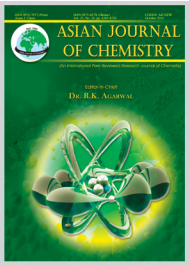
Asian Journal of Chemistry; Vol. 24, No. 4 (2012), 1429-1431

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Optimization of Activity of Bromelain

RUCHITA DUBEY[1], SOUMYA REDDY[2] and N.Y.S. MURTHY[2,*]

[1]Nitza Biologicals Research Labs Pvt. Ltd., Chandra Towers, Neredmet 'X' Road, Secundrabad-500 056, India
[2]Malla Reddy Engineering College, Maisammguda, Secunderabad-500 014, India

*Corresponding author: E-mail: nysrim@yahoo.co.in

(*Received*: 1 January 2011; *Accepted*: 15 November 2011) AJC-10682

Bromelain is a crude protein extract obtained from pineapple fruit and stem, which comprises a variety of proteolytic enzymes. It applied for reduction of muscle and tissue inflammation, alleviation of bronchitis and sinusitis, as an aid in digestion and for relieving symptoms of gout. The most important application of bromelain includes meat tenderization. Pineapple was peeled and crushed to extract the juice containing soluble bromelain enzyme. Further processing includes precipitation, dialysis and ion exchange chromatography of enzyme. Ion exchange elutes (pure enzyme) reveal the activity of bromelain by titrimetric enzyme assay method. Further, the enzyme was immobilized for commercial uses. Finally, the enzyme was applied for meat tenderization to check the effectiveness of extracted bromelain. It exhibits potential therapeutic activities and other industrial uses.

Key Words: Bromelain, Proteolytic enzyme, Immobilized enzyme and bioevents.

INTRODUCTION

Bromelain is a general name for a family of sulfhydryl containing, proteolytic enzymes obtained from Ananas comosus, the pineapple plant. It can function at a pH range 3 to 9 but once it is combined with substrate, the activity is no longer susceptible to the effect of the pH. The effective temperature range is 40-65 ºC with the optimum being 50 ºC. Bromelain can be activated by calcium chloride, cysteine, bisulfate salt, NaCN, H_2S, Na_2S and benzoate. Bromelain is inhibited by Hg^{2+}, Ag^{+}, Cu^{2+}, α-1-antitrypsin, estatin A and B, idoacetate. First introduced as a therapeutic compound[1] in 1957, bromelain's actions include (1) inhibition of platelet aggregation[2-4], (2) fibrinolytic activity[5], (3) antiinflammatory action[6], (4) antitumor action[7], (5) modulation of cytokines and immunity[8], (6) skin debridement properties[9], (7) enhanced absorption of other drugs[10], (8) mucolytic properties[11], (9) digestive assistance[12], (10) enhanced wound healing[13] and (11) cardiovascular and circulatory improvement[14,15]. After the extraction, purification (salt precipitation, dialysis, ion exchange chromatography) and estimation (Folin-Lowery's method) of the enzyme, the activity of the enzyme was explored at different pH, temperatures and substrate concentrations. The effect of immobilization activator and inhibitor effects are also investigated. Further the application of bromelain in meat tenderization was also studied.

EXPERIMENTAL

Pineapple was purchased from local fruit market in Hyderabad (A.P., India). The major equipment used in the present research work includes U.V spectrophotometer (microprocessor (EI) model 1371), Ion exchange chromatograph (borosil), gel filtration chromatograph (borosil), cooling centrifuge (remi). Sodium acetate buffer was prepared by dissolving 25.5 g of sodium acetate and 24.5 mL of acetic acid in water and the solution is made up to 200 mL to get a pH of 4.5. Gelatin solution (5 %) was prepared by heating 5 g of gelatin in 100 mL of water at 80 ºC for 20 min. 3 % Hydrogen peroxide was prepared by dissolving 3 mL of H_2O_2 in 100 mL water.

