ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 8, pp. 12860-12865, August, 2016

International Journal of Recent Scientific Research

Research Article

AN OVERVIEW OF PURIFICATION AND ACTIVITY OF BROMELAIN

Vinal Pardhi[1]., Prajkta Chivte[2]., Amruta Kothare*[3] and Tejashree Shanbhag[4]

[1,2]National Facility for Biopharmaceuticals, G.N.Khalsa College, Matunga, Mumbai-400019
[3,4]Department of Life Sciences, K.C College, Churchgate, Mumbai 400020

ARTICLE INFO

Article History:

Received 06th May, 2015
Received in revised form 14th June, 2016
Accepted 23rd July, 2016
Published online 28th August, 2016

Key Words:

Bromelain, Purification, Specific activity, Recovery.

ABSTRACT

Bromelain is a mixture of enzymes found in pineapples (*Ananas comosus*) that digests proteins (proteolytic). The active factors involved are biochemically characterized only in part. The mixture of the enzyme can be purified using various techniques such as Precipitation, ATPS (Aqueous Two-Phase System), RMS (Reverse Micellar System), IEX (Ion Exchange Chromatography) etc. The current review discusses methods in relation with enzyme recovery and specific activity of Bromelain enzyme. The implications will be helpful to researchers for selection of suitable purification methods. These methods should be precise, cost effective and reproducible. Based on the said criteria Ammonium sulphate precipitation followed by Dialysis and subsequently by IEX were observed to have minimum loss of activity and higher yields of product were obtained. Bromelain has earned growing acceptance and compliance among patients as a phyto-therapeutical drug due to its efficacy after oral administration, its safety and lack of undesired side effects. This review will help to strategize the purification process for Bromelain and thus use it for various applications.

INTRODUCTION

Bromelain is a crude, aqueous mixture of thiol proteolytic enzymes from *Ananas comosus*, (Linn.) Merr. The pineapple plant belongs to Bromeliaceae family. The reason for synthesis of Bromelain in pineapple plants is still a mystery in plant science. The pineapple plant grows as an epiphyte in the forest. The rosette like arrangements of pineapple leaves develop funnel type rain water reservoirs called phytotelmata which are always filled with water, nitrogen and phosphorous suppliers. It is hypothesized by few scientists that the Bromelain production is the stimuli to various stress conditions confronted by the plant. (Bhattacharyya 2008).

Bromelain is accumulated in the entire pineapple plant in different proportions and with properties depending on its source. (Abdulrahman Ali 2015). Mostly it is distinguished as either Fruit Bromelain (EC 3.4.22.33) or Stem Bromelain (EC 3.4.22.32), with all commercially available being derived from the stem. The Stem Bromelain further possesses two types of proteases namely Ananain (EC 3.4.22.31) and Comosain. The molecular weight of the purified proteases was found within the range of 23.4-35.7 kDa, 31.00 kDa, 23.4 kDa and 23.5-24.5 kDa of Stem, Fruit, Ananain and Comosain respectively. Similar proteases are also present in other part of pineapple such as peel, core, crown and leaves which are considered as pineapple wastes. However, maximum proteolytic activity was detected in the crown extracts of pineapple plant.(Arshad *et al*. 2014)

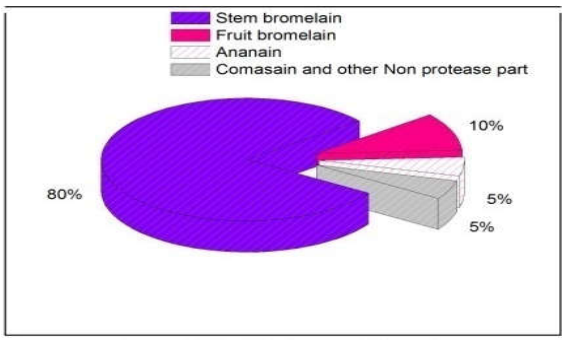

Figure 1 Percentage constitution of proteases in pineapple plant

The percentage constitution of major components of Bromelain proteases and their types is shown in Fig.1. Among the non-protease components of Bromelain are the phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates. (Nadzirah *et al*. 2013)

Separation or purification of bio molecules such as proteins, enzymes, DNA, lipids and other metabolites from the complex mixtures always remains as a major concern for researchers as well as industry personnel. Moreover, with respect to proteins and especially enzymes, it is complicated because of its structural complexities and structure-function relation.

