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Batch and Continuous Extraction of Bromelain Enzyme from pineapple juice (*Ananas comosus*)

Sree Nikitha K, Prithika V.S, Akassh V.S Geethanjali K[1], Divya Nair[1], Anitha Shree D[1], & Bindhu J[1]

[1]Department of Biotechnology, Sri Shakthi Institute of Engineering & Technology, Coimbatore– 641062
*Corresponding author, Email ID:bindhubt@siet.ac.in,

Abstract: Bromelain is one of the vegetal proteases found in pineapple plant. It has numerous applications in food and pharmaceuticals. This study was focused on industrial large-scale bromelain purification technique (Reversed micelles) which will assist in determining the effect of processing conditions on the purification efficacy. The purification was carried out in batch extraction and a COCO simulation for continuous extraction. The cationic micellar solution was made of CTAB as a surfactant, isooctane as a solvent and hexanol as a co-solvent. For the batch process, a best purification factor at the values of surfactant agent, co-solvent and KBr concentrations, pH of the back and forward extractions were, 152.18 mM, 10.53 % v/v, 1.24 M, 2.67 and 7.90, respectively. For the continuous operation, optimal process was determined using simulation. This optimal point led to a productivity of 96.128% and a purification fold of 10.87.

Keywords: Reversed micelles, Bromelain, pineapple, Optimization, Response surface, COCO, Purification fold.

Bromelain is one of the protease enzymes found in the pineapple plant (*Ananas comosus*). Stem bromelain is the major protease present in extracts of pineapple stem while fruit bromelain is the major enzyme fraction present in the juice of the pineapple fruit (Kelly et al., 1996). Some other minor cysteine endopeptidases (ananain, comosain) are also present in the pineapple stem bromelain (SBM). Although the fruit bromelain (FBM) was discovered much earlier than stem bromelain, the biochemical characterization of the latter enzyme has been described in more detail (Harrach et al., 1998). The numerous industrial and therapeutic applications of enzymes necessitated their production. In fact, they represent about 60% of all commercial enzymes worldwide. They are widely used in food, pharmaceutical and detergent industries (Feijoo-Siota et al., 2010). It has been extensively used in food industry for meat tenderization, baking processes, protein hydrolysate production, and beer clarification, as food supplement and in prevention of browning of apple juice (Tochi et al., 2008). Similarly, SBM is a highly accepted phyto therapeutic agent. In fact, it had obtained universal acceptability as therapeutic drug. This is owing to its history of effectiveness and safety. It was firstly introduced as a therapeutic compound in 1957. It is not a licensed medical product, thus, it, is freely available to the general public in health food stores and pharmacies in the USA and Europe (Brien et al., 2004). Additional clinical applications of bromelain include modulation of tumor growth, third degree burns and improvement of antibiotic action. It is also used as a drug for the oral systemic treatment of inflammatory, blood-coagulation-related diseases and some 3 malignant diseases. Furthermore, bromelain is involved in the reversible inhibition of platelet aggregation, sinusitis, bronchitis, angina pectoris, thrombophlebitis, surgical traumas, pyelonephritis and improved absorption of drugs especially antibiotics (Maurer et al., 2001). Bromelain has also been successfully used as digestive enzyme in many intestinal disorders. It has been shown that the enzyme serves as adequate replacement of pepsin and trypsin in case of deficiency(Wu et al., 2012).

