International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
OPEN ACCESS
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST

Isolation and Comparative Kinetics Study of Bromelain from Different Parts of Pineapple

Ishita Bhattacharya
Department of Life Science, Binod Bihari Mahto Koyalanchal University, Dhanbad

ARTICLEINFO

Article History:

Accepted: 10 Nov 2023
Published: 24 Nov 2023

Publication Issue
Volume 10, Issue 6
November-December-2023

Page Number
556-567

ABSTRACT

Bromelain is a general name for a family of sulfhydryl l containing Proteolytic enzymes obtained from Ananas comosus, the pineapple plant. It is present in large quantities in fruit, leaves and stems of several varieties of pineapple. Pineapple is a good source for manganese and contains significant amount of vitamins c and B1.it contains the proteolytic enzymes Bromelain which is used as a meat tenderizing agent. Bromelain obtained from the stems of the pineapple plant contains all the soluble components of the pineapple stem in their original properties, which may involve malignant cell growth, thrombus formation, inflammation, control of diarrhea, dermatological and skin debridement. The present research investigation is focused towards the comparative study of Bromelain and its enzymatic activity which are present in fruit, peel and leaf part of pineapple. Bromelain extract was used to determine the rate at which gelatin was degraded. Activity of one-gram Bromelain is equivalent to 1,200 GDU. Gelatin was chosen as the substrate for the analysis of activity of Bromelain. In case of enzymes isolated from fruit of pineapple elute 1 had the highest activity with 560 units/mL (Table 5). Like wise in case of peel and leaf extracts of Bromelain were highest with 560 units/mL and 583 units/mL respectively.

Keywords : Bromelain, GDU, Gelatin, Kinetics

Introduction

Bromelain is a general name for a family of sulfydryl containing Proteolytic enzymes obtained from *Ananas comosus*, the pineapple plant. It is present in large quantities in fruit, leaves and stems of several varieties of pineapple.

In biochemistry since the 1940, Enzymes have provided the basis for the field of clinical chemistry. The use of enzymes in the diagnosis of disease is one of the important benefits derived from the intensive research.(**Bennett, T. P.,1969**)

Enzymes are biological catalysts that promote the transformation of chemical species in living systems. This molecule consisting of thousand of atoms in precise arrangements. They have been a key component in human activities especially food processing. (**Stryer,L 1995**).

enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.Like all catalysts, enzymes work by lowering the activation energy ($E_a^{\ddagger}$) for a reaction, thus dramatically increasing the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alters the equilibrium of these reactions. Enzymes do differ from most other catalysts in that they are highly specific for their substrates. Enzymes are known to catalyze about 4,000-biochemical reaction.(**Bairoch A, 2000**)

The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical change, which goes on continually in the living organism. Build-upof new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials, reproduction - all the activities that we characterize as "life."

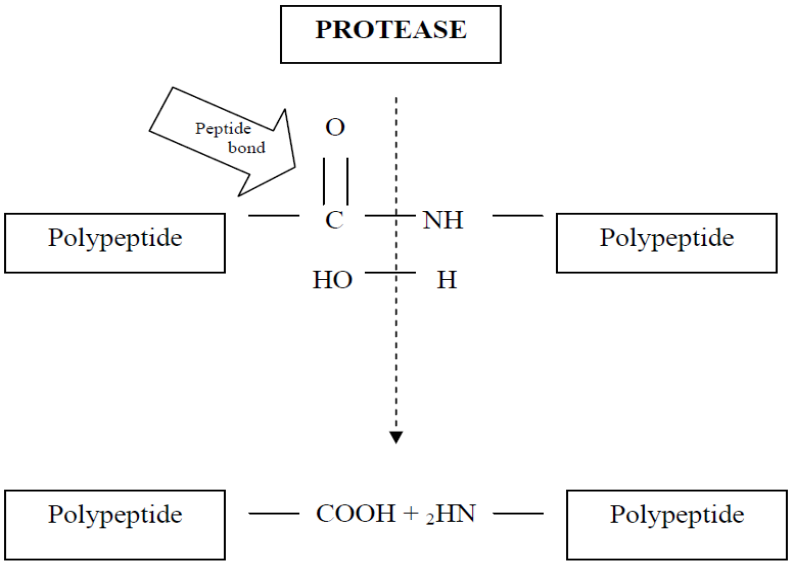

Fig.1. Action of protease

Pineapple (*Ananas comosus*), a tropical plant with edible multiple fruit consisting of coalesced berries, named for resemblance to the pine cone, is the most economically important plant in the *Bromeliaceae* family.(**Coppens d'Eeckenbrugge et al., 2003**)