*Corresponding author: **Amruta Kothare***
Department of Life Sciences, K.C College, Churchgate, Mumbai 400020

Thus, this review aims to build an insight towards the methods that are significant to purify the target protein i.e Bromelain, without hindering its activity potential.

As enzymes are liable to either structural modification or aggregation, it is of paramount importance to strategize the purification process. Enzymes vary in their activity pattern at different physico-chemical parameters such as pH, temperature, ionic strength etc. Therefore it is necessary to optimize the process considering the properties of the enzyme and its working conditions. The optimized process should get extrapolated conveniently at large scale with minimum loss of target proteins/enzymes with respect to yield and activity both.
For Bromelain purification the literature has described the use of ammonium sulphate precipitation, organic solvents precipitation, reverse micellar systems, conventional polymer-salt, polymer-polymer aqueous two-phase systems (ATPS), and various chromatographic techniques.(F. A. Vicente *et al*. 2015) Bromelain has been widely used in food industry for tenderizing meat and chill proofing beer, leather tanning process and in latex manufacturing, skin care product, and pharmaceuticals. (Mulyono *et al*. 2013)

Clinical applications of Bromelain particularly modulation of tumour growth, blood coagulation, improvement of antibiotic action and anti-inflammatory properties are well known. Also, Bromelain is absorbed well in human intestine and shows no negative impact on health even after prolonged use. Studies have shown that Bromelain may help in the treatment of several disorders like bronchitis, angina pectoris, sinusitis, thrombophlebitis, pyelonephritis and cancer. (Abdulrahman Ali 2015)

Extraction, concentration and purification methods

The solubility of globular proteins increases upon the addition of salt (<0.15 M), an effect termed salting-in. At higher salt concentrations, protein solubility usually decreases, leading to precipitation; this effect is termed salting-out. The mechanism of salting-out is based on preferential solvation due to exclusion of the co-solvent (salt) from the layer of water closely associated with the surface of the protein (hydration layer).(Wingfield 2016)

Although Bromelain proteases are the major constituents of the pineapple and its extraction from the fruit is easy, the concentration of the target molecule is necessary in order to purify and further study its properties.

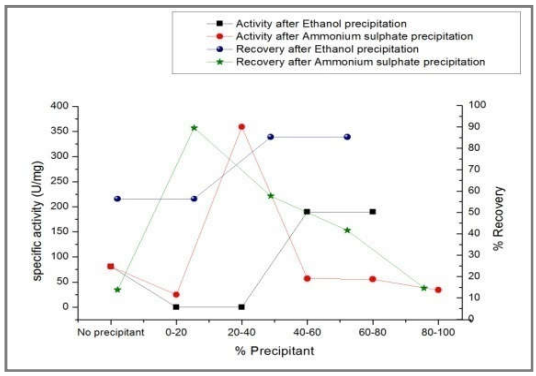

Figure 2 Bromelain activity and Recovery profile at various concentration of precipitant

Attempts were made since many years to extract Bromelain proteases from pineapple with suitable recovery and activity using various methods like Salt and organic precipitation, aqueous two phase extraction and reverse micellar system.

Figure 2 describes activity and recovery profile of Bromelain w.r.t. salt (Ammonium Sulphate) and organic (Ethanol) precipitation methods. The highest activity and recovery was obtained at salt concentration within the range of 20-40%. However, as the salt concentration increased further, decrease in the activity and recovery was observed. At low concentration of ethanol the enzyme activity was low, as the concentration increases the activity and recovery increased.(Bresolin *et al*. 2013), (Soares *et al*. 2011)

The fold of purification was observed to be 4.4 fold after salt precipitation at 20-40% and w.r.t organic precipitation at 30-70% concentration 2.34 fold. No precipitation was detected when PEG (Polyethylene glycol) was used as a precipitant.(Soares *et al*. 2011)

Aqueous Two-Phase System (ATPS)

It is important to design an economic and efficient process for isolation and purification of Bromelain which would be devoid of any contaminants and can be applicable at a large scale, retaining the biological activity of the bio-product. Aqueous two phase systems (ATPS) have overcome these demands and emerged as a powerful method for the downstream processing of bio-products. (Ratanapongleka 2010). Aqueous two-phase extraction (ATPE) is a unique liquid-liquid extraction, involving transfer of solute from first aqueous phase to another. Proteins can be recovered using ATPE by polymer-polymer type and polymer-salt type systems. The protein must be recovered in a highly purified form in order to improve its quality, decrease energy consumption, reduce waste and minimize costs.(Hong Yang 2013)

Ferreira *et al*. (2006)studied the enzyme characterization and recovery present in the pineapple stem and peel, by ATPS liquid-liquid extraction. In the year 2013, De Lencastre Novaes *et al*. (2013), focused on the Bromelain extraction from waste part using PEG/polyacrylic acid (PAA) ATP system and attained very high yield and purification fold too, which was found to be significant with support of statistical analysis.