Bromealin being a proteolytic enzyme acts on gelatine and degrades it into amino acids and oligo peptides. Hydrogen peroxide is used to stop the reaction after 20 min. It helps in oxidation. When pH is adjusted to 6.9 there will not be any amino acids. Formaldehyde breaks the amino group of amino acids to release H^+ ions. Number of H^+ ions is proportional to amino acids. Sodium hydroxide is added to neutralize H^+ ions. Amount of NaOH needed for titration proportional to number of H^+ ions, this is proportional to number of amino acids, proportional to activity of enzyme. Volume of NaOH needed for test is greater than blank (containing no enzyme) indicates test tube containing enzyme shows activity.

Effect of temperature: Ten test tubes were labeled and separated into 5 groups (1-test, 2-blank; 3-test, 4-blank…. 9-test, 10-blank). Then 2.5 mL of gelatin was added to both test and blank test tubes. These are incubated at different temperatures (25.35, 45, 55 and 65 ºC) for 10 min. Then 0.1 mL of enzyme was added to all the test tubes and incubated at 25, 35, 45, 55 and 65 ºC for 20 min. Then 10 µL of H_2O_2 is added to all the test tubes. The pH is adjusted to 6.9 and then 1 mL of formaldehyde is added. These are titrated against NaOH to get a pH of 7.8 and the readings are noted. While studying the effect of pH, substrate concentration, activators and inhibitor the following procedure was adopted.

Out of 6 test tubes, five are labeled as test and one test tube as blank. Then 2.5 mL of gelatin was added to all the test tubes. The test tubes were incubated at 45 ºC for 10 min. Enzyme (0.1 mL) was added to each test tube and incubated at 45 ºC for 20 min. Then the variation in the pH, different concentrations of the substrate, activator (magnesium chloride) and inhibitor (mercuric chloride) were maintained. Later 10 µL H_2O_2 is added to all test tubes. The pH is adjusted to 6.9 and then 1 mL of formaldehyde was added. The pH of each test tube was adjusted to 7.8 by titrating with NaOH. The readings are noted.

Immobilization of bromelain: 1 mL of ion exchange elute (of the enzyme) was added to 10 mL of sodium alginate solution and mixed well. This solution was added drop by drop into calcium chloride, which leads to the formation of beads. Number of beads was counted which were formed while adding the solution. Thus the enzyme was immobilized.

Number of beads formed = 70 per 1 mL of elute and hence, 0.1 mL of elute contains 7 beads.

Enzyme assay of immobilized bromelain: Two test tubes were marked as test and blank. Gelatin (2.5 mL) was added in each test tube and incubated at 45 ºC for 10 min. Seven immobilized beads were added in test and mixed well, incubated the test tubes at 45 ºC for 20 min. Then10 µL of hydrogen peroxide was added in both test and blank and mixed well. pH of both test and blank was adjusted to 6.9 by using acid (HCl) and base (NaOH). Formaldehyde (1 mL) was added in both the test tubes. Then pH of each test tube was adjusted to 7.8 by titration with NaOH.

Meat tenderization: Beef (top round post rigor meat, *i.e.*, 72 h postmortem) was purchased from a local abattoir and used fresh. Samples of 100 g were weighed and injected with 411.5 MCU/mg of enzyme solution with injection needles being about 1 inch apart. Another 100 g of sample weighed and injected with 4117.5 MCU/mg of enzyme solution + activator (1% $CaCl_2$), with injection needles being about 1 inch apart. pH was checked for both the samples. It was noted as 6. The meat was incubated for 4-5 h in refrigerator (4 ºC) after, which samples were again monitored for the change in pH. The pH was decreased to 4. Then the samples were sliced into portions of 1 gm and ground in the presence of 10 mL of 0.1 M sodium acetate buffer.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of temperature on enzyme activity.