Pineapple helps in the digestive system as just protein compounds which are broken down have the chance of being absorbed through the intestinal walls into the bloodstream. In the intestines and stomach, pineapples are known to kill potentially harmful bacteria.

Manganese is a mineral required in our bodies for bone building and connective tissues. Pineapples are rich in manganese and provide almost ¾ of the daily recommended manganese intake.

Vitamin C is known as a regular antioxidant which mainly prevents the human body from some radical damages and also known to boost the immunity of humans. The role of Vitamin C in the body include helping in metabolizing fats and cholesterol, synthesizing amino acids and collagen as well as absorbing irons. For the skin to look healthy and clear, the body requires collagen which is also a primary building block for bones and

cartilage.Mainly from its stem, pineapple contains a proteolytic enzyme namely Bromelain, which breaks down protein.

Bromelain is probably the most valuable and the most studied component from the pineapple It has been investigated since 1894 and was first identified in 1891 by Marcano It is a crude extract of pineapple that contains, among other components, various closely related proteases demonstrating, *in vitro* and *in vivo*, antiedematous, anti-inflammatory, antithrombotic fibrinolytic activities and has potential as an anticancer agent.(**Chobotova *et al.*, 2009**).

The protein, Bromelain, from pineapple fruit will is isolated and characterized. Bromelain is a protein which functions as an enzyme and belongs to a subclass of enzymes known as proteolytic enzymes (proteases). The function of proteases is to catalyze the hydrolysis of proteins to give amino acids. Proteolytic enzymes often catalyze self-degradation, but this usually occurs at a slower rate than the breakdown of other proteins.

Bromelain obtained from the stems of the pineapple plant contains all the soluble components of the pineapple stem in their original properties, which may involve malignant cell growth, thrombus formation, inflammation, control of diarrhea, dermatological and skin debridement (**Taussig and Batkin,1988**)

Bromelain concentrations is high in pineapple stems necessitating its extraction and use as a phytomedical compound unlike the pineapple fruit which is normally used as food, the stems are a waste by-product and inexpensive.The main proteolytic constituents contained inpharmacological preparations or food supplements of Bromelain (stem Bromelain,fruitBromelainandananain are also present in the pineapple fruit. Bromelain primary component is a Sulfhydryl proteolytic fraction. Bromelain also contains aPeroxidase, acid Phosphatase, several protease inhibitors and organically bound calcium (**Bitang Nipa tochi et al., 2008**)

Bromelain activity is stable over a wide pH range (**Cooreman *et al.,* 1976**) Therefore it may not be necessary to enteric-protect the protease from acid conditions in the stomach. It may be administered with a buffering agent, for example bicarbonate or in water or in a solution containing nutrients to assist with absorption of fluid and nutrients (**Mynott *et al.,* 1999**)

Pineapples (*Ananas comosus* from the family of Bromeliaceae), constituting an unusually complex mixture of called Bromelain. It is prepared from cooled pineapple juice bycentrifugation, ultra filtration and lyophilization. The processyields a yellowish powder, the enzyme activity ofwhich is determined with different substrates such ascasein (FIP units), gelatin (gelatin digestion units).Bromelain is an aqueous extract of pineapple that contains a complex mixture of thiol protease and non-protease components.

Protease constitute the major components of Bromelain and include stem Bromelain (80%), fruitBromelain (10%) and ananain (5%).

Nonprotease components are phosphtases, Peroxidase, cellulases, glycoprotein, and carbohydrates.(**H.R. Maurer 58,2001**)

Bromelain can be absorbed in human intestines without degradation and without losing its biological activity.