Reverse Micellar System (RMS)

Micelle is an aggregate of molecules possessing both polar and non-polar regions. Reverse micelles are thermodynamically stable, minute surfactants that hold water in their interior surrounded by organic phase. Only protein of interest is entrapped in micelle whereas other impurities remain in organic phase. Reverse micellar system is an encouraging strategy for downstream processing. It is ideal method to extract biomolecules through diluted samples. Reverse micellar system possesses higher sample loading capacity, are specific and easy to operate.(Manzoor *et al*. 2016)

It has been reported that the RMS of CTAB (cetyltrimethylammonium bromide)/isooctane/hexanol/butanol and AOT (Dioctyl sulfosuccinate sodium salt)/isooctane were used for the extraction and primary purification of Bromelain from crude aqueous extract of pineapple wastes. The effect of forward as well as back extraction process parameters was

studied. The optimized conditions for the extraction from core resulted in forward and back extraction efficiencies of 45% and 62%, respectively, using CTAB. Recovery (106%) and purification (5.2-fold) of Bromelain was obtained under these conditions. RMS extraction from peel, extended stem and crown using CTAB system resulted in purification folds of 2.1, 3.5, and 1.7, respectively.(Nadzirah *et al*. 2013)

Dhaneshwar *et al*.(2014) applied RMS for the purification of stem Bromelain using sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/iso-octane system. Maximum forward extraction efficiency of 58.0% was obtained at 100 mM AOT concentration, aqueous phase pH of 8.0 and 0.2 M NaCl. Back extraction studies on altering stripping phase pH and KCl concentration, addition of counter-ion and iso-propyl alcohol (IPA) and mechanical agitation with glass beads indicated that IPA addition and agitation with glass beads have significant effects on extraction efficiency. The protein extraction was higher (51.9%) in case of the IPA (10% v/v) added system during back extraction as compared to a CTAB (100 mM) added system (9.42%).

Study of Wan *et al*. (2016), focused on extracting Bromelain from pineapple peel by using reverse micelles. It was found that gemini surfactant C_{12}-8-C_{12}2Br (octamethylene-a,x-bis(dimethyldodecylammonium bromide)) showed distinctive advantage over its mono-meric counterpart DTAB (dodecyl trimethyl ammonium bromide); under optimized condition. The optimum specific activity of the recovered Bromelain was 11,097 CDU/mg when gemini surfactant was used and 5774 CDU/mg when DTAB was used. Therefore, it can be concluded that gemini surfactant was more efficient than monomeric surfactant.

Chaurasiya *et al*. (2014), used Reverse micellar extraction (RME) for the separation and purification of Bromelain from pineapple core and compared the efficacy of RME purified Bromelain (RMEB) with commercially available Stem Bromelain in tenderization of beef meat in which they observed that RME resulted in reasonably high Bromelain activity recovery (85.0 %) and purification fold (4.0).

Hebbar *et al*. (2011), came up with slight modification in the RME extraction of Bromelain with the use of CTAB/Iso-octane/1-hexanol/1-butanol as organic phase with equimolar ratio of aqueous phase. Extract obtained from pineapple core employing the conditions indicated in materials and methods, had pH value 4.2, protein content 15.5 mg/g of core with Bromelain activity of 162.0 U/g of core Bromelain activity recovery and purification fold were found to be as 85.0 % and 4.0, respectively.(Chaurasiya *et al*. 2014)

Aqueous Two-Phase Micellar System (ATPMS)

Two-phase aqueous micellar systems can be exploited in separation sciences for the extraction/purification of desired biomolecules.(Rangel-Yagui *et al*. 2004). As described earlier in this review, both RMS and ATPS could be the best techniques for extraction of Bromelain from pineapple, not only from fruit or stem but also from waste parts of it. Thus, thought was given to use the combination of both such powerful techniques and effectively extract active Bromelain at maximum yield.

