. This process is widely used in chemical and pharmaceutical industries, such as in the recovery of antibiotics or organic acids from fermentation broths.

Nevertheless, the application in the purification of proteins is still limited mainly due to the possibility of protein denaturation if in contact with organic solvents, yielding a useless product [14-17]. The ATPS (aqueous two-phase systems) are created by the addition of aqueous solutions of two hydrophilic polymers such as PEG (polyethylene glycol) and dextran or a polymer and a salt, such as PEG and potassium phosphate.

These substances provide the appropriate and convenient method for extracting substances of biological origin, as the formation of phases between 70% and 90% of water provides a bland environment to work with biologically active compounds, preserving their molecular stability and thus, allowing their processing on this medium [18].

The pH in the medium, besides influencing in the maintenance of enzymatic activity, also affects the charge distribution on the surface of the protein. Thus, adjusting the pH of the medium to values close to the IP (isoelectric point) of the protein (pH value where the molecule presents electric charge near zero), the effects that act over the partition in ATPS are reduced [19].

Ferreira [20] analyzed the purification of the bromelain enzyme extracted from the stem of the pineapple (*Ananascomosus*) in ATPS (aqueous two-phase system) PEG/potassium phosphate and got better results using polyethylene glycol 4000, rather than polymer with lower molecular weight (PEG 1500).

Thus, this work had as objective obtaining PF (purification factors) of the bromelain enzyme derived from white variety of Curauá (*Ananaserectifolius LB Smith*) through the ATPS PEG 4000/potassium phosphate.

2. Materials and Methods

PEG with molecular weight of 4000 was obtained from Sigma (Switzerland). Milli-Q-quality distilled water was used. Potassium hydrogen phosphate, comassie brilliant blue G, di-sodium hydrogen phosphate, sodium hydroxide were obtained from Synth (São Paulo, Brazil).

The following equipments were used: Mars electronic scale AL 200; an Arno multiprocessor; pH meter Analyser pH 300; thermostatic bath FANEM; automatic micropipettes and spectro-photometer UV/VIS Spectronic 21D.

The concentration values of PEG and phosphate for each tie line were reproduced according to Ref. [20].

Curauá's leaves were collected from field at São Manoel Experimental Farm in Botucatu, São Paulo. Upon arriving on lab, they were washed with distilled

water, dried with paper towel and stored in plastic bags, refrigerated until use.

2.1 Samples Preparation

Buffer and Curauá's leaves were weighted in 1:1 proportion; then, they were grinded using a high efficiency multiprocessor and the extract was filtered using nylon filter removing fibers and other present particles.

2.2 Preparation of Reactive Biuret

It was dissolved in 500 mL of distilled water, 1.5 g of copper pentahydrate and 6.0 g of tartarate of sodium and potassium sulphate, and it was added 300 mL sodium hydroxide solution 10% under constant agitation, and finally added distilled water for a 1 L solution.

2.3 Determination of Bromelain Activity

In order to determine the activity of bromelain, we used the Biuret reagent and a solution to 5 g/L BSA (bovine serum albumin), this method is based on the reaction of the reactive Biuret, consisting of a mixture of copper and sodium hydroxide with a complexing that stabilizes copper in solution. Readings were taken in a spectrophotometer at 540 nm, and finally calculated the enzymatic activity, according to Eq. (1).

$$AE = \frac{Vreactor(l)\times 10^6(\mu\frac{mol}{l})}{MM_{BSA}\times V_{enzyme}(ml)\times time_{reaction}(\text{min})} \quad (1)$$

where, $V_{reactor}$ is the reactor volume; MM_{BSA} is the molar mass of PEG and V_{enzyme} is the enzyme volume used for the analysis.

Total protein in samples of bottom and top phases were determined by the Bradford method [21].

2.4 Preparation of Buffers

Buffer solutions were prepared in pH 7.0, 8.0 and 9.0, from a solution of mono potassium phosphate dibasic to 15% (w/w).

