International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 2 Feb 2022, pp: 623-628 www.ijaem.net ISSN: 2395-5252

Investigation on Two Phase Aqueous Extraction of Bromelain from Pineapple

Logeshwaran K S[1],Madhankumar S[2],Sarannath S[3],Harikaran M[4]

IV Year Chemical Engineering,Adhiyamaan College Of Engineering
Yuvaraj S[5] ,Assistant Professor, Department of chemical Engineering, Adhiyamaan College Of Engineering
Corresponding Author:logesh1661@gmail.com

Submitted: 01-02-2022 Revised: 07-02-2022 Accepted: 10-02-2022

ABSTRACT: It is possible to find bromelain in pineapple juice and its stems.Its properties include antibiotic,anti inflammatory,anti cancer and anti thrombotic.Sometimes bromelain used as a medicine.It is used for pain,treatment of sinusitis,surgicaltrauma,angina pectoris.Pineapple core has a tougher structure and is one of the most common waste products in the pineapple industry,from these wastes of pineapple Bromelain is extracted.At the pH of 8,Bromelain has its highest relative activity at the temperature of 60◦C.The enzyme activity is 17 percent greater in the top phase. 20 percent MgSO4 in PEG-5000.Here Bromelain was extracted from pineapple core using an aqueous two-phase extraction method. It is a simple method for separating proteins from vast amounts of crude suspensions. After separating the two phases in an aqueous two-phase extraction, analyzed volumes of the top and bottom phases.Upper phase is Poly ethelyne glycol enriched and lower base is Salt enriched.Increase in protein concentration and molecular weigth of protein decrease their separation in the upper phase.

KEYWORDS: Bromelain enzyme,Pineapple,Two phase aqueous extraction.

I. INTRODUCTION:

All fresh pineapples contain bromelain, an enzyme extracted from the pineapple stems, although it may be found in any part of the fruit.Bromelain is a natural remedy for lowering pain and swelling after surgery or injury, particularly in the nose and sinuses, gums, and other body regions. It's also used to treat osteoarthritis, cancer, digestive issues, and muscular pain.From Pineapple only 40% is utilized as juice,jams,squash,pickles and jellies,The rest 60% of the fruit Pineapple is waste which is described as core,peel,crown and stems.These wastes have been discovered to have potential applications as valuable goods.In these wastes bromelain is extracted

By the technique of two phase aqueous extraction,bromelain is extracted from Pineapple.Aqueous two phase extraction is a economically feasible and effective method for the separation of Bromelain from Pineapple.ATPs remove some of the system's unwanted byproducts, such as pigments, interfering proteins, and unidentified polysaccharides, which reduce enzyme function. When compared to other technologies, aqueous two phase extraction offers several advantages, including cheap cost, environmental friendliness, and continuous operation.In this present study Protein determination and stability of the Bromelain enzyme is studied.

II. MATERIALS AND METHODS:

Materials

The pineapples utilised in this investigation were bought from a local market. To reduce variance in fruit quality, there was just one supply of fruit that had fruit that was almost ripe at the same time. Prior to peeling the fruit, the crown and peel were properly washed in the tap.The core piece of the apple was scraped out and utilised in the experiments.

Methods

Sample Preparation:

To take off the dust particles from pineapple,it is washed with water.The wastes of the pineapple like core,peel were taken out and weighed .It is crushed using phosphate buffer and filtered twice.Thefilterate is centrifuged for few minutes to remove suspended imprities.This extract is further used in two phase aqueous extraction.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 2 Feb 2022, pp: 623-628 www.ijaem.net ISSN: 2395-5252

Enzyme extraction:

Cold extraction buffer (0.1 M sodium phosphate buffer, pH 7, with 1% polyvinyl pyrolydine) was used to homogenise the frozen pineapple chunks. At 10,000 rpm and 4 degrees Celsius, the filtrate was centrifuged for 20 minutes to obtain the "crude enzyme extract."

