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A N I N T E R N A T I O N A L J O U R N A L

Viability in the Production of a Drug Extracted from *Ananas comosus* by a Flat Membrane System

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ABSTRACT

The aim of this work was to study the production of e bromelain from the Ananas comosus L. Merril, by determining the process conditions using flat membranes. The production system modeling generated a hyperbolical curve and the optimization by response surfaces showed an influence of the transmembrane pressure higher than the pH influence. The cost of the production of bromelain from A. comosus was estimated 9 to 13 times lower than Sigma's retail sales price and 6.5 to 8.5 times lower than when this enzyme was obtained through a liquid-liquid extraction, which showed the economical feasibility of the process.

Key-words: *Ananas comosus*, bromelain, flat membrane, production cost, optimization

INTRODUCTION

The bromelain (EC 3.4.22.5), protease (molar mass 31 kDa) is similar to ficin and to papain. However, contrary to papain and ficcin, bromelain could be considered to be a glycoprotein which is formed in the groups of proteic carbohydrates with simple distinctions (Yasuda 1970).
According to Reguly (2000), vegetable proteinases such as ficcin, papain, bromelain and some microbial proteinases contain the –SH group, sulphidrila group, in the active site, which is essential for its proteolytic activity. Proteolytic enzymes account for 60% of enzymes commercialized, including microbial proteases. The bromelain is obtained from *Ananas comosus* L. MERRIL stems after the harvest of the fruit, although the leaves and the proper fruit also contain it, but in lesser quantity.
The production of bromelain from pineapples has been studied since 1894. According to Doko (1991), bromelain applications are numerous, having major usage in the food, medical, and pharmacology industry.
The purification level of an enzyme depends primarily on the usage to which it is intended. The desired purification depends on the number of phases employed during the process and on the usage to which the final product refers to, which include academic usage, industrial usage, therapeutic usage, etc. There is activity loss at each purification phase, thus, to increase the efficiency, a minimal number of phases must be performed. This way, the choice of the method

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depends on the protein properties and on the purity degree desired (Biazus et al., 2006, 2007, 2009 and 2010; Ferreira et al., 2011; Silveira et al., 2009).

Microfiltration (MF) and ultrafiltration (UF) membranes are widely used in tangential filtrations which aim at concentrating and purifying the enzymatic concentrates. The membranes (MF) are not suitable to retain the macromolecules in solution, if the globular proteins or polysaccharides are too big; however, they are still suitable to recover the cells, if the dimensions are smaller than around 0.3 μm. Membranes (UF) retain colloids and particles in suspension, with proteins and carbohydrates of a molar mass bigger than 300 Da (Malleaville, 1998; Severo Júnior et al., 2007; Lopes, 2005; Passarini et al., 2012).

The work aimed at studying the development of process conditions for production of bromelain from pineapple using a flat membrane system and determining its production cost.

MATERIALS AND METHODS

The concentrated juice from the pineapple pulp (*Ananas comosus* L. MERRIL) was prepared under ambient pressure and temperature conditions using a 650g pulp, which was initially filtered with a thin cotton cloth to retain the scattered solids. The medium pH was adjusted to certain experimental conditions by using a phosphate buffer. The solution volume was adjusted to 1 liter (Severo Júnior et al., 2007; Lopes, 2005; Passarini et al., 2012).

The total protein concentration and the enzymatic activity was determined in the permeate and concentrate by using the methods described by Bradford (1976), Murachi (1976) and Baldini et al. (1993), respectively. The enzyme recovery index $\%Y$ was obtained according to Equation 1, where: $AC_{initial}$ and AC_{final} are the specific enzymatic activities of the initial and final concentrates, respectively.

$$\%Y = \left(\frac{AC_{initial} - AC_{final}}{AC_{initial}} \right) * 100$$

The membrane rejection index (R) to the pineapple proteins was calculated by Equation 2, where: Cp_f and Cp_P are the total protein concentrations in the circulated fluid and in the permeated (Ferreira et al., 2011; Filleti et al., 2009 and 2010; Silveira et al., 2009).

$$R = 1 - \frac{Cp_P}{Cp_f} \quad (2)$$

The experiments were performed in a module of membranes constituted by the flat supports, bearing a membrane sheet on both sides, and separators that were put alternately between the supports, with a feeding flux circulating tangentially to the surface of the membrane (Lopes, 2005). The membranes were made from polyvinyl which had an area of 0.0225 m^2 and pore size area equal to 0.1 µm.

To have a better evaluation of the experiment responses, a type 2^2 experimental planning was made, with two factors (transmembrane pressure and pH) and two levels (-1 and +1). The level -1 as represented by 0.05 bar and pH 7.0 and the level +1 by 0.15 bar and pH 7.5 (Biazus et al., 2007; Curvelo-Santana et al., 2010; Ferreira et al., 2007; Severo Júnior et al., 2007; Silva et al., 2008).