Materials and Methods

Healthy and disease free Pineapple fruit was collected from a local market in Dhanbad, India and washed with tap water. 200 g of fruit and 80g of peel and leaf was weighed and then cut into small pieces.2.82 gm of di-sodium hydrogen phosphate, and 3.12 gm of sodium dihydrogen phosphate was weighed and dissolved in 200 ml of Distilled water. The pH was adjusted to 7. Juice was collected from its fruit, peel and its leaf parts by homogenization in the presence of phosphate buffer (pH 7, 0.1M) and filtered.100 ml of juice was collected and added in sodium benzoate gradually. After filtration, the sample was called as “crude extract”.Then the crude extract was centrifuged at 2000 rpm for 5 min. Supernatant was transferred into a fresh tube and incubated

overnight at 4`c. In crude extract various types of proteins and other molecules are present, purification of enzyme was necessary for obtaining the desired enzyme. Mainly three step of purification are performed.

Enzyme Purification: - Ammonium salt precipitation

Principle - When salt is added to the extract, it dissolves to gives ions that become hydrated by hydrogen bonding and it disturbs the interaction of water molecule with the protein molecule.This will expose the hydrophobic patches of proteins and result in the hydrophobic interaction between protein molecules.This finally leads to the aggregation of proteins molecule and their precipitation.

Protocol:-

1. Weigh 44grams of Ammonium sulphate (for 100ml of suspension)
2. Add pinch by pinch Ammonium sulphate in to the enzyme suspension in ice cold conditions, on magnetic stirrer for 1 hour.
3. Keep it in ice-cold conditions for overnight
4. After ammonium sulphate precipitation , take the enzyme suspension in to centrifuge tubes.Centrifuge at 10000rpm for 10minutes.
5. Collect the pellet and dissolve it in 10ml of 10mM Tris-HCl Buffer.

Dialysis

Principle:-dialysis works by diffusion a process that results from movement of molecules in solution and leads to the movement from areas of higher to lower concentration. Dialysis is a separation technique that facilitates the removal of small,unwanted compounds from macromolecule in solution by selective and passive diffusion through a semi permeable membrane.

1. Take 100ml of distilled water and boil it.
2. Add the dialysis membrane in to boiling water.
3. Boil it for 10 minutes.
4. Add 2% Sodium bi Carbonate, boil it for 10minutes.
5. Take another 100ml of distilled water and boil it.
6. Transfer the dialysis membrane in to this boiling water and boil it for 10minutes.
7. Take out the membrane with the help of forcepsis.
8. Tie the one side of the membrane.
9. Add 10ml of enzyme suspension in to the dialysis membrane.
10. Tie another side of the dialysis membrane.
11. Place it in distilled water containing beaker.
12. Place the beaker on magnetic stirrer for 2- 3Hours (or) keep it in refrizirator for overnight.

Ion exchange chromatography

Principle:-Ion exchange chromatography based on the reversible exchange of ions in solution with ions electrostatiscally bound to an insoluble support media there can be two types of functional groups covalently attached to the support beads. These are called anion exchangers (resin with positive functional groups) or cation exchangers (resin with negative functional groups) separation on ion exchange chromatography column is based on charge density.

Column preparation: 2 gm of DEAE cellulose weighed and it dissolved in 50 ml of phosphate buffer pH-7, then poured in column and equilibrated with phosphate buffer, pH 7.

Procedure:

1. Prepare 6 elutes with 25mM Tris-HCl, 25mM,50mM,75mM,100mM,125mM,150mM NaCl in 6 different test tubes.
2. Column preparation: Wash the column with ethanol, then wash with distilled water.
3. Add DEAE Cellulose to the column leave it for settling.
4. Elute the buffer.
5. Add enzyme in to the column leave it for settling.
6. Collect the sample in to the test tube.
7. Add **Elute1** in to the column leave it for settling, collect the sample in to the same test tube.
8. Add **Elute2** in to the column leave it for settling, collect the sample in to the same test tube.
9. Like this, perform the elution process for Elutes 3,4,5 & 6 also.

Enzyme Assay of Bromelain enzyme :-

Enzyme assay is a method for the measuring the activity of enzyme. Bromelain extract was used to determine the rate at which gelatin was degraded. Activity of one-gramBromelain is equivalent to 1,200 GDU.Gelatin was chosen as the substrate for the analysis of activity of Bromelain.