Two phase aqueous extraction:

Using Nitsawang's technique et al. With a little tweaking, centrifuge tubes of 50ml capacity were used for aqueous two-phase extraction. Different salt concentrations were used to study the effect of salts on Bromelain partitioning. (13,17,20 percent,w/w) of ($(NH_4)2SO_4$), $MgSO_4$, and K2HPO4 were mixed with 18 percent (w/w) of PEG 1000. To investigate the impact of Poly Ethylene Glycol concentrations (14,15, and 18 percent, w/w) of PEG (1000, 2000, and 3000 Da) Bromelain (10g), which was then blended with distilled water to produce a 20 g combination. The mixture was continuously blended for 3 minutes. Centrifugation at 7000xg for 20 minutes at 4°C achieved phase separation.Purification factor (PF), volume ratio (VR), protein partitioning coefficient (Kp), specific activiy (SA), and Bromelain recovery were among the metrics calculated (percent yield). For characterisation, the phase with the maximum Bromelain recovery was chosen.

Protein determination:

By the method of Bradford method,the protein is determined.Firstly 100 microgram of protein extract was taken.Using extraction buffer, dilute two distinct strengths of the extract (20 microliter and 5 microliter) to a volume of 100 microlitre. 5mL colour reagent was added and well mixed. Bovine Serum Albumin should be prepared in separate tubes containing 5, 10, 20, 30, 40, 50, and 100 µl at the same time.Fill each tube halfway with extraction buffer to get a total volume of 100 l. Add 5mL dye reagent to these tubes and mix well by vortexing. After 5 minutes and before 1 hour, compare the absorbance at 595nm to a reagent blank (100 l of extraction buffer with 1 ml of dye reagent). using the BSA standard curve to determine the protein content in the extract, calculate the protein concentration in the extract. Prepare a more appropriate dilution if the diluted extract is too high or too low. Different proteins have a wide range of dye-binding capabilities, resulting in a wide range of experiment results. Bovine serum albumin, in particular, has a high value and so is not completely representative of proteins. It's utilised with complete extract since it's more convenient.

SDS-PAGE:

SDS PAGE was used to evaluate the molecular weight distribution of the extracted materials using the Laemmli technique. In a 1: 1 ratio, The sample buffer was added to the sample (0.5 M Tris-HCl, pH 6.8, 4 percent SDS and 20 percent glycerol). Adding 10% ME to the sample buffer solved the issue. Polyacrylamide gels were loaded with 20 g of protein and electrophoresed at 15 mA per gel. In order to identify the protein, a staining solution was used (0.02 percent Coomassi Brilliant Blue R-250)and washed with an acetic-methanol solution after separation of the extract sample.

Activity staining:

Using the Garcia Carreno approach, protein separated by electrophoresis was assayed for bromine using activity staining. For 45 minutes at 4oC, the gel was submerged in 50 ml of 2 percent (w/v) Casein in 50 mM Tris-HCL buffer at pH 7.5 with steady stirring. For 30 minutes, the gel was incubated at 50°C with continual stirring. The clear zone on the blue background shows the action of bromelain, which can be seen.

pH Stability:

Samples were incubated at different pH levels to determine the stability of bromelain. There were five different buffers used: 0.05M Glycine HCl (pH 2.0-3.0), 0.05M Na acetate (4.0-5.0), 0.05M Na phosphate (6.0-7.0), and 0.05M tris HCl (8.0-10.0). (11.0-12.0). After incubation, the enzyme's proteolytic activity was evaluated.

Determination of thermal stability:

By the incubation of enzyme sample(200µl) at 90◦C for 0-60 minutes,Studying the effect of temperature on bromelain's activity was done.Bromelain's residual activity was determined.

Measurement of salt stability:

Bromelain was incubated for 20 minutes at room temperature in the presence of NaCl varying from 0-3.0% (w/v) in a 1:1 (v/v) ratio. Bromelain's residual activity was assessed.

III. RESULT AND DISCUSSION:

Two-phase aqueous extraction may have certain negative effects on the extraction of Bromelain from Pineapples.

- **Protein Concentration:**

If there is a increase in protein concentration and molecular weight of protein decrease their separation in the upper phase.Because the viscosity of the phase is significant in this case, a high protein content is recommended for extreme partition.

- **Effect of Temperature:**

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 2 Feb 2022, pp: 623-628 www.ijaem.net ISSN: 2395-5252

With a little concentration of Poly ethylene glycol (PEG) and salt, it is possible to generate a two-phase system at high temperatures.At lower temperature Poly ethylene glycol system will be formed.