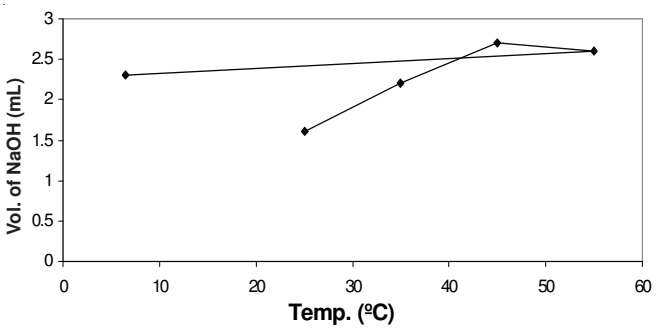


Fig. 1. Effect of temperature on enzyme activity

Five sets of test tubes (10) containing one blank and one test, maintained at varying temperatures were added with ion exchange chromatography elute and tested for activity of gelatine. Maximum activity was found at 45 ºC. This result could be attributed to the thermal inactivation of the enzyme at higher temperatures.

Maximum enzymatic activity was found at pH 4.5. It can be observed from graph that the activity of enzyme increases with the increase in pH up to 4.5 and then decreases (Fig. 2).

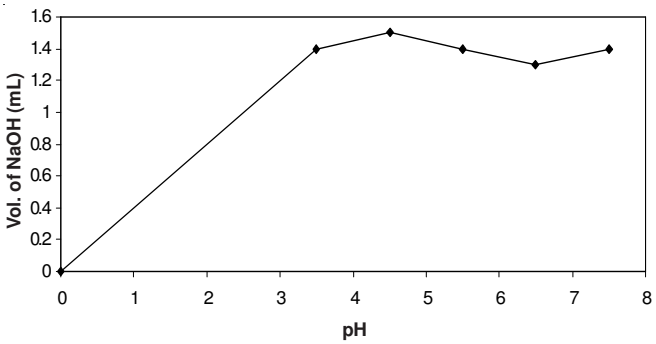


Fig. 2. Effect of pH on the enzyme activity

Maximum activity of the enzyme was found when the substrate concentration of 4.5 mL (Fig. 3). As substrate concentration increases, the rate of reaction (enzyme activity) also increases up to certain point. An enzyme is saturated when the active site of all molecules are occupied, most of the time. At the saturation point, reaction will not speed up.

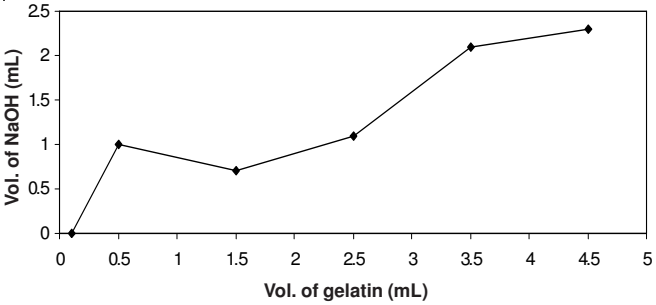


Fig. 3. Effect of substrate concentration on enzyme activity

Ion exchange chromatographic elutes are added to six test tubes along with varying volumes of activators $MgCl_2$. Maximum activity was founded for 1.0 mL of $MgCl_2$ (Fig. 4).

Least activity of the enzyme was found by using 1 mL of mercuric chloride (Fig. 5).

TABLE-1
ENZYME ASSAY OF IMMOBILIZED ENZYME

Test tube no.	Vol. of gelatin (mL)	Incubation for 10 min at 45 °C	Vol. of enzyme (mL)	Incubation for 20 min at 45 °C	Vol. of hydrogen peroxide (µL)	Adjust the pH to 6.9	Vol. of formaldehyde (mL)	Adjust the pH to 7.8	Vol. of NaOH (mL)
Blank	2.5		-		10		1		1.0
Test	2.5		0.1(7 beads)		10		1		1.4