Pulp, stem and Peels of Pineapple was crushed, filtered and the filtrate was obtained. The filtrate contains the crude bromelain enzyme. The filtrate samples were deep frozen at -5ºC. The cationic micellar solution was made of CTAB (surfactant), isooctane/butanol as a solvent and hexanol as a co-solvent (Hasmann, 2000). The solution for backward extraction should contain the counter ions for CTAB. So, potassium bromide (KBr) was used as counter ion and their concentration used for the process was based on the design of experiments. The samples of pineapple juice were subjected to determine bromelain enzyme activity and the total protein concentration. Pineapple juice and micellar solution were evenly mixed (5 mL each). The mixture was stirred in a glass tube until homogenization (emulsion) occurs. The separation of the phases was performed by centrifuging it at 8000 rpm for 5 minutes. The light phase (micellar) 22 was taken for the backward extraction of the bromelain (Hasmann et al., 1999 and 2000). COCO (CAPE-OPEN to CAPE-OPEN) is a CAPE-OPEN compliant steady state simulation environment. Based on results of the batch extraction process, the input values (Table 4) were entered for continuous extraction process on simulation tool.RMS extracted solution was freeze dried at -20atm for 5hours using ALPHA 1-2 LD plus freeze dryer. The dry sample of BML was subjected for obtaining maximum absorbance using ultra-violet spectrophotometer (LAMBDA 35). The peaks were found at 280nm. Protein content by Lowry's method (Lowry et al., 1951) at 280 nm. A FT-IR spectrometer with 2 cm−1 resolution was used for sample analysis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with standard/extracted enzymes (bromelain). A 20 µL aliquot of the sample obtained from RMS extraction was subjected to analytical high performance liquid chromatography (HPLC) performed on an Agilent Technologies ® apparatus with a UV/vis LC-20A detector. The overall continuous extraction of bromelain form pineapple juice was desinged using coco (cape open to cape open) simulation. The input for the continuous extraction was obtained from the best fit of the RSM of batch extractions. The conditions were optimized in the continuous system using various tools of coco simulation.

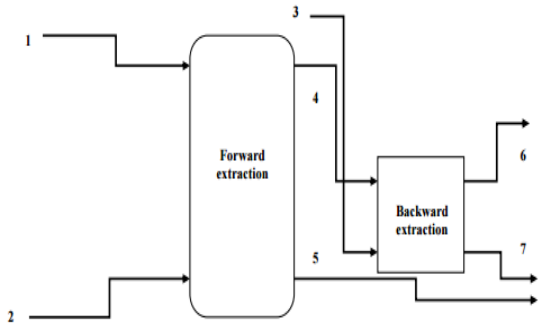

Figure 1: Block diagram for continuous bromelain extraction Where, 1- Pineapple juice; 2- Micellar solution inlet; 3- backward extraction solution inlet; 4- Light phase outlet (raffinated); 5- Heavy phase outlet (extracted); 6- solvent waste; 7- bromelaine outlet.

In order to get maximum yield theoretically, the necessary conditions were altered in coco simulation software. The efficiency of the product varies as the process changes from batch to continuous mode and it decreased considerably in the due course of conversion (Hebbar et al., 2011, Hebbar et al., 2012). But, on using the coco simulation tool of extraction design, the conversion efficiency can be considerably increased. By 38 altering the conditions, the required output efficiency can be altered and the result shows that the increase in efficiency of the continuous process. It was obtained as 96.128% on comparing the cent batch process. The following UV visible spectro photometric graph shows the presence of peak for both the extracted and standard bromelain at same wavelength (280nm). (Swaroop et al., 2013)

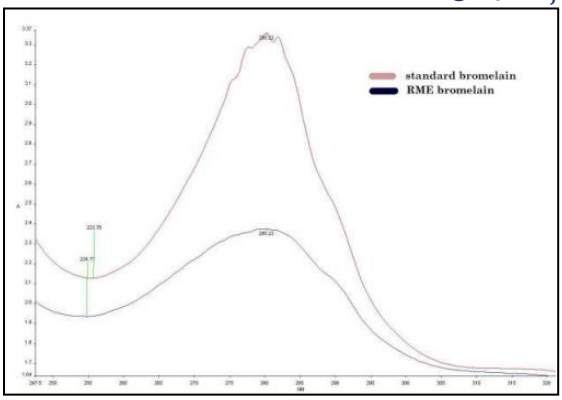

Figure 2: UV-Visible spectrophotometric result

The FTIR spectrum of freeze-dried bromelain is shown in Figure . The characteristic C–N stretch vibration frequencies of mono alkyl guanidinium are assigned to the observed IR bands at 1638, 1425– 1256 and 1053 cm−1 . The band at 1760-1670 cm−1 (s) shows the presence of C=O groups (amides at ~1639 cm−1). This confirms the presence of amino acids that contain amine groups in their side chain, i.e., aspargine and glutamine (Devakate et al., 2009). Bands at 3428 cm−1 for valine are assigned to the N-H stretching mode.