Method – Titrimetric

Condition –pH 4.5,temp. 45^0

Principle -

Gelatin + H_2O ⟹ Amino Acid + Oligopeptide

Procedure -

ENZYMATIC ASSAY FOR BROMELAIN ENZYME:

Preparation of reagents

1. 5.0% (w/v) Gelatin Solution, pH 4.5 at 45°C.
2. Add distilled water Stir until the gelatin is dissolved, then place in an 80°C
3. Water bath for about 20 minutes. Cool to 45°C.

Table 1: Enzymatic assay for Bromelain enzyme

Sample	Vol.of gelatin(ml)	Equilibriate to 45°C in waterbath	Crude Enzyme	Incubate in water bath at 45°C for 20 minutes	Distill Water	Vol.of H_2O_2 (ml)	Adjust the pH to 6.9 with 0.05 N NaOH	Vol.of formaldehyde	Titrate to pH 7.8 with 0.05N NaOH	Volume of NaOH run down (ml)
Blank	2.5ml.		---		0.1ml	0.01ml.		1 ml.		ml.
Test	2.5ml.		0.1ml		---	0.01ml.		1 ml.		ml.

Actual amount of NaOH run down =Volume of Test–Volume of Blank

Calculation of Bromelain Enzyme (Crude) activity :-

Units /ml enzyme = (Volume of Test – Volume of Blank)(N) (14) (1000)
mg enzyme / RM

Enzyme Activity (units/gm) = (vol. of test – vol. of blank) x N x 14 x 1000
Mg. of enzyme/ RM

Where

N= Normality of NaOH.

Mg of enzyme = Quantity of enzyme in each sample

RM= Reaction mixture (Volume of gelatin + Volume of enzyme/distill water + Volume of hydrogen peroxide +Volume of Formaldehyde.

RESULT AND DISSCUSSIONS

Assay for Crude extract of Bromelain was used to determine the rate at which gelatin was degraded. After the titration with NaOH the enzyme activity of peel Bromelain was higher(466 units) observed than fruit and leaf extract.(Table 3, figure 1)

Table 3. Enzyme Assay for crude extracts of different parts of pineapple

S.No.	Sample name	volume of NaOH run down (in ml)	Enzyme Activity(units/mL)
1.	Peel	1.2	466
2	Leaf	1.6	110
3.	fruit	1.5	420

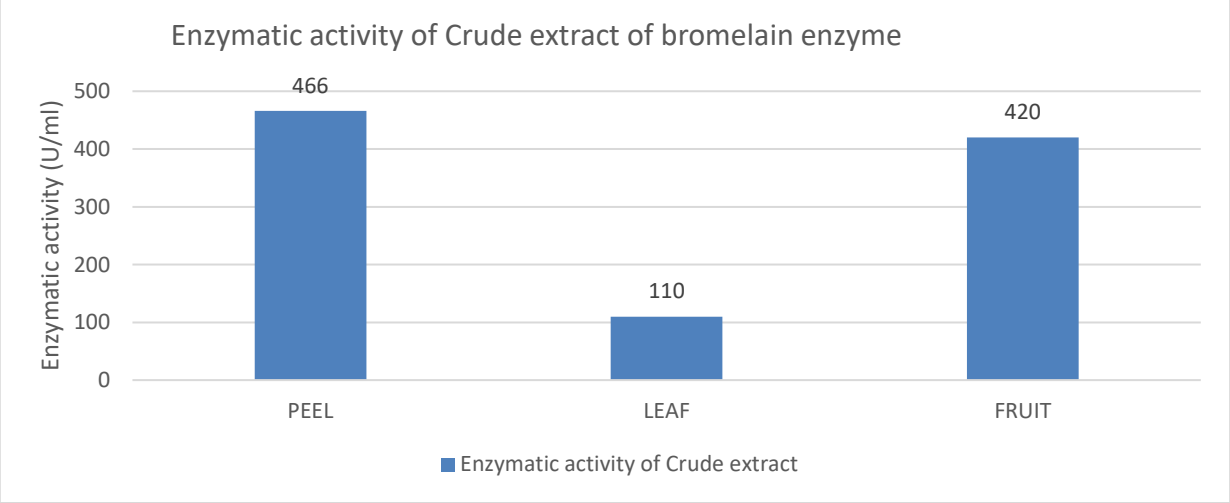

Figure 1 : Enzymatic activity of crude extracts of Bromelain isolated from different part of pineapple.

