Original article

Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant

S. S. Gautam[1], S. K. Mishra[1], V. Dash[1], Amit K. Goyal[2] and G. Rath[2*]

[1]Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela, Orissa, India
[2]Department of Pharmaceutics, ISF College of Pharmacy, Moga (Punjab), India
**Corresponding author: E-mail address: goutamrath@rediffmail.com*

Abstract:

Bromelain is a major proteinase, isolated from pineapple *(Ananas comosus).* In the plant, bromelain is accumulated in the entire plant to different extent and properties depending on its source. The objective of present study was to compare the amount and activity of bromelain present in stem and fruit of the plant. Bromelain was isolated from stems and fruit of adult pineapple plants by buffered aqueous extraction. Purification of enzyme was done by centrifugation, salt precipitation technique, dialysis, ion-exchange chromatography and estimation by Lowry's method. Bromelain was assayed for its activity by hydrolysis of gelatin, represented by using gelatin digestion unit. The homogeneity of bromelain was confirmed by SDS-PAGE (sodium dodecylsulphate-polyacrylamide gel electrophoresis) analysis. It was found that stem bromelain had a better activity than fruit bromelain in gelatin digestion unit analysis. Moreover, ion exchange chromatography using diethylaminoethyl cellulose (DEAE) anion exchangers maintained the structural integrity of purified bromelain and thereby the product exhibited better proteolytic activity then crude extract.

Keywords: Fruit bromelain; Gelatin digestion unit; Ion exchange chromatography; Lowry's method; Stem bromelain

Introduction

Bromelain is a mixture of protein-digesting (proteolytic) enzymes found in pineapples *(Ananas comosus).* Pineapple has been used for centuries to treat indigestion and reduce inflammation [1]. In pineapple plant, bromelain is accumulated in the entire part with different extent and properties depending on its source. Bromelain, which is derived from the stem and juice of the pineapple, was first isolated from the plant in the late 1800s. It is usually distinguished as either fruit bromelain or stem bromelain depending on its source, with all commercially available bromelain being derived from the stem. The stem bromelain has the EC Number EC 3.4.22.32 and that of the fruit bromelain is EC 3.4.22.33. It is approved to treat swelling and inflammation following surgery, particularly sinus surgery. Bromelain can be useful in treating a wide range of conditions, but it is particularly effective in reducing inflammation associated with infection, sinusitis, osteoarthritis and cancer [2-7].

The primary component of bromelain is a sulfhydryl proteolytic fraction. It also contains a peroxidase, acid phosphatase, several protease inhibitors, and organically-bound calcium. It is made up of 212 amino acids and the molecular weight is 33 kDa [8, 9]. Bromelain is stable at pH 3.0-6.5 and once it has combined with its substrate, the activity is no longer susceptible to the effect of the pH. The effective temperature range is 40˚C-65˚C with the optimum being 50˚C-60˚C. Bromelain can be activated by calcium chloride, cysteine, bisulphate salt, NaCN, H_2S, Na_2S and benzoate. However, bromelain is usually sufficiently active without the addition of activators. Bromelain is inhibited by Hg^{++}, Ag^{+}, Cu^{++}, antitrypsin, estatin A and B, iodoacetate [10].

Isolation, separation and purification of enzymes/ proteins can be performed using variety of chromatography, electrophoretic, ultrafiltration, precipitation and other procedures. Ion exchange chromatography is often very useful in protein purification [11, 12]. This chromatographic procedure uses the net charges of the molecules to achieve their separation [13, 14]. Two commonly employed ion exchange resins are the cationic and anionic resins, carboxymethyl (CM) and diethylaminoethyl cellulose (DEAE). Several works on ion exchange chromatography have contributed to better understanding of the effects of ionic resins, effect of velocity and elution solution for the recovery of biomolecules [15].

In the present work, purification of bromelain-containing juice extracted from pineapple *(Ananas comosus)* plant stems and fruits were studied by centrifugation technique at different rotational speed and DEAE cellulose bed based ion-exchange chromatography techniques. The effect of separation and purification methods of bromelain activity and purity was studied by gelatin digestion unit assay and SDS-PAGE (sodium dodecylsulphate-polyacrylamide gel electrophoresis) analysis.

