Enzymatic Studies on Crude Papain from Papaya Peels

Nwe Mar Lin[1], Khin Sandar Linn[2], Yee Mun Than[3], Myat Kyaw Thu[4]

Abstract

Papain (EC 3.4.22.2) is the most important plant proteolytic enzyme present in the latex, leaves, roots and fruits of the papaya plants. In this research, partially purified papain enzyme was isolated from matured green papaya peels by ammonium sulphate precipitation (20 % - 70 %) method. The partially purified papain was qualitatively examined by skim milk powdered test for its milk clotting activity. Papain activity was determined by spectrophotometric method using tyrosine as standard and casein as substrate at 273 nm. Protein content was determined by Biuret method using Bovine Serum Albumin (BSA) as standard at 560 nm. Specific activities, protein recovery and degree of purification were determined in each purification steps. Papain was purified 8.6 fold over crude extract and protein recovery was found to be 31.50 %. Specific activity of papain in crude extract was 0.0045 μmol min^{-1} mg^{-1} and increased to 0.0387 μmol min^{-1} mg^{-1} after 70 % ammonium sulphate precipitation. The optimum pH of the papain - catalyzed reaction was determined by using different pH values (6 - 8) and the maximum papain activity was found at 7.4. The optimum temperature of the papain - catalyzed reaction was conducted at different temperature in the range of (27°C - 87 °C) and the highest papain activity was observed at 67 °C.

Keywords: papain, papaya peels, ammonium sulphate precipitation method, milk clotting activity, spectrophotometric method

Introduction

Papaya plant (*Carica papaya* L.) is a source of carotenoides, vitamin C, thiamine, riboflavin, niacin, vitamin B_6 and vitamin K (Bari *et al*., 2006). Papaya plant is a large, single-stemmed herbaceous perennial tree. The leaves are very large, palmate lobed or deeply incised with entire margins and stems are hollow, light green to tan brown in colour (Yogiraj *et al*., 2014). The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has attained amber to orange hue (Aravind *et al.,* 2013). Papain activity from the latex of the unripe fruit or other parts of the plant, and also in the quantification of papain present in the pulp (Tripathi *et al*., 2011).

The most important proteolytic enzymes found in fruits are papain (EC 3.4.22.2), ficin (EC 3.4.22.3), bromelain (EC 3.4.22.4) and actinidin (EC 3.4.22.14) found in papayas, figs, pineapples, and kiwis, respectively (Azarkan *et al*., 2004). *Carica papaya* contains biologically active compounds. The two most common important compounds are papain and chymopapain (Milind and Gurditta, 2011). They have multiple applications in the food industry as meat tenderizers, for chill proofing of beer, in the manufacture of cheeses, and in bread making where they modify gluten properties (Hewitt *et al*., 2000).

[1] Assistant Lecturer, Department of Chemistry, Maubin University
[2] Assistant Lecturer, Department of Chemistry, University of Yangon
[3] Lecturer, Department of Chemistry, University of Yangon
[4] Professor, Department of Chemistry, University of Yangon

Scientific classification

Kingdom	: Plantae
Division	: Spermatophyta
Class	: Angiospermae
Order	: Caricales
Family	: Caricaceae
Genus	: *Carica*
Species	: *papaya*
Botanical name	: *Carica papaya* L.
Common name	: Papaya, Paw Paw
Myanmar name	: Thimbaw
Part used	: Peels

Figure 1 Papaya Fruits

Papain Enzyme

Enzymes are biological catalyst that increase the rate of otherwise slow reactions by decreasing the reactions activation energy, without undergoing any neat change in there structures at the end of a reaction (Trivedi *et al*., 2013). When an enzyme is present, the reaction occurs at a much higher rate and the enzyme itself is not consumed in the activity (Nordin, 2010). Enzymes are useful in various areas of applications like manufacturing of food and feedstuff, cosmetics, medicinal products and as a tool for research and development. Enzymes can be used in chemical analysis and as a research tool in the life sciences (Binod *et al.,* 2013).

Papain may be obtained from bacteria, plants, vegetables and invertebrates. Papain, a cysteine protease is usually obtained from the latex of *Carica* papaya. However it is not only the latex that contains this valuable enzyme. In fact the whole parts of the papaya tree including its leaves, stems and petioles can be utilized for the extraction of papain except its root (Sanchez *et al*., 2009).

Papain is used to treat commercial beer, to degum natural silk, as a meat tenderizer and in the production of chewing gums. Cosmetically it is used in shampoos and a number of face - lifting operations (Aravind *et al.,* 2013). Enzyme papain is being used in several industries.

The aim of this study was to isolate and partially purify papain from *Carica papaya* L. peels and to study its kinetic properties.

Materials and Method

Papaya peels sample (*Carica papaya* L.) were collected from the compound of Maubin University. Papain was extracted from papaya peels by using ammonium sulphate precipitation (20 % and 70 % saturation) method. The prepared enzyme was qualitatively examined for