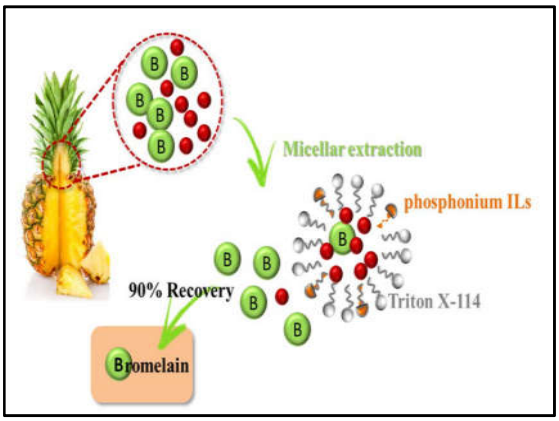

Figure 3 Representation of Bromelain Micellar extraction using Triton X-114(F. a. Vicente *et al*. 2015)

Spir *et al*. (2015), studied the recovery of Bromelain from pineapple peel by liquid–liquid extraction in ATPMS, using Triton X-114 (TX-114) and McIlvaine buffer. AMTPS have been proposed as alternative purification techniques when proteins and/or enzymes are involved, principally due to their outstanding ability to maintain the native conformations and biological activities of the target molecules. One of the study describes the use of ATPMS composed of ioninc liquids as a cosurfactants in the extraction of Bromelain from the pineapple stem. The main results showed that Bromelain partitions preferentially towards the micelle-poor phase, with enzyme recoveries above 90%. (F. A. Vicente *et al*. 2015)

Chromatographic techniques

Various purification strategies were implemented to purify and separate Bromelain from mixture of proteins. Techniques such as precipitation, Dialysis followed by Ion exchange based separartion, and in some studies further fractionation was done on Gel Filtration Chromatography. Mohapatra *et al*.(2013), purified Bromelain from waste parts of the pineapple (Peel, Pulp and Stem) using DEAE cellulose, an Anion exchanger, at pH 8.0, using counter ions containing buffer as eluent. The eluted fractions were assayed for the activity. The purification fold after ion-exchange chromatography was 6.27, 9.89 and 4.48 while the percentage purification was 1193.5%, 1423.29% and 2185.50% for Stem, Pulp and Peel respectively. Babagana *et al*.(2015), used three step purification strategies to enhance the percent yield of purification. They carried out ATPS using PEG followed by salt precipitation followed by ion exchange (DEAE) purification. The purification fold achieved for stem and fruit Bromelain was 0.23 and 4.64 while their percentage yields were 23.4% and 463% respectively. The outcome of their study was higher purification fold of stem Bromelain. It could probably be due to the structural modifications of the enzyme active sites in the presence of an unidentified component in the purification step. The fraction of crude pineapple stem powder soluble in sodium acetate, pH 5.0, was eluted from an S-Sepharose column with a step gradient of NaCl. The fraction containing Ananain and Comosain was applied to a second S-Sepharose column and eluted with NaCl in sodium acetate, pH 5.0, after the column had been washed with Na_2CO_3 pH 9.0. The composition of the starting material and products was analysed by cation-exchange chromatography on a Mono S column.(Rowan *et al*. 1990)

High Speed Counter Current Chromatography (HSCCC) is another technique, in which the stationary phase is liquid instead of solid, provides a lot of advantages over other chromatographic techniques. Advanced centrifugal partition technology is used to hold the liquid stationary phase in the column, while the liquid mobile phase is pushed through it, which provides high yield and purity. It is applied for separation of dipeptides and proteins, flavonoids, alkaloids, etc, proves the versatile and dynamic nature of the technique. (Sethi *et al*. 2009). The crude protein in pineapple fruit was purified by high-speed counter-current chromatography(HSCCC).The results showed that pure Bromelain with one band analysed by SDS–PAGE was successfully separated from pineapple fruit using the aqueous phase with a pH of 6.0, 7.0 and 8.0, respectively.(Yin *et al*. 2011)

Costa *et al*. (2014) performed two step purification of Bromelain and obtained a better fold in proteolytic activity. Initially, cation exchange purification followed by gel filtration chromatography outcome of which was 16.93 fold purification with 89% recovery of target protein.