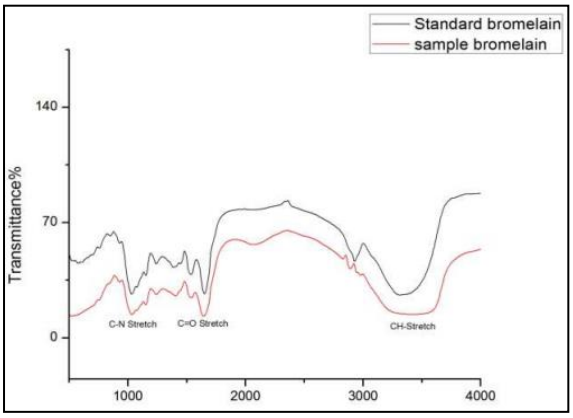

Figure 3: FTIR spectrum of freeze-dried purified bromelain powder

Thus, the crude pineapple juice was subjected to batch extraction in order to obtain bromalein. The presence of bromelain was confirmed using necessary qualitative techniques and the amount of the enzyme along with its activity was estimated. After the execution of batch process, the extraction was subjected to continuous process using the same inputs. Based on the method of coco simulation, it was found that the efficiency of the continuous extraction was nearly equal to that of batch process and the obtained result was 96.128% of the cent batch process. This shows increased efficiency of the continuous process of bromelain extraction when compared to the currently existing methods.

References:

[1].Brien, S., Lewith, G., Walker, A., Hicks, S.M. And Middleton, D. (2004) 'Bromelain as a Treatment for Osteoarthritis: a Review of Clinical Studies' Journal of Evidence-Based Complementary and Alternative Medicine, Vol.1, No.3, pp.251-257.

[2]. .Devakate, R.V. Patil, V.V.Waje, S.S. and x Thorat, S.S. (2009) 'Purification and drying of bromelain', Journal of Separation and Purification Technology, Vol.64, pp.259-264

[3]. .Feijoo-Siota, L. and Villa, T. G. (2010) 'Native and biotechnologically engineered Plant Proteases with Industrial applications', Journal of Food and Bioprocess Technolology, Vol.4, pp.1066-1088.

[4]. Harrach, T., Eckert, K., Maurer, H.R., Machleidt, I., Machleidt, W., and Nuck, R. (1998) 'Isolation and Characterization of Two Forms of an Acidic Bromelain Stem Proteinase', Journal of Protein Chemistry, Vol.17, No.4, pp.351-361

[5]. Hebbar, U. H., Sumana, B., Hemavathi, A. B. and. Raghavarao K. S. M. S (2012) 'Separation and Purification of Bromelain by Reverse Micellar Extraction Coupled Ultrafiltration and Comparative Studies with Other Methods' Journal of Food and Bioprocess Technology, Vol.5, pp.1010-1018

[6].Kelly, G.S. (1996) 'Bromelain: A Literature Review and Discussion of its Therapeutic Applications', Alt. Med. Rev. Vol.1, No.4, pp.243-257

[7]. Maurer, H.R. (2001) 'Bromelain: Biochemistry, Pharmacology and Medical Uses', journal of Cellular and Molecular Life Sciences, Vol.58, pp.1234-1245.

[8]. Tochi, B.N., Zhang, W., Ying, X. and Wenbin, Z. (2008) 'Therapeutic Application of Pineapple Protease (Bromelain): A review', Pakistan Journal of Nutrition, Vol.7, No.4, pp.513-520.

[9]. Wu, S., Hu, W., Zhang, B., Liu, S., Wang, J. and Wang, A. (2012) 'Bromelain ameliorates the wound microenvironment and improves the healing of firearm wound', Journal of Surgical Research, pp.11-1027.

[10]. .Zhang, C, Zhang, K., Zhang, C. and Miao, W. (2009) 'Immobilization of bromelain by embedding in sodium alginate', Chinese journal of bioprocess engineering, pp.2-5