The crude sample was then subjected to ammonium salt precipitation step where the protein was precipitated out of the crude extract. The salt precipitation was then centrifuged and the pellet after centrifugation was dissolved in 100mM Tris HCl buffer.

Enzyme Assay of Bromelain sample

The enzymatic activity of dialysis purified sample was carried out. The dialysis sample of peel Bromelain was decreasing (233 unit)whereas the activity of fruit and leaf Bromelain activity was remain almost constant. (Table 4, Figure 2)

Table 4. Enzyme Assay for Dialyzed extract of different parts of pineapple

S.No.	Sample name	volume of NaOH run down in ml)	Enzyme Activity(units/mL)
1.	Peel	1.3	233
2.	Leaf	1.6	110
3.	fruit	1.4	420

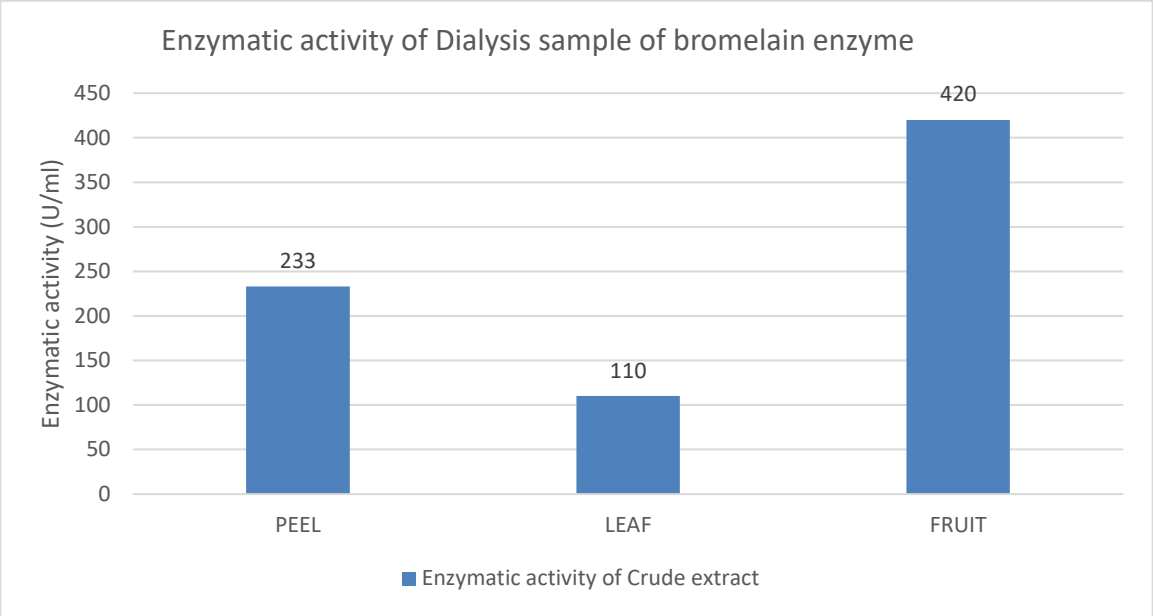

FIGURE 2: Enzymatic activity of dialysis samples of Bromelain isolated from different part of pineapple.

Enzyme assay of ion exchange Elutes

After the dialysis, the sample was later subjected to ion exchange chromatography where the different elutions were made by ion exchange chromatography procedure. The presence of enzyme is detected by the higher activity. In case of enzymes isolated from fruit of pineapple elute 1 had the highest activity with 560 units/mL (Table 5). Like wise in case of peel and leaf extracts of Bromelain elute 2 (Table 6) and elute 1 (Table 7) were highest with 560 units/mL and 583 units/mL respectively.