Experimental

Materials

Pineapple stem was collected from Kerala Agricultural University, Kerala, India. Herbarium of the same was prepared and maintained at KMIPS, Rourkela, Orissa bearing the voucher no. KMP-AN-COM-98. Temperatures of 7 to 12˚C (45 to 55˚F) were maintained for storage of pineapples for 14 to 20 days at 85 to 95% of relative humidity. DEAE cellulose (GeNei India Ltd., Bangalore) and all other reagents (Hi-Media Labs Ltd., Mumbai) used were of highest commercially available purity.

Extraction

Fresh pineapple stems parts were collected, washed with 0.1% hydrogen peroxide solutions, peeled off, cut into small pieces and weighed. The weighed mass was found out to be 1,700 gm. Juice was collected from the fresh pineapple stem part by homogenization, in the presence of sodium acetate buffer solution and was filtered. Five-hundred ml of filtrate were collected. Benzoic acid/sodium benzoate was added as a preservative at a concentration of 1 gm. per kg of stem. The filtrate obtained was called as “crude extract”, and used as source of “stem bromelain”.

Purely ripe pineapple fruits were taken, cleaned and made into small slices. The weighed mass was 600 gm. The juice was extracted using a homogenizer,

collected into a beaker and filtered. The filtered mass was about 300 ml and 0.6 gm of sodium benzoate was added. The filtrate was called as "crude extract", and used as source of "cruit bromelain".

Selection of substrate

Bromelain can be assayed by measuring digestion action on gelatin and is expressed as GDU (Gelatin digestion unit). Activity of one gram of bromelain is approximately equivalent to 1,200 GDU. Gelatin was chosen as the substrate for the analysis of activity of bromelain [16].

Assay for crude stem bromelain by gelatin digestion unit analytical method

Crude stem bromelain extract was used to determine the rate at which gelatin (substrate) was degraded. Various reagents were prepared such as gelatin (5%) (reagent A) used as substrate, hydrogen peroxide solution (3%) (reagent B), formaldehyde solution (37%) (reagent C), 0.05N sodium hydroxide (reagent D), 100 mM sodium acetate buffer with 2.6 M sodium chloride (reagent E).

One ml of crude stem extract was taken in a beaker, designated as test solution. The pH was maintained to 6.0 with 0.05 N NaOH. Twenty-five ml of reagent A was added to the test solution and equilibrated at 45 °C in a water bath. After 20 minutes of incubation at 45 °C, 0.1 ml of reagent B was added and swirled. The solution was incubated for an additional 5 minutes. The beaker was removed from the water bath and pH was adjusted to 6.0 with 0.05 N NaOH. Ten ml of reagent C was added with constant stirring. Titration was done to pH 9.0 with 0.1 N NaOH. The titration volume of the test solution was recorded. The blank solution was run concurrently with the test solution. Firstly, 25 ml of reagent A and 0.1 ml of reagent B was added and equilibrated at 45 °C in water bath. After 20 minutes of incubation at 45 °C, 1.0 ml of bromelain solution was added, swirled and incubated for an additional 5 minutes. The beaker was removed from the water bath and pH 6.0 was adjusted with 0.05 N NaOH. Ten ml of reagent C was further added with constant stirring and the titration was done to pH 9.0 with 0.1 N NaOH. The titration volume of the blank solution was recorded (Table 1). The bromelain content was calculated for the crude enzyme [16].

$$\text{Units/gm enzyme} = \frac{(\text{Volume of test-Volume of blank})\ (N)\ (14)\ \times 1000}{\text{mg enzyme/RM}} \quad (1)$$

where N is normality of NaOH, 14 = mg nitrogen per millimole nitrogen, mg enzyme = amount per concentration of bromelain enzyme present in 1 ml of crude extract, RM = reaction mix. The concentration of enzyme was found out to be 0.7 mg/ml of crude extract.

Table 1 Assay for crude stem and fruit bromelain

Sample	Reagent A (ml)	Crude enzyme (ml)	Crude enzyme (ml)	Reagent B (ml)	Reagent C (ml)	Volume of NaOH run down (ml)
Stem bromelain						
Blank	25	---	1	0.1	10	7.00
Test	25	1	---	0.1	10	8.05
Fruit bromelain						
Blank	25	---	1	0.1	10	9.40
Test	25	1	---	0.1	10	10.55
Bromelain standard						
Blank	25	---	1	0.1	10	7.00
Test	25	1	---	0.1	10	8.05

Purification

The crude extracts of stem and fruit bromelain were centrifuged for 10 minutes at 2,000 rpm, 4,000 rpm and 6,000 rpm consecutively at 4 °C. The superna tant was collected and enzyme assay was performed as above (Table 2). Finally, samples were taken for purification by using ammonium sulfate precipitation, dialysis, and ion exchange chromatography.