The model was evaluated through the analysis of variance (ANOVA), which made this a statistical analysis of the models validity based on the Gauss curve deviations. The optimization was evaluated by the generation of response surfaces following the methodology of Barros Neto et al. (2007).

The calculations for bromelain production cost were done as described by Giraçol et al., (2011); Martins (2006) and Sbruzzi (2010). of the cost of the pineapple, chemicals, water, dollar rate and energy were those at SEAGESP (2009), Sigma (2006), Vetec (2009), SABESP (2009), Uol-Economia (2009) and ELETROPAULO (2009), respectively.

RESULTS AND DISCUSSIONS

Generally during the tangential filtering through the flat membranes (MF), the permeated fluxes of the process increased with the increase on trans-membrane pressure and it reached at an almost stationary state after 50 minutes of filtering. However, the trans-membrane pressure increased the membrane hydraulic resistance and the resistance due to the fouling, causing the total or partial obstruction of the membrane pores (Lopes, 2005).

The increase of trans-membrane pressure can provoke a modification in the structure conformational of the enzymes, through the membrane pores, decreasing the specific enzymatic activity (Lopes, 2005; Severo Júnior et al. 2007).

While recovering the macromolecules, the recovery index (Y) is normally used as an indicator of the separation degree that occurs in the process. In the bromelain micro-filtration the enzyme was forced into the membrane pores. The membrane rejection average index was 30%, indicating that 70% of the bromelain had passed through the membrane pores.

Results in Table 1 show the statistical analysis of the models validity, based on the experimental results obtained by Lopes (2005) and by using the variance analysis method, described by Barros Neto et al. (2007), for obtaining the empirical model which describes the behavior system in the study.

Table 1 showed that the hyperbolical empirical model had a high multiple correlation and a high rate between the Fcal / Ftab (~155), which, as described by Barros Neto (2007), Benvenga el al. (2011), Biazus et al. (2007), Curvelo-Santana et al. (2010a and 2010b), Evangelista et al. (2010), Ferreira et al. (2007), Severo Júnior et al. (2007) and Silva et al. (2008), has to be of at least 10. This showed that the hyperbolical model was significant and that it had good data to be able to adjust to being used as the empirical form to describe the trans-membrane pressure influence behavior and the pH over the bromelain percentage recovery index (Y%) in the flat membranes system.

Table 1 - Hyperbolical model variance analysis for the bromelain enzyme recovery optimization.

Source	Quadratic Sum	Freedom Degree	Quadratic Average	Fcalc	Ftab
Regression	5483.112	3	1827.704		
Residues	19.885	8	2.486	735.32	4.07
Total	5522.024	11			
% of explained variance =			99.29		
Determination Coefficient (R^2) =			0.9929		

Equation 3 represents the hyper flat model which describes the behavior of the recovery index (Y%) under the influence of factors x_1 (pH) and x_2 (Pressure), after regression by the minimal squares method, described by Barros Neto (2007); Curvelo-Santana et al. (2010a and 2010b); Ferreira et al. (2007); Severo Júnior et al. (2007); Silva et al. (2008). This model shows the response behavior under separate factors and also considers its behavior under multiple influences of the factors.

$$Y\% = -200{,}4211 + 41{,}8652.pH + 529{,}64400.\Delta P_{TM} - 780{,}6720.pH.\Delta P_{TM} \quad (3)$$

Figure 1 shows the level curve generated by the model. The recovery index (Y%) increased significantly with smaller trans-membrane pressure values.

This resulted from the fact that the system pressure elevation caused a rupture of modification in the protein structures, either through the membrane pores passing, or through the rude contact with formed *fouling*. This caused a reduction in the specific activity as it was related to the total protein concentration.

The pH effect was less expressive when they were used in the large enzyme range as described by Lima et al. (2001). To analyze the production costs, survey to the bromelain pharmaceutical product using the MF through flat membranes, expenses with the insurance, depreciation, general material and indirect costs were not taken into consideration (Martins, 2006).

In a study performed on the *SABESP* website (2009) in January, 2009, it was observed that the cost of water consumption and sewage treatment from 11 to 20 m^3 in the city of São Paulo-SP was R$ 3.10/m^3 and R$ 2.46/m^3, respectively.

The values of the reagents were acquired from *Vetec Química Fina Ltda* (Duque de Caxias – RJ) in January 2009, as well as the amount of these reagents spent per liter of the prepared solution. Table 2 shows the cost of the survey per hour and per day. The pineapple cost survey was made through the *CEAGESP* website (2009) in the month of January and there was a cost variation of 2.06-3.14 R$ per pineapple mass (in kg).

Table 2 shows the calculations for the cost calculation of purified proteic material, which contained in part, bromelain enzymes, considering salaries and prices of the product and service values obtained until January, 2009. The concentrated enzyme value was stipulated as 125 mL per hour, that is, 1 L per day. The total cost was elaborated based on the acquisition of one liter of concentrated enzymes; thus, all other expenses with reagents, raw material and services were calculated based on this daily quantity.