Table 5. Enzyme Assay for ion exchange elutes of fruit extract

S. No.	Fruit extract	Vol. of NaOH run down(ml)	Enzyme Activity(units/mL)
1.	Elute 1	1.6	560

2.	Elute 2	1.0	280
3.	Elute 3	0.8	420
4.	Elute 4	0.6	280
5.	Elute 5	0.5	140
6.	Elute 6	0.3	140

Table 6. Enzyme assay for ion exchange elutes of peel Bromelain

S. No.	Peel extract	Vol. of NaOH run down (ml)	Enzyme Activity(units/mL)
1.	Elute 1	4.5	175
2.	Elute 2	1.5	583
3.	Elute 3	1.5	175
4.	Elute 4	1.4	175
5.	Elute 5	1.5	116
6.	Elute 6	1.6	116

Table 7. Enzyme Assay for ion exchange elutes of leaf Bromelain

S. No.	leaf extract	Vol. of NaoH run down (ml)	Enzyme Activity(units/mL)
1.	Elute 1	1.0	184
2.	Elute 2	0.8	73.6
3.	Elute 3	0.5	73.6
4.	Elute 4	1.0	73.6
5.	Elute 5	0.7	73.6
6.	Elute 6	0.7	110

Table 9: Protein quantification of different enzyme samples.

S.No.	Sample name	O.D. at 660 nm	Concentration Of protein(µg/µl)
1.	Fruit(crude)	**0.46**	**92.0**
	Dialyzed enzyme	**0.45**	**89.5**

	Elute 1	**0.41**	**79.5**
2.	Peel(crude)	**0.47**	**94.5**
	Dialyzed	**0.32**	**57.0**
	Elute 2	**0.43**	**84.5**
3.	Leaf (crude)	**0.16**	**17.0**
	Dialyzed enzyme	**0.48**	**97.0**
	Elute 1	**0.46**	**92.0**

Enzyme kinetics

Effect of temperature: The highest activity for different extracts of Bromelain was measured at 45^{0}C (Table 10)

Table 10. Effect of temperature on different extracts of Bromelain

Temperature	Sample name	Vol. of NaOH run down (in ml)	Enzyme Activity(units/mL)
25^{0}C	Peel	1.2	116
	Leaf	1.0	58.3
	Fruit	0.9	280
35^{0}C	Peel	1.3	175
	Leaf	1.2	73.6
	Fruit	1.2	280
45^{0}C	Peel	1.8	350
	Leaf	2.0	110.5
	Fruit	1.6	420
55^{0}C	Peel	1.4	233.3
	Leaf	1.5	110.5
	fruit	1.5	112.0
65^{0}C	Peel	1.5	291.6
	Leaf	1.3	73.6
	fruit	1.3	280

Effect of pH: The highest activity for different extracts of Bromelain was measured at pH = 4.5 (Table 11, Graph 2)

Table 11.Effect of pH on different extracts of Bromelain

pH	Sample name	Vol. of NaOH run down (in ml)	Enzyme Activity(units/mL)
2.5	Peel	1.4	233.3
	Leaf	0.3	21.2
	Fruit	1.3	280
3.5	Peel	2.0	175
	Leaf	0.5	50.63
	Fruit	1.4	320
4.5	Peel	2.3	233.3
	Leaf	0.6	73.6
	Fruit	1.5	420
5.5	Peel	2.5	212.3
	Leaf	0.6	42.2
	fruit	1.0	280
6.5	Peel	1.3	202.9
	Leaf	0.4	39.3
	fruit	0.4	140

Graph 2: Effect of pH on different parts of pineapple

Effect of substrate concentration: The highest activity for different extracts of Bromelain was measured at substrate concentration= 5%. (Table 12)

Table12: Effect of substrate concentration on different extracts of Bromelain

Substrate concentration	Sample name	Vol. of NaOH run down in ml	Enzyme Activity(units/mL)
3%	Peel	0.5	116.6
	Leaf	0.4	36.8
	Fruit	0.5	105
4%	Peel	1.0	233.3

	Leaf	0.6	73.6
	Fruit	1.0	108
5%	Peel	1.5	245.6
	Leaf	0.8	81.33
	Fruit	1.4	119.6
6%	Peel	1.6	249.8
	Leaf	1.2	83.33
	fruit	1.7	121.6
7%	Peel	1.8	250.5
	Leaf	1.3	84.2
	fruit	2.1	122.2

CONCLUSION

Bromelain has a wide range of therapeutic benefits, but the mode of its action is not properly understood. It is proved that bromelain is well absorbed in body after oral administration and it has no major side effects, even after prolonged use. All the evidences reviewed in this paper suggest that bromelain can be used as an effective health supplement to prevent cancer, diabetes, and various cardiovascular diseases in the long run.Bromelain can be a promising candidate for the development of oral enzyme therapies for oncology patients. It is clear from this paper that bromelain is a multiaction enzyme; however, more research is required to understand the proper mechanism of action of bromelain so that the multiaction activities of bromelain can be harnessed efficiently.