Salt precipitation/salting out

For stem bromelain, ammonium sulfate precipitation was carried out by adding 6.6 gm of ammonium sulfate salt, pinch by pinch, to 15 ml supernatant taken after centrifugation under ice cold conditions with continuous stirring on a magnetic stirrer for 45 minutes. For fruit bromelain, similar activity was carried out for same time period. Stem and fruit bromelain sample solutions were

Table 2 Enzyme assay of centrifugated fraction and ion exchange eluate of stem and fruit bromelain

Sample	Reagent A (ml)	Enzyme	Enzyme	Reagent B (ml)	Reagent C (ml)	Volume of NaOH run down (ml)
Stem bromelain (2000 rpm)						
Blank	25	---	1	0.1	10	7.50
Test	25	1	---	0.1	10	8.50
Stem bromelain (4000 rpm)						
Blank	25	---	1	0.1	10	7.60
Test	25	1	---	0.1	10	8.60
Stem bromelain (6000 rpm)						
Blank	25	---	1	0.1	10	7.40
Test	25	1	---	0.1	10	8.42
Fruit bromelain (2000 rpm)						
Blank	25	---	1	0.1	10	7.50
Test	25	1	---	0.1	10	8.47
Fruit bromelain (4000 rpm)						
Blank	25	---	1	0.1	10	7.80
Test	25	1	---	0.1	10	8.62
Fruit bromelain (6000 rpm)						
Blank	25	---	1	0.1	10	7.7
Test	25	1	---	0.1	10	8.43
Stem bromelain (2^{nd} eluate)						
Blank	25	---	1	0.1	10	7.90
Test	25	1	---	0.1	10	8.75
Stem bromelain (4^{th} eluate)						
Blank	25	---	1	0.1	10	7.40
Test	25	1	---	0.1	10	8.35
Fruit bromelain (2^{nd} eluate)						
Blank	25	---	1	0.1	10	7.40
Test	25	1	---	0.1	10	7.95
Fruit bromelain (4^{th} eluate)						
Blank	25	---	1	0.1	10	7.90
Test	25	1	---	0.1	10	8.50

incubated overnight at 4 °C. After incubation, the precipitated enzymes were centrifuged at 10,000 rpm for 10 minutes at 4 °C. The pellet was collected and dissolved in 10 ml of 10 mM Tris HCl buffer which was later subjected to dialysis.

Dialysis

The above obtained solution was placed in a dialysis bag and checked for the leakage of the sample in it. The dialysis bag was then suspended in a beaker containing 100 mM phosphate buffer-NaCl solution. This setup was kept in refrigerator/cool conditions overnight. This entire process was carried out for both stem and fruit bromelain.

Ion exchange chromatography of purified bromelain on DEAE cellulose

DEAE cellulose bed, of 1 cm thickness, was prepared in a chromatography column and equilibrated with 0.5 M sodium phosphate buffer solution (pH-8.0) followed by eluting buffer '1' i.e. 25 mM Tris HCl and 25 mM NaCl. The dialyzed sample of stem and fruit bromelain was poured onto the column, from the sides, without disturbing the DEAE cellulose bed and allowed to settle. Enzyme was eluted using the first eluting buffer i.e. 25 mM Tris HCl and 25 mM NaCl. Eluate was collected in test tube. Elution was done at a flow rate of 1 ml/min. The same process of elution was carried out using solutions 2, 3, 4, 5 and 6 containing 50 mM, 75 mM, 100 mM, 125 mM and 150 mM NaCl, respectively. Besides the variable concentration of NaCl, all eluates contained 25 mM Tris HCl. The dialyzed enzyme sample was poured onto the column. The enzymes were then eluted using eluting buffer '2' (10 ml of 25 mM Tris HCl and 50 mM NaCl). Eluates were collected in the same test tubes. The process of elution was continued using eluting buffers 3, 4, 5 and 6 contained 75 mM, 100 mM, 125 mM and 150 mM of NaCl, respectively. Finally, ion-exchange eluates of stem and fruit bromelain were assayed for their activity (GDU assay) as reported above for crude isolates of bromelain for stem and fruits.