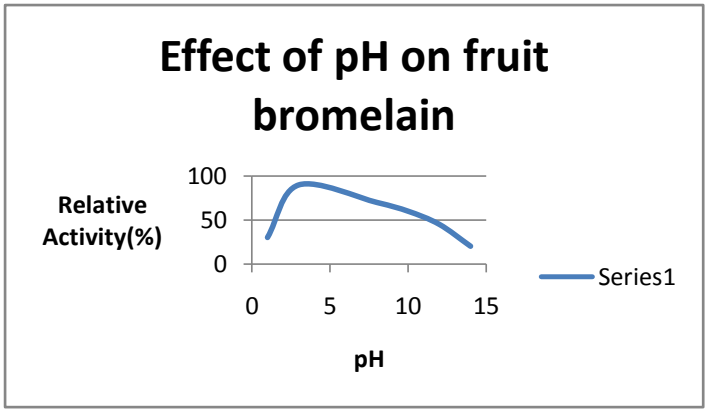
- **Effect of pH:**

In Poly ethylene glycol(PEG), protein's positive or negative charge changes when the pH changes from acidic to basic.Negative charge proteins prefer the top phase, i.e. the Poly ethylene glycol(PEG) layer, due to repulsion by salt anion.

- **Salt effects:**

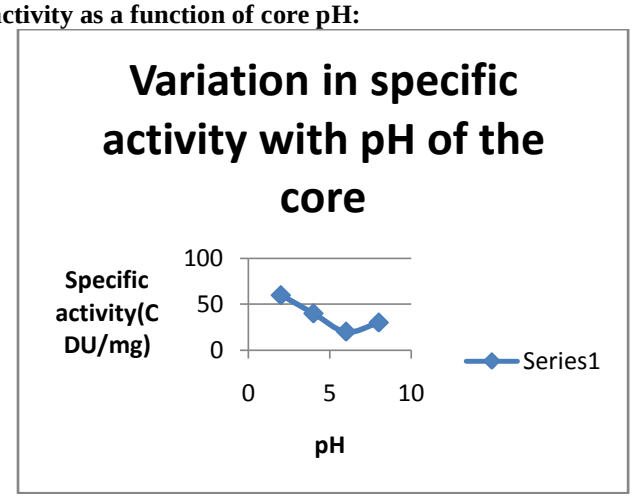
The distribution of salt ions across the phases is uneven, resulting in a variation in electrical potential across the phases of the system.This electrical potential difference would be independent of salt content, but linearly dependent on ion partition behaviour. Poly

Effect of pH on fruit Bromelain:



The graph above depicts the impact of pH on fruit bromelain, with the horizontal axis representing pH and the vertical axis representing relative activity (percent).The maximum Relative activity attains at a pH of 2.9,the enzyme is less active at a range of 2.7-3.1 pH

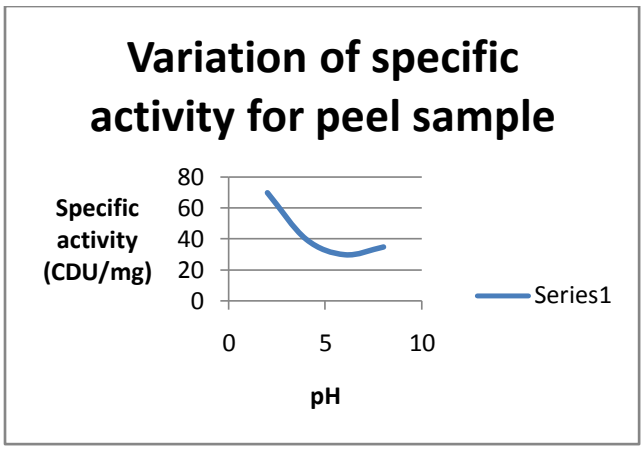
Variation in particular activity as a function of core pH:



This graph depicts the fluctuation in specific activity of the core extract as a function of pH.If the enzyme is stored for a longer period,itlooses its specific activity.The minimum specific activity is at a pH of 5.8

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 2 Feb 2022, pp: 623-628 www.ijaem.net ISSN: 2395-5252

Variation of specific activity for peel sample:



The above graph shows the Variation of specific activity for peel sample.The bromelain enzyme is mostly found in the fruit's crown and peel.The minimum specific activity for peel sample is at a pH of 6.