References

1. Ana Maria Frattini Fileti etal., Batch and Continuous Extraction of Bromelain Enzyme by Reversed Micelles Vol.52, n. 5: pp. 1225-1234, September-October 2009
2. Atul upadhyayet al., utilization of pineapple waste : A review2010
3. Bitange Nipa Tochi etal.,Therapeutic Application of Pineapple Protease (Bromelain): A Review Pakistan Journal of Nutrition 7 (4): 513-520, 2008
4. Bennett, T. P., and Frieden, EModern Topics in Biochemistry, .(1969): pg. 43-45
5. Carola Metzig etal., Bromelain Proteases Reduce Human Platelet Aggregation in Vitro,Adhesion to Bovine Endothelial Cells and Thrombus Formation inRat Vessels in Vivo ,Institute of Pharmacy, Free University of Berlin, Germany in vivo 7 1 - 7 - 12 (1 9 9 9)
6. Faergeman NJ, Knudsen J etal.,. "Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling"1997
7. Doble B. W., Woodgett J. R.etal., "GSK-3: tricks of the trade for a multi-tasking kinase"(2003).
8. Carr C. M., Kim P. S. "A spring-loaded mechanism for the conformational change of influenza hemagglutinin".(2003).

9. Sookkheo B, Sinchaikul S, Phutrakul S, Chen ST..Purification and characterization of the highly thermostable proteases from Bacillus stearothermophilus TLS33. 2000 Nov;20(2):142-51
10. Gupta et alBacterial alkaline proteases and molecular approaches and industrial application. .,(2002)
11. Coppens d'Eeckenbrugge, Geo; Freddy Leal ("Chapter 2: Morphology, Anatomy, and Taxonomy".2003).
12. Cooreman, W.M., S. Scharpe, J. Demeester and A. demmestere et al Bromelain, biochemical and pharmacological properties. (1976)
13. Maurer, H.R., Bromelain: Biochemistry,pharmacology and medical use. Cell. Mol. Life Sci., 2001
14. Mynott, T.et al Bromelain the enzyme complex of pineapple proteolytically blocks activation of extracellularregulated kinase-2 in T cells. (1999)
15. S. Batkin Bromelain, the enzyme complex of pineapple (Ananas comosus) and its antitumor property. ,(1985)
16. Klaue In: Bromelain:, pharmacology and medical use. ,(1979)
17. Thomson et al.,small bowel review physiology part 1 digestive science 46, 2567-2587
18. Knill - Jones, (1970.)Comparative trial of Nutrizym in chronic pancreatic.
19. Wen, et al(2006.) Bromelain improves decrease in defecation in postoperative rats:
20. B. López-García1, et al.,2012Volume 55, Issue 1, pages 62–67, July 2012 Bromelain, a cysteine protease from pineapple (Ananas comosus) stem, is an inhibitor of fungal plant pathogens.
21. B. Ravindra Babu, et al., 2007 Liquid–liquid extraction of Bromelain and polyphenol oxidase using aqueous two-phase system
22. Gregory S. Kelly, et al.,1996 Bromelain: A Literature and Review
23. S. S. Gautam1,et al.,2010Comparative study of extraction, purification and estimation of Bromelain from stem and fruit of pineapple plant.
24. Soares paulo et al., 2012 Purification of bromelain from pineapple wastes by ethanol precipitation Volume 98, issue (September 19, 2012), p. 389-395.
25. Sarah Brien 2004Bromelain as a Treatment for Osteoarthritis: a Review of Clinical Studies 2004 December; 1(3): 251–257.Published online 2004 October 6.