DISCUSSION AND CONCLUSION

Precipitation provides ease to concentrate the target molecule in the solution containing other proteins or contaminants. The concentration of the precipitant is an essential parameter in protein chemistry. Salt precipitation commonly utilises Ammonium sulphate, yields in better activity and recovery. In Fig.2.0, it can be seen that the Bromelain precipitates at 20-60% ammonium sulphate with specific activity of 100-350 U/mg which is maximum than higher salt concentrations. Furthermore, the recovery profile drops down at higher salt concentration. 90% Recovery was obtained when Bromelain precipitation was performed at 20% of salt which makes it a more profitable process for large scale purification. Salt precipitation facilitates the concentration of Bromelain yields in the 4.4 fold purification. Hence, 20% of ammonium sulphate for Bromelain precipitation could be the optimum concentration.

Bromelain also precipitated with Organic solvents such as Ethanol, Acetone etc. In Fig.2.0, Specific activity profile of Bromelain post-precipitation is showing increasing pattern from low to high Ethanol concentration, numerically, 0 U/mg at 0-20% to 150 U/mg at 40-60% of Ethanol. But the recovery profile shows a reverse pattern i.e. at low concentration of ethanol recovery is highest and it drops down with slight change from 20-80% of Ethanol. At a concentration of 30-70%, 2.34 fold purification is achieved with Ethanol. Hence, the optimum range for Ethanol precipitation of Bromelain could be 30-70% of Ethanol Concentration which results in better recovery and specific activity.

Bromelain can also be extracted from waste parts of pineapple in a convenient and cost-effective way with ATPE system formed by PEG/PAA. Bromelain partitioned preferentially to the top/PEG-rich phase under optimum conditions, achieved a yield of 335.27% with a purification factor of 25.78. The parameters which could affect the Bromelain yield and purification were PEG and PAA concentrations, PEG and PAA molar mass, salt (Na_2SO_4) concentration and temperature and thus considering them to be significant to the process.

Proteins precipitate with polyelectrolytes (PAA), and thus to prevent precipitation of Bromelain, it becomes mandate to use and optimize the salt ions concentration which will decrease electrostatic interaction and thus prevents precipitation of target molecule. Usually, high salt concentration will prevent precipitation even in presence of polyelectrolyte. Parameters like temperature, polymer concentration were optimized based on the stability of the molecule as described in the literature earlier. Molar mass of PEG used in the extraction has a negative effect on Bromelain extraction using ATPE system. Bromelain extraction recovery and purification is also affected by the molecular weight of the PEG, used for the extraction. Amongst the various molecular weights PEG ranging from 2000-6000 Da and concentrations (10-18%), 18% concentration of PEG 6000 along with 15% Ammonium sulphate results into 62% recovery. As the molecular weight of the PEG increases, the possibility of Bromelain binding to the polymer increases, which could be the probable reason to get higher recovery than lower molecular weight PEG based extractions.

Nadzirah *et al*. (2013) obtained 106% recovery and 5.2 fold of purification when Bromelain from core was extracted using RMS of CTAB/Isooctane/Hexanol/Butanol and AOT/Isooctane under optimized conditions. Recently, Dhaneshwar *et al*. (2014), applied RMS for the purification of stem Bromelain using sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/iso-octane system, in which the focus was on extraction of Bromelain but using mechanical agitation with glass beads which improves the back extraction efficiency. Wan *et al*. (2016) compared the efficiency of two Micellar systems for the extraction of Bromelain. Out of the Gemini surfactant (octamethylene-a,x-bis(dimethyldodecylammonium bromide)) and DTAB (dodecyl trimethyl ammonium bromide); the first one gives superior activity of 11,097 CDU/mg as compared to other 5774 CDU/mg, hence, Gemini surfactant proved to be more efficient. Chaurasiya *et al*. (2014), achieved high Bromelain activity recovery (85.0 %) and purification fold (4.0) using RME. Hebbar *et al* (2008) came up with slight modification in the RME extraction of Bromelain with the use of CTAB/Iso-octane/1-hexanol/1-butanol as organic phase with equimolar ratio of aqueous phase. Use of ATPMS system gives high grade results for Bromelain extraction from waste part of pineapple.