Quantitative estimation of stem and fruit bromelain by Lowry's method

Concentration of proteins (bromelain) in stem and fruit was determined by Lowry's method as reported previously with minor modifications [17]. Different dilutions of BSA solution were prepared by taking BSA solution (100 µg/ml) and distilled water as a standard in the test tube. The final volume in each of the test tubes was 1 ml. The BSA range was 0.02 to 0.1 mg/ml. The 0.1 ml of crude enzymes of stem and fruit bromelain, ion exchange eluate 2^{nd} and 4^{th} of stem and fruit bromelain enzymes were taken into test tubes 7, 8, 9, 10, 11 and 12 respectively and the final volume was made up to 1 ml with distilled water. These were unknown samples. Five ml of alkaline copper sulphate reagent was added to each tube and mixed well. These solutions were incubated at room temperature for 10 mins. Then 0.5 ml of FC (Folin Ciocalteau) reagent was added to each tube and incubated in dark for 30 min. The spectrophotometer analysis was performed and the optical density i.e. absorbance was measured at 660 nm for all the samples. The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of unknown sample was checked and the concentration of the unknown sample was determined.

Gel electrophoresis of isolated enzymes

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with different isolated/extracted enzymes (bromelain). The extracted enzymes were concentrated and loaded onto a 3.5% stacking gel and subjected to electrophoresis on a 12% separating gel at 200 V (BioRad, Hercules, California, USA) until the coomassie dye stained protein band reached the gel bottom. SDS PAGE photograph of isolated bromelain was obtained.

Results

Bromelain is a mixture of enzymes found naturally in the juice and stems of pineapple. In the present experiments, purification of bromelain-containing juice extracted from pineapple stems and fruits by known procedures comprising in general crushing the stems and fruits in roll presses followed by pressing the crushed mass to extract pineapple stem and fruit juice. Juice was extracted from stem and fruit of pineapple plant which were called as crude extract of the enzymes and the activity of these crude extracts was checked by the hydrolysis of gelatin which was estimated and represented in the form of gelatin digestion units (GDUs). Fruit-and stem-isolated bromelain activities were determined and the comparative analysis is shown in Table 4 and Fig. 1. The enzymatic activity of crude extracts was found out to be 2,100 units/gm for stem bromelain, whereas for fruit bromelain it was 1,450 units/gm (Fig. 1).

The crude extract was subjected to ammonium sulfate precipitation to precipitate the enzyme. The pellet was dissolved in 10 mM Tris HCl buffer and subjected to dialysis to remove the salt and other ions bound to the enzyme. Then the enzymes were purified by anionic ion exchange chromatography, where the resin used was DEAE cellulose. The stem and fruit bromelain enzymes were eluted using different concentrations of NaCl and Tris HCl buffer. From the enzymatic assay of ion exchange eluates, it was observed that maximum amount of NaOH was required for eluate 2^{nd} and 4^{th} of stem and fruit bromelain.

The protein concentration (bromelain) of crude extract, centrifuged fraction and ion-exchage eluates of stem and fruit were determined by Lowry's method (Table 3). Protein concentrations of stem bromelain were found to be 0.7 mg/ml, 0.08 mg/ml, and 0.14 mg/ml for crude extract, eluate 2^{nd} and 4^{th}, respectively. Protein concentration for centrifuged fractioned of stem

Table 3 Quantitative estimation of stem and fruit bromelain by Lowry's method

Sample	sample (ml)	Distilled water (ml)	Alkaline $CuSO_4$ (ml)	F.C. reagent (ml)	O.D. at 660 nm
Blank	---	1.0	5	0.5	---
BSA (20 µg/ml)	0.2	0.8	5	0.5	0.135
BSA (40 µg/ml)	0.4	0.6	5	0.5	0.292
BSA (60 µg/ml)	0.6	0.4	5	0.5	0.400
BSA (80 µg/ml)	0.8	0.2	5	0.5	0.535
BSA (100 µg/ml)	1.0	---	5	0.5	0.635
Stem crude	0.1	0.9	5	0.5	0.475
Fruit crude	0.1	0.9	5	0.5	0.756
Stem bromelain (2000 rpm)	0.1	0.9	5	0.5	0.268
Stem bromelain (4000 rpm)	0.1	0.9	5	0.5	0.308
Stem bromelain (6000 rpm)	0.1	0.9	5	0.5	0.421
Fruit bromelain (2000 rpm)	0.1	0.9	5	0.5	0.536
Fruit bromelain (4000 rpm)	0.1	0.9	5	0.5	0.482
Fruit bromelain (6000 rpm)	0.1	0.9	5	0.5	0.362
Stem eluate 2	0.1	0.9	5	0.5	0.052
Stem eluate 4	0.1	0.9	5	0.5	0.096
Fruit eluate 2	0.1	0.9	5	0.5	0.258
Fruit eluate 4	0.1	0.9	5	0.5	0.088