Bromelain extraction using an aqueous two-phase system:

Effect of salts on ATP partitioning of bromelain:

Bromelain was separated from nang le pineapple peel in numerous bypass systems using 18 percent PEG-1000 and several salts ((NH4) 2SO4, MgSO4, and K2HPO4) at varied concentrations (14, 17, and 20 percent). Salts are often used in ATPs in order to enhance the dispersion of the target molecules between the stages.The PEGrich top grid and saline bottom grid were obtained after grid separation. Bromine was the most easily broken down of all the ATPs examined in the polymer phase, owing to the hydrophobic features of enzymes. The distribution of biomolecules in PEG-saline systems is influenced by the polymer's polymerization phase impact and the salinity in the saline phase. Separation variables (VR, Kp, SA, PF, and yield) are affected by salt and enrichment. With the exception of K2HPO4, the VR of the examined systems decreases as salinity rises. With just a modest quantity of PEG molecule, a water layer developed around the cation, generating an extremely compact structure.

Table 1 shows the effect of salts on bromelain partitioning from pineapple peel.

Phase Composition	VR	Kp	SA	PF	Yield %
18% PEG1000-14%(NH4)2SO4	2.22	7.51	2.22	11.95	66.35
18% PEG1000-17%(NH4)2SO4	1.53	8.34	2.31	12.43	71.98
18% PEG1000-20%(NH4)2SO4	1.33	8.57	2.50	13.55	80.30
18% PEG1000-14%K2HPO4	1.38	7.15	2.56	13.97	72.97
18% PEG1000-17%K2HPO4	0.93	6.41	2.66	14.45	20.65
18% PEG1000-20%K2HPO4	1.07	10.75	2.82	15.12	77.50
18% PEG1000-14% MgSO4	4.25	8.37	2.85	15.50	51
18% PEG1000-17% MgSO4	3.05	8.52	3.67	19.77	59.03
18% PEG1000-20% MgSO4	1.42	4.93	4.55	24.67	74.15

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 2 Feb 2022, pp: 623-628 www.ijaem.net ISSN: 2395-5252

VR: volume ratio ; KP: partition coefficient of protein; SA: specific activity (unit/mg protein); PF:Purification factor.

Kp reports on the protein distribution in ATPs. According to the findings, the Kp of all ATPs varied between 4.9 and 10.77, with an average of 8. KB maximum (10.77) In the K2HPO4 system, 18 percent PEG-1000-20 percent was identified. High Kp values suggest that the majority of the protein in the sample was only separated at the lower or higher stages. The Kp of an 18 percent PEG-1000-20 percent MgSO4 phase compound is low (4.93). It was pointed out that it causes the contaminating protein nucleic acid and other undesired components to be converted to a lower stage. Thus, the extraction conditions used led to the enrichment of SA and PA.

A phase with 18% PEG-1000 and 20% MgSO4 gave the maximum SA (4.53 units / µ gProtein) and PF (24.67). The percentage of bromine recovered using ATPs in the research ranged from 52 to 80 percent. The system produced a maximum output of 80.30 percent, which included 18 percent PEG-1000 and 20 percent (NH4) 2SO4.This means that it is not just too much Bromelain is portioned in upper phase, but other proteins also.18% PEG-1500 and 20% K2HPO4 provided maximum activity recovery.20% PEG-1000 with 20% MgSO4 provided maximum SA and PF.

In addition, the data demonstrate an increase in salt content (14 to 20%), which leads to an increase in Kp, SA, and Bromelain recovery.Solubility during the salinity phase of the biological molecules decrease with increasing salt concentration,which results in a reduced distribution to the upper phase of these molecules.As a result, the specific activity can be seen to increase with increasing salt concentration. However, in terms of maximum SA and PF of 20% MgSO4, this codition is selected for the study of the impact of PEG on bromine partitions.

Effect of PEG on ATP partitioning of bromelain:

At 20 percent MgSO4, bromelain dispersion was investigated in ATPs with various molecular weights (MW) and PEG concentrations**.** The distribution of bromine is strongly dependent on the concentration ofmolecular weight and Polyethyleneglycol. The VR varies from 0.7 and 1.7. The majority of systems have a VR value close to 1.0, indicating that the volume fraction is divided into two stages. All of the Kp values were larger than 1, showing that the bromine was separated preferentially for the upper phase. Exclusion from the protein domain caused Polyethyleneglycol's molecular weight to rise, reducing the intended connection between PEG and protein.Low quantities of bromine salt might result as a result of this. The primary impact of block exclusion, rather than salting, is responsible for the rise in Bromelain content. In ATP testing, the composition of 15% PEG-3000 and 20% MgSO4 efficiently separated the bromine from the PEG-rich phase and the salt from the bottom phase beneath the undesired proteins.