Most of the studies have been performed using Anion exchanger for Bromelain purification but it can also be separated using Cation exchanger at different pH followed by Gel filtration. Although multistep purification gives higher specific activity it affects the total recovery of the enzyme as each additional step accounts for loss for enzyme. HSCCC is one of the recent methods applied for Bromelain purification in addition to above listed methods.

The review gives an insight to various methods applied for Bromelain purification. Basic requirement for any purification technique should be efficient product recovery, cost effectiveness and minimum loss of enzyme activity. On this account, the preferred concentration method is ammonium sulphate for concentration of Bromelain. ATPE is the most suitable process for extraction of Bromelain. Anion exchange chromatography can be used to purify Bromelain. The purified

Bromelain can be then applied in various preparations, nutraceuticals and therapies etc

Acknowledgements

We duly acknowledge National Facility for Biopharmaceuticals, G.N.Khalsa College, Matunga, Mumbai-19 for infrastructure support and kind co-operation throughout the research of the review process.

References

Abdulrahman Ali, Ali. 2015. "Antimicrobial Effects of Crude Bromelain Extracted from Pineapple Fruit (Ananas Comosus (Linn.) Merr.)." *Advances in Biochemistry* 3(1):1.

Arshad, Zatul Iffah Mohd *et al*. 2014. "Bromelain: An Overview of Industrial Application and Purification Strategies." *Applied Microbiology and Biotechnology* 98(17):7283–97.

Babagana, Kamaluddeen and Muntari Bala. 2015. "Comparative Study on The Extraction and Purification The Stem and Fruit Bromelain From Pineapple (Ananas Comosus)." *Journal of Natural Sciences Research www* 5(18):2225–2921.

Bhattacharyya, Barun K. 2008. "Bromelain: An Overview." *Indian Journal of Natural Products and Resources* 7(4):359–63.

Bresolin, Iara Rocha Antunes Pereira, Igor Tadeu Lazzarotto Bresolin, Edgar Silveira, Elias Basile Tambourgi, and Priscila Gava Mazzola. 2013. "Isolation and Purification of Bromelain from Waste Peel of Pineapple for Therapeutic Application." *Brazilian Archives of Biology and Technology* 56(6):971–79.

Chaurasiya, Ram Saran, P. Z. Sakhare, N. Bhaskar, and H. Umesh Hebbar. 2014. "Efficacy of Reverse Micellar Extracted Fruit Bromelain in Meat Tenderization." *Journal of Food Science and Technology* 52(6):3870–80.

Costa, Helber B., Patricia M. B. Fernandes, Wanderson Romão, and José a. Ventura. 2014. "A New Procedure Based on Column Chromatography to Purify Bromelain by Ion Exchange plus Gel Filtration Chromatographies." *Industrial Crops and Products* 59:163–68. Retrieved (http://dx.doi.org/10.1016/j.indcrop.2014.04.042).

Dhaneshwar, Amrut D., Ram Saran Chaurasiya, and H. Umesh Hebbar. 2014. "Process Optimization for Reverse Micellar Extraction of Stem Bromelain with a Focus on Back Extraction." *Biotechnology Progress* 30(4):845–55.

Ferreira, Juliana Ferrari, Iara Rocha P. Bresolin, Edgar Silveira, and Elias Basile Tambourgi. 2006. "Purification of Bromelain from Ananas Comosus by PEG / Phosphate ATPS." *Enzyme*.

Hebbar, H. Umesh, a. B. Hemavathi, B. Sumana, and K. S. M. S. Raghavarao. 2011. "Reverse Micellar Extraction of Bromelain from Pineapple (Ananas Comosus L. Merryl) Waste: Scale-Up, Reverse Micelles Characterization and Mass Transfer Studies." *Separation Science and Technology* 46(10):1656–64.

Hong Yang, Arafat M. Goja. 2013. "Aqueous Two-Phase Extraction Advances for Bioseparation." *Journal of Bioprocessing & Biotechniques* 04(01):1–8. Retrieved (http://www.omicsonline.org/aqueous-twophase-extraction-advances-for-bioseparation-2155-9821.1000140.php?aid=21538).

De Lencastre Novaes, Let??cia Celia, Val??ria De Carvalho Santos Ebinuma, Priscila Gava Mazzola, and Adalberto Pessoa J??nior. 2013. "Polymer-Based Alternative Method to Extract Bromelain from Pineapple Peel Waste." *Biotechnology and Applied Biochemistry* 60(5):527–35.