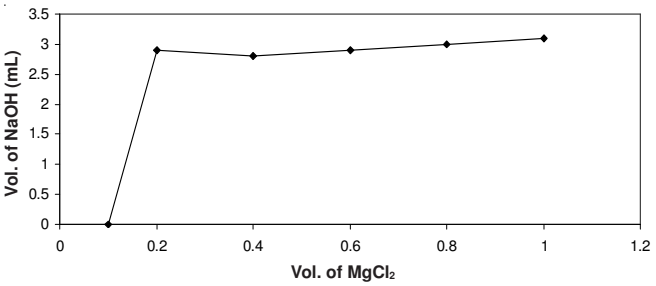


Fig. 4. Effect of activators on enzyme activity

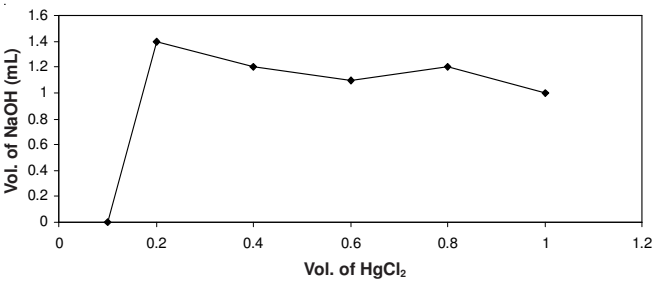


Fig. 5. Effect of inhibitors on enzyme activity

Test tube containing 7 beads (Table-1) of immobilized bromealin enzyme (Fig. 6) showed higher activity. It is evident from the amount of sodium hydroxide consumed.

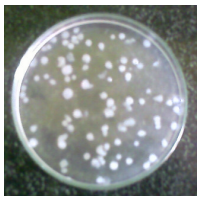


Fig. 6. Immobilized bromelain enzyme

Tenderized beef (Fig. 7) was observed after 4-5 h of incubation. It gives softy appearance and tenderness is felt on touching.

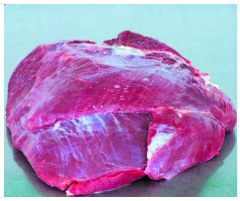


Fig. 7. Tenderized beef

Conclusion

The enzyme exhibited the maximum activity at 45 ºC, the optimum pH for the activity is 4.5, maximum activity of the enzyme was found when the amount of the substrate used was 4.5 mL, the optimum activity of activator ($MgCl_2$) is observed when 1 mL is used and while the optimum activity for inhibitor ($HgCl_2$) is observed when 0.1 mL of the inhibitor is used.

ACKNOWLEDGEMENTS

The authors thank Dr. Sunakar Ketan Nayak of Nitza Biologicals, Hyderabad and to the Principal and Management of Malla Reddy Engineering College, Secunderabad for their support in carrying out this research work.

REFERENCES

1. R.M. Heinicke and W.A. Gortner, *Econ. Bot.*, **11**, 225 (1957).
2. R.M. Heinicke, L. van der Wal and M. Yokoyama, *Experientia,* **28**, 844 (1972).
3. A.H. Morita, D.A. Uchida and S.J. Taussig, *Arch. Inter. Phar. Ther.*, **239**, 340 (1979).
4. M. Livio, M.P. Bertoni and G. De Gaetano, *Drugs Expt. Clin. Res.*, **4**, 49 (1978).
5. G.E. Felton, *Med. Hypotheses,* **6**, 1123 (1980).
6. B. Seligman, *Angiology*, **13**, 5082 (1962).
7. G. Kelly, *Alt. Med. Rev.*, **1**, 243 (1996).
8. S. Brien, G. Lewith and A. Walker, *Evidence-based Complement. Alternat. Med.*, **1**, 251 (2004).
9. S. Taussig and S. Batkin, *J. Ethnopharmacol.*, **22**, 191 (1988).
10. G. Renzinni and M. Varengo, *Arzneim-Forsch*, **22**, 410 (1972).
11. A. Schafer and B. Adelman, *J. Clin. Inves.*, **75**, 456 (1985).
12. R.P. Knill-Jones, J. Pearce and H. Batten, *Br. Med. J.*, **4**, 21 (1970).
13. H.R. Maurer, *Cell. Mol. Life Sci.*, **58**, 1234 (2001).
14. H.A. Nieper, *Acta Med. Empirica.,* **5**, 274 (1978).
15. S.J. Taussig and H.A. Nieper, *J. IAPM*, **6**, 139 (1979).