bromelain at 2,000, 4,000 and 6,000 rpm were found to be 0.87 mg/ml, 0.72 mg/ml and 0.52 mg/ml, respectively whereas protein concentration of centrifuged fractioned for fruit bromelain at 2,000, 4,000 and 6,000 rpm were found to be 0.40 mg/ml, 0.51 mg/ml and 0.63 mg/ml respectively. Protein concentration of fruit bromelain was determined as 1.11 mg/ml, 0.39 mg/ml and 0.14 mg/ml for crude extract, 2^{nd} and 4^{th} eluate of fruit bromelain, respectively.

Enzymatic activity of crude extract for stem and fruit bromelain obtained from centrifugation at different rpm demonstrated that the maximum enzymatic activity was observed at 2,000 rpm (GDU 3,500 units/gm) for stem bromelain ($P > 0.001$), while maximum enzymatic activity of fruit bromelain was found to be at 6,000 rpm (GDU 1,965 units/gm). Similarly, enzymatic activity of 2^{nd} eluate for stem bromelain was 14,875 units/gm and that of eluate 4^{th} was 9,500 units/gm ($P > 0.001$). For fruit bromelain, eluate 2^{nd} was 1,974 units/gm and that of eluate 4^{th} was 6,000 units/gm. Comparative study of proteolytic activity of different fractions from stem and fruit bromelain showed stem bromelain was found to be superior to fruit bromelain in hydrolyzing the gelatin (Fig. 1).

The SDS-PAGE analysis showed that extracted protein (bromelain) was electrophoretically pure and stable during in-process isolation steps (Fig. 2). The estimated molecular weight of bromelain was around approximately 30 kDa (Fig. 2), as in previous reports [8, 9].