For this, it was prepared standard solutions of monobasic potassium phosphate (solution A) and bibasic potassium phosphate (solution B) mixing them into a beaker containing silver chloride electrode for measuring the pH, until the desired pH.

2.5 Determination of the Purification Factor

To calculate the *PF* it was used the Eq. (2).

$$PF = \frac{EA}{EA_{crude}} \quad (2)$$

where, EA_{crude} is crude enzymatic activity (U) and *EA* is enzymatic activity of top and bottom phase.

3. Results and Discussions

Tables 1-3 demonstrate the influence of the pH and the concentration of the PEG 4000/potassium phosphate system regarding the purification of the bromelainenzyme derived from white variety of Curauá.

The ATPS that presented better result in the purification of the bromelain enzyme derived from white Curauá was the PEG 4000/potassium phosphate system in pH 7.0 ("tie line" 2 and 3); the PEG 4000/potassium phosphate system in pH 9.0 ("tie line" 1, 2 and 3) also presented considerable values.

These results are based on the values of enzymatic activity and purification factor.

Analyzing the behavior of the three "tie lines" of the ATPS PEG 4000/potassium phosphate in pH 7.0,

Table 1 Partition of the bromelain enzyme derived from leaves of white Curauá using the ATPS PEG 4000/potassium phosphate in pH 7.0.

Tie line		Proteins (μg/mL)	*EA* (U)	*PF*
1	Top phase	18.73	0.004	2.81
	Bottom phase	32.57	0.004	2.82
2	Top phase	22.02	0.006	3.70
	Bottom phase	35.75	0.006	3.80
3	Top phase	30.62	0.007	4.53
	Bottom phase	54.72	0.007	4.29

Table 2 Partition of the bromelain enzyme derived from leaves of white Curauá using the ATPS PEG 4000/potassium phosphate in pH 8.0.

Tie line		Proteins (μg/mL)	*EA* (U)	*PF*
1	Top phase	21.64	0.004	1.36
	Bottom phase	47.88	0.004	1.30
2	Top phase	22.23	0.008	2.23
	Bottom phase	37.74	0.006	1.84
3	Top phase	32.32	0.005	1.41
	Bottom phase	87.74	0.004	1.28

Table 3 Partition of the bromelain enzyme derived from leaves of white Curauá using the ATPS PEG 4000/potassium phosphate in pH 9.0.

Tie line		Proteins (μg/mL)	*EA* (U)	*PF*
1	Top phase	35.39	0.008	2.39
	Bottom phase	113.04	0.007	2.23
2	Top phase	28.97	0.008	2.47
	Bottom phase	74.14	0.008	2.42
3	Top phase	30.65	0.007	2.22
	Bottom phase	68.16	0.008	2.31

it is possible to notice the increasing trend of the purification factors as the length of the mooring lines increases; however, the ATPS PEG 4000/potassium phosphate in pH 8.0presented the inverse behavior; higher values for the purification factors were obtained in lower concentrations of the components of the system.

Sbruzzi [22] showed in her study about the costs of this process (ATPS), using the fiber of curauá, purple and white variations, and the estimated sale price, showing its economic viability, whether it is for use as a pre-process in traditional chromatographic systems or direct commercialization, as an alternative for the bromelain of pineapple.

4. Conclusions

The values of the purification factor of the bromelain enzyme derived from white Curauá were affected by concentrations of the polymeric and saline phases, as well as by the pH value in the medium.

The best result was obtained working with the lowest pH studied in this work but with highest polymeric concentration (“tie line” 2 and 3) of the ATPS PEG 4000/potassium phosphate.

The technique of the aqueous two-phase PEG 4000/potassium phosphate systems proved being promising in the purification of the bromelain enzyme derived from the white variety of the Curauá.

It can be used to extract this proteolytic enzyme during the extraction of the white Curauá from automotive industry, for instance, maximizing profit, reducing environmental impact and the waste.

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