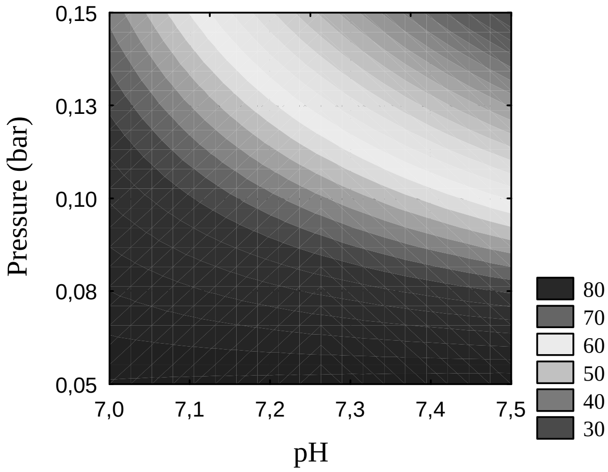

Figure 1 - Level curves generated by the great model, to describe the recovery index behavior (Y%) under influence of the transmembrane pressure and of the pH.

Table 2 - Pineapple bromelain production cost survey.

Item	Price per Unity	Quantity (per h)	Cost (R$/h)	Drug Cost (R$/ L)
NaOH	11.20 R$/kg	0.020 kg	0.22	1.79
FMP	39.49 R$/ kg	~0.005 kg	0.19	~1.50
FDS	32.80 R$/kg	~0.005 kg	0.16	~1.30
Water and sewage	47.17 R$/month	0.001 m^3	0.27	2.14
Perola Pineapple	2.06-3.14 R$/kg	0.325 kg	0.67-1.02	5.36-8.16
Technician*	4.54-6.82 R$/h	1	4.54-6.82	36.36-54.54
Chemical Eng. **	18.18-22.73 R$/h	1	18.18-22.73	145.45-181.82
Energy***	0.27 R$/kW.h	1	0.27	2.15
Total	-	-	**25.16-31.79**	**196.94-254.39**

*Considering 2-3 salaries to the technical level (800-1200 R$/month) and a month of 22 business days.
** Considering 8-10 salaries to the Chemical Engineering (3200-4000R$/month).
***According to *Eletropaulo* (2009) the fare is 0.27 R$/kW (with the financial burdens).

The two last columns in this table referred to the process cost per hour (R$ 25.16 to 31.79) and to the acquisition of a liter of products, that is, the cost per day was (R$ 196.94 to 254.39). Evidently the acquisition of pineapple bromelain obtained through the membrane separation processes appeared to be of low cost.

Table 3 shows bromelain jar prices commercialized by Sigma (2006) to each quantity that the user wishes to buy. The value of 1kg was used as reference for the comparisons in this work, and which was between R$ 2,335.65 and R$ 2,582.07.

Comparison of the data shown in Tables 2 and 3 (showed that the bromelain prices obtained by the membrane separation processes were 9 to 13 times lower than the same enzymes sold by Sigma (2006). The cost prices obtained in this work were also from 6.5 to 8.5 times smaller than the ones obtained by César (2006) that costed R$ 1,650/ kg of bromelain obtained through a liquid-liquid extraction. Sbruzi (2010) found a price of 114R$/L of bromelain from Curauá (*Ananas erectifolius* L. B. Smith) purified by PEG/Phosphate ATPS.

It is also necessary to emphasize that as the membrane quantity in the process increases the product price will decrease abruptly. Using seven membranes, the production cost would decrease to a bit more than R$ 125/L. Hence, this process seemed economically feasible as the cost seemed almost similar to the value reported by Sbruzzi (2010).

Table 3 - Price of the pineapple obtained bromelain. The product contains 30% of total protein (measured by Biuret), with an activity between 3-7 U/mg measured under pH 4.6 and 25°C.

Packing with	Value (U$)
10g	28.70
25 g	47.10
100 g	317.40
500 g	648.50
1 kg	1075.40

*A unity of enzymatic activity (U) corresponds to 1 µmol of paranitrophenol per minute under pH 4.6 and 25°C.
**The Dollar price oscillated from 2.18 to 2.41 in January, 2009 (Uol-Economia, 2009).
Source: Sigma (2006).

CONCLUSIONS

The recovery optimization of the bromelain enzyme by the flat membranes showed the smallest trans-membrane pressure values most suitable for the process, which include pH 7.0-7.5 and pressure if 0.05 bar.

The cost for the acquisition of pineapple bromelains was from 9 to 13 times lower than the price commercialized by Sigma and 6.5 to 8.5 times lower than when the bromelain was produced through a liquid-liquid extraction, which indicated the economic feasibility of the flat membrane separation process to obtain pineapple bromelains.

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