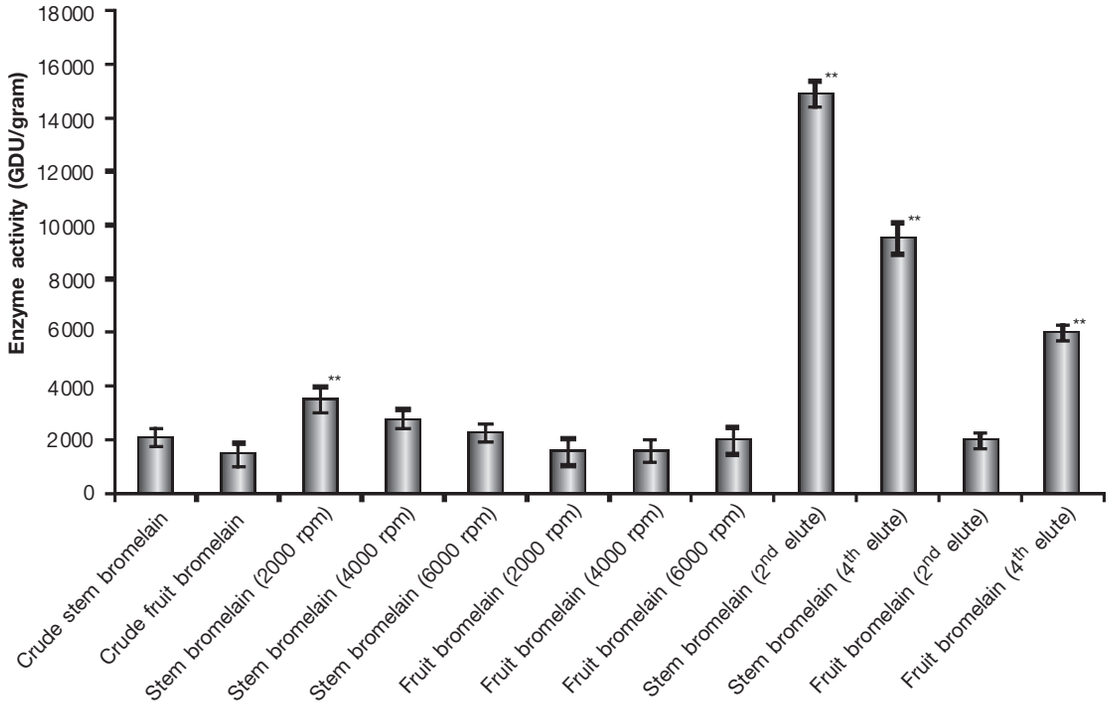

Figure 1 Proteolytic activity of isolated bromelain from stem and fruits of *Ananas comosus.* Significance was tested using one way ANOVA and Tukey-kremer post test by comparing all the isolated extracts with crude stem bromelain. **indicates the most significant methods ($p < 0.01$) for bromelain isolation.

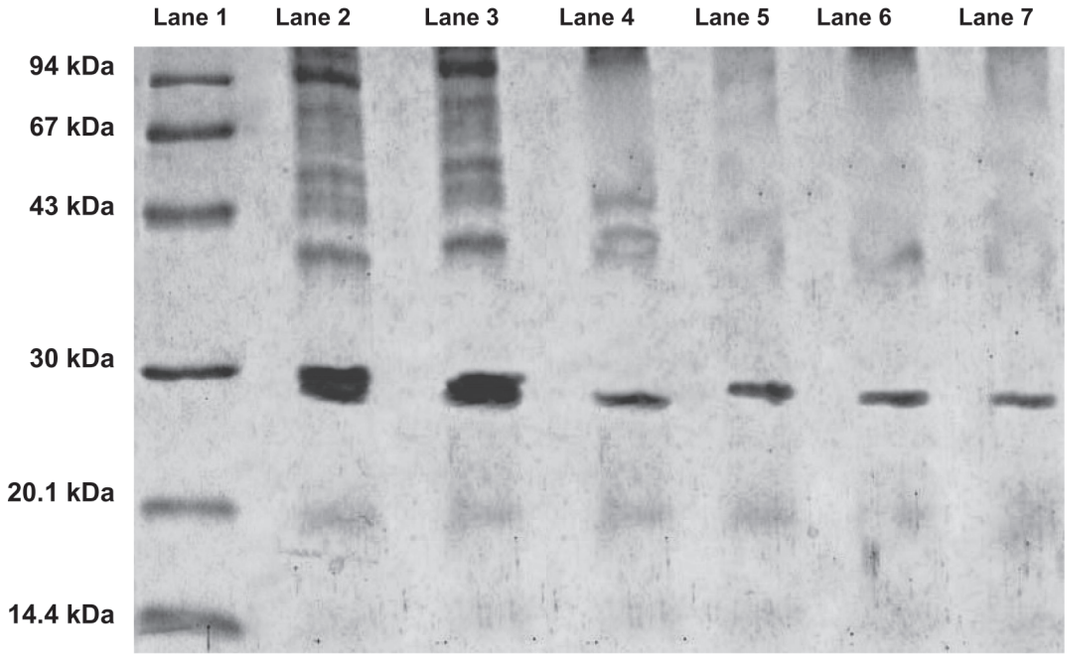

Figure 2 SDS-PAGE electrophoresis of isolated bromelain. Lane 1: standard of molecular weight, from top to bottom, phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and alpha lactoalbumin (14.4 kDa); Lane 2: crude stem bromelain; Lane 3: crude fruit bromelain; Lane 4: 2^{nd} eluate stem bromelain; Lane 5: 4^{th} eluate stem bromelain; Lane 6: 2^{nd} eluate fruit bromelain; Lane 7: 4^{th} eluate fruit bromelain.

Table 4 Proteolytic activity of isolated bromelain from stem and fruits of *Ananas comosus*

Sample	Actual amount of NaOH rundown (ml)	Normality of NaOH (N)	Conc. of enzymes (mg/ml)	Enzyme activity (GDU/gram) (n=3)
Crude stem bromelain	1.05	0.1	0.70	2,100 ± 340
Crude fruit bromelain	1.15	0.1	1.11	1,450 ± 450
Stem bromelain (2000 rpm)	1.00	0.1	0.40	3,500 ± 480
Stem bromelain (4000 rpm)	1.01	0.1	0.51	2,772 ± 350
Stem bromelain (6000 rpm)	1.02	0.1	0.63	2,266 ± 330
Fruit bromelain (2000 rpm)	0.97	0.1	0.87	1,560 ± 500
Fruit bromelain (4000 rpm)	0.82	0.1	0.72	1,594 ± 500
Fruit bromelain (6000 rpm)	0.73	0.1	0.52	1,965 ± 400
Stem bromelain (2^{nd} eluate)	0.85	0.1	0.08	14,875 ± 500
Stem bromelain (4^{th} eluate)	0.95	0.1	0.14	9,500 ± 600
Fruit bromelain (2^{nd} eluate)	0.55	0.1	0.39	1,974 ± 280
Fruit bromelain (4^{th} eluate)	0.60	0.1	0.14	6,000 ± 290