In the upper phase, 108.45 percent of the enzyme is recovered at this stage.

Bromelain enzyme activity recovery increased as the molecular weight of Polyethyleneglycol rose. This resulted in an increase in PF because bromelain was less distributed in the lower phase than other proteins.PWG-3000 (15%) MW with a high concentration of MGSO4 (20%) gave the best purity and recovery of bromelain from pineapple peel, according to these findings.

Table 2. PEG's influence on Bromelain partitioning

Phase Composition	**VR**	**Kp**	**SA**	**PF**	**Yield %**	
12% PEG1000-20%MgSO4	0.74	2.06	1.42	7.58	43.66	
15%PEG1000-20%MgSO4	1.17	2.93	1.03	5.43	30.06	
18%PEG1000-20%MgSO4	1.41	4.92	4.55	24.66	74.12	
12%PEG2000-20%MgSO4	1.05	1.95	2.65	14.44	86.05	
15%PEG2000-20%MgSO4	1.09	2.45	2.87	15.25	91.53	

18%PEG2000-20%MgSO4	1.72	2.75	1.91	10.23	50.15
12%PEG3000-20%MgSO4	1.03	2.13	2.72	14.92	94.81
15%PEG3000-20%MgSO4	1.60	2.93	5.25	28.25	108.42
18%PEG3000-20%MgSO4	0.85	1.22	4.77	25.92	45.22

IV. CONCLUSIONS:

Bromelain enzyme was extracted from the fruit Pineapple by aqueous two phase extraction method.The highest Specific activity and Bromelain is 15% PEG3000-20%MgSO4 from top phase.It also gives high stability at neutral Ph.Upper phase is Poly ethelyne glycol enriched and lower base is Salt enriched.Bromelain activity is reduced by 50% when it is incubated at 90°C for 5 minutes.

REFERENCE:

[1]. B.Ravindrababu,N.K.Rastogi,K.S.M.S.Raghavaro : "Liquid liquid extraction of bromelain".

[2]. Ram Saran Chaurasiya,H.UmeshHebbar: "Extraction of bromelain from pineapple core and purification by RME method".

[3]. Chen Cui,ChangfengZeng,Chongqing Wang and Lixiong Zhang: "Complex emulsion by extracting water from homogeneous solutions comprised of aqueous three phase systems".

[4]. Goncalo Vale,SarahA.Martin,MatthewA.Mitsche,BonneM.Thompson,KaitlynM.Eckert and Jeffrey G.McDonald: "Three phase liquid extraction"

[5]. Carlos A.Corzo,KrzysztofN.Waliszewski,JorgeWeltiChanes: "Pineapple fruit bromelain affinity to different protein substrates".

[6]. Benucci, B., Liburdi, K., Garzillo, A. M. V., &Esti, M. (2011). Bromelain from pineapple stem in alcoholic-acidic buffers for wine application. Food Chemistry, 124, 1349–1353.

[7]. Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of dye binding. Analytical Biochemistry, 72, 248–254.

[8]. Brien, S., Lewith, G., Walker, A., Hicks, S. M., & Middleton, D. (2004). Bromelain as a treatment for osteoarthritis: A review of clinical studies. Evidence-based Complementary and Alternative Medicine, 1, 251–257.

[9]. A.D. Rowan, D.J. Buttle, A.J. Barrett, The cysteine proteinases of the pineapple plant, Biochem. J. 266 (1990) 869–875.

[10]. A. Jeung, Encyclopedeia of Common Natural Ingredients Used in Foods, Drugs, and Cosmetics, John Wiley & Sons, New York, 1980. pp. 74–76.

[11]. B.K. Bhattacharyya, Bromelain – an overview, Nat. Prod. Radian. 7 (2008) 359–363.

[12]. J. Choe, A.A. Vandernoot, R.J. Linhardt, J.S. Dordick, Resolution of glycoproteins by affinity-based reversed micellar extraction and separation, AIChE J. 44 (1998) 2542–2548.