Manzoor, Zoya, Ali Nawaz, Hamid Mukhtar, and Ikram Haq. 2016. "Bromelain : Methods of Extraction , Purification and Therapeutic Applications." 59(December):1–16.

Mohapatra, Anannya, V. Madhava Rao, and Mala Ranjan. 2013. "Comparative Study of the Increased Production & Characterization of Bromelain from the Peel, Pulp & Stem Pineapple (Anannus Commas)." 2(8):249–79.

Mulyono, Noryawati *et al*. 2013. "Quantity and Quality of Bromelain in Some Indonesian Pineapple Fruits." *International Journal of Applied Biology and Pharmaceutical Technology* 4(2):235–40.

Nadzirah, K. Z., S. Zainal, a. Noriham, and I. Normah. 2013. "Efficacy of Selected Purification Techniques for Bromelain." *International Food Research Journal* 20(1):43–46.

Rangel-Yagui, C. O., a. Pessoa-Jr, and D. Blankschtein. 2004. "Two-Phase Aqueous Micellar Systems - an Alternative Method for Protein Purification." *Brazilian Journal of Chemical Engineering* 21(04):531–44.

Ratanapongleka, Karnika. 2010. "Recovery of Biological Products in Aqueous Two Phase Systems." *International Journal of Chemical Engineering and Applications* 1(2):191–98. Retrieved (http://www.ijcea.org/show-23-296-1.html).

Rowan, a D., D. J. Buttle, and a J. Barrett. 1990. "The Cysteine Proteinases of the Pineapple Plant." *The Biochemical journal* 266(3):869–75.

Sethi, Neha *et al*. 2009. "High Speed Counter Current Chromatography: A Support-Free LC Technique." *Journal of Pharmacy And Bioallied Sciences* 1(1):8.

Soares, Paulo *et al*. 2011. "Studies on Bromelain Precipitation by Ethanol, Poly (Ethylene Glycol) and Ammonium Sulphate." *Chemical Engineering Transactions* 24:979–84.

Spir, Lívia Genovez *et al*. 2015. "Application of an Aqueous Two-Phase Micellar System to Extract Bromelain from Pineapple (Ananas Comosus) Peel Waste and Analysis of Bromelain Stability in Cosmetic Formulations." *Biotechnology progress*. Retrieved (http://www.ncbi.nlm.nih.gov/pubmed/25919128).

Umesh Hebbar, H., B. Sumana, and K. S. M. S. Raghavarao. 2008. "Use of Reverse Micellar Systems for the Extraction and Purification of Bromelain from Pineapple Wastes." *Bioresource Technology* 99(11):4896–4902.

Vicente, Filipa A., Luciana D. Lario, Adalberto Pessoa, and Sónia P. M. Ventura. 2015. "Recovery of Bromelain from Pineapple Stem Residues Using Aqueous Micellar Two-Phase Systems with Ionic Liquids as Co-Surfactants." *Process Biochemistry*. Retrieved (http://dx.doi.org/10.1016/j.procbio.2016.01.004).

Vicente, Filipa a., Luciana D. Lario, Adalberto Pessoa, and Sónia P. M. Ventura. 2015. "Recovery of Bromelain

from Pineapple Stem Residues Using Aqueous Micellar Two-Phase Systems with Ionic Liquids as Co-Surfactants." *Process Biochemistry*. Retrieved (http://dx.doi.org/10.1016/j.procbio.2016.01.004).

Wan, Jing, Jingjing Guo, Zhitong Miao, and Xia Guo. 2016. "Reverse Micellar Extraction of Bromelain from Pineapple Peel - Effect of Surfactant Structure." *Food Chemistry* 197:450–56. Retrieved (http://dx.doi.org/10.1016/j.foodchem.2015.10.145).

Wingfield, Paul T. 2016. "Protein Precipitation Using Ammonium Sulfate." 1–10.

Yin, Lianhong *et al*. 2011. "Preparative Purification of Bromelain (EC 3.4.22.33) from Pineapple Fruit by High-Speed Counter-Current Chromatography Using a Reverse-Micelle Solvent System." *Food Chemistry* 129(3):925–32. Retrieved (http://dx.doi.org/10.1016/j.foodchem.2011.05.048).

****

How to cite this article:

Vinal Pardhi *et al*.2016, An Overview of Purification and Activity of Bromelain. *Int J Recent Sci Res.* 7(8), pp. 12860-12865.