Discussion

Bromelain is a general name for a family of sulfhydryl-containing, proteolytic enzymes obtained from *Ananas comosus.* The primary component of bromelain is a sulfhydryl proteolytic fraction. It also contains a peroxides, acid phosphatase, several protease inhibitors and organically-bound calcium [18]. It is a cysteine endopeptidase which specifically cleaves peptide bonds at the carbonyl group as found in arginine or in aromatic amino acids like phenylalanine or tyrosine. In the present work comparative study of stem bromelain and fruit bromelain has been performed. Juices were extracted from stem & fruit of pineapple plant, which contained the enzymes cysteine endopeptidase in stem bromelain and aspartic endopeptidase in fruit bromelain [19]. The juice extracted was called as crude extract of the enzymes and the activity of these crude extracts was estimated by the hydrolysis of gelatin and represented in the form of gelatin digestion units (GDUs).

Earlier reports on structural and kinetic analyses revealed that stem bromelain differed markedly in their enzymatic activity from fruit bromelain. From the results, it was concluded that the stem bromelain showed more enzymatic activity than fruit bromelain. Crude fraction of stem bromelain obtained from centrifugation at 2,000 rpm possess better proteolytic than fruit bromelain collected at 6,000 rpm. After that the crude extract was subjected to ammonium sulfate precipitation to precipitate out the enzyme. The pellet was dissolved in 10 mM Tris HCl buffer and subjected to dialysis to remove the salt and other ions bound to the enzyme. Then the enzymes were purified by anionic ion exchange chromatography using DEAE cellulose.

The stem and fruit bromelain enzymes were eluted using different concentrations of NaCl and Tris HCl buffer. From the enzymatic assays of ion exchange eluates, it was observed that maximum amount of NaCl was required for eluate 2^{nd} and 4^{th} of stem and fruit bromelain. Thus, ion exchange eluate 2^{nd} of stem bromelain and eluate 4^{th} of fruit bromelain showed more activity ($P > 0.001$). Proteolytic activity of different fractions from stem and fruit bromelain follows the sequence as follows, 2^{nd} eluate stem bromelain > 4^{th} eluate stem bromelain > 4^{th} eluate fruit bromelain > stem bromelain at 2,000 rpm > crude stem bromelain > crude fruit bromelain. From the above results we concluded that stem bromelain was found to be superior to fruit bromelain in hydrolyzing the gelatin.

However, it was possible to obtain pure biological products, such as bromelain, with lower operational cost using DEAE-cellulose resins, thus, decreasing overall process cost. Moreover, single SDS-PAGE band of ion exchange isolated bromelain protein also inferred the integrity and purity of bromelain protein. Results of poly-acrylamide gel electrophoresis of purified bromelain components supports the earlier findings revealed, the molecular weight of bromelain was approximately 30 kDa [20].

It has now been found that stability and activity of purified solution of the enzyme is important, not merely from the standpoint of purity or concentration of the product, but also because presence of impurities is accompanied by a decrease in enzymatic activity. The present study provides ion exchange process producing a purified bromelain having not only the advantages of reduced ash and increased specific activity, but also stability in solution which in turn means stability of specific activity.

Conclusion

This work exhibited that the purification of bromelain by ion exchange chromatography using DEAE anion exchanger, served as an economical means for the purification of bromelain from stem and fruit of pineapple plant. Recovered extract maintain the structural integrity and showing better proteolytic activity then crude extract and centrifugal fraction. Study result inferred that the stem bromelain possess better GDU activity over fruit bromelain. Centrifugal fraction exhibited better enzymatic activity over crude extract but lesser in comparison to ion exchange eluates, suggested that this technique needs to be optimized to produce bromelain with better proteolytic activity. Moreover a lot of attempts are required to be made to develop a simple, economical and effective technique to produce bromelain of ultrapure grade.

Acknowledgement

The authors are indebted to the staff members of the Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela, Orissa, India and Dr. (Mrs.) M.R. Vishnu Priya, Nitza Biologicals, Hyderabad, India for providing us the guidance and required facilities for the research work. The authors gratefully acknowledge to chairman of ISF College of Pharmacy, Moga to provide facilities for carry out part of research work.

References

[1] S. Gregory, and N. D. Kelly. Bromelain: a literature review and discussion of its therapeutic applications, *Alt. Med. Rev.* 1(4): 243-257 (1996).

[2] M. T. Murray, and J. E. Pizzorno. Bromelain. In: J. E. Pizzorno, and M. T. Murray (eds.), *Textbook of Natural Medicine,* Vol 1. (2nd ed.), Churchill Livingstone, Edinburgh, 1999, pp. 619-622.

[3] S. J. Taussig, and S. Batkin. Bromelain, the enzyme complex of pineapple *(Ananas comosus)* and its clinical application: an update, *J. Ethnopharmacol.* 22: 191-203 (1988).

[4] J. N. Moss, C.V. Frazier, and G.J. Martin. Bromelains, the pharmacology of the enzymes, *Arch. Int. Pharmacodyn.* 145:168 (1963).

[5] R. Guo, P. H. Canter, and E. Ernst. Herbal medicines for the treatment of rhinosinusitis: a systematic review, *Otolaryngol. Head Neck Surg.* 135(4): 496-506 (2006).

[6] G. Uhlig, and J. Seifert. The effect of proteolytic enzymes (traumanase) on posttraumatic edema, *Fortschr. Med.* 99: 554-556 (1981).

[7] A. Gutfreund, S. Taussig, and A. Morris. Effect of oral bromelain on blood pressure and heart rate of hypertensive patients, *Hawaii Med. J.* 37(5): 143-6 (1978).

[8] R. M. Heinicke, and W. A. Gortner. Stem bromelain-a new protease preparation from pineapple plants, *Econ. Bot.* 11(3): 225-234 (1957).

[9] T. Murachi, and H. Neuratii. Fractionation and specificity studies on stem bromelain, *J. Bio. Chem.* 235(1): 99-107 (1960).

[10] T. Harrach, K. Eckert, H. R. Maurer, I. Machleidt, W. Machleidt, and R. Nuck. Isolation and characterization of two forms of an acidic bromelain stem proteinase, *J. Protein Chem.* 17(4): 351-61 (1998).

[11] D. M. Bollag. Ion-exchange chromatography, *Methods Mol. Biol.* 36: 11-22 (1994).

[12] B. Paull, and P. N. Nesterenko. Novel ion chromatographic stationary phases for the analysis of complex matrices, *Analyst* 130(2):134-46 (2005).

[13] P. R. Levison. Large-scale ion-exchange column chromatography of proteins comparison of different formats, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 790(1-2): 17-33 (2003).

[14] J. J. Holthuis, and R. J. Driebergen. Chromatographic techniques for the characterization of proteins, *Pharm. Biotechnol.* 7: 243-99 (1995).

[15] G. L. Volkov, S. I. Andrianov, E. S. Gavriliuk, T. V. Goroshnikova, and A. Slominskii. Purification of biomolecules by the method of the expanded bed adsorption chromatography. III. Method optimization Applications, *Ukr Biokhim Zh.* 77(2): 26-57 (2005).

[16] P. Moodie, *Gelatin digestion unit analytical method.* Enzyme Development Corporation, New York, 2001.

[17] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R.J. Randall. Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193: 265 (1951).

[18] A. D. Napper, S. P. Bennett, M. Borowski, M. B. Holdridge, M. J. Leonard, E. E. Rogers, Y. Duan, T. Richard, R. A. Laursen, B. Reinhold, and S. L. Shames. Purification and characterization of multiple forms of the pineapple-stem-derived cysteine proteinases ananain and comosain, *Biochem. J.* 301: 727-735 (1994).

[19] A. D. Rowan, D. J. Buttle, and A. J. Barrett. The cysteine proteinases of the pineapple plant, *Biochem. J.* 266(3): 869-75 (1990).

[20] J. Scocca, and Y. C. Lee. The composition and structure of the carbohydrate of pineapple stem bromelain, *J. Biol. Chem.* 244(18): 4852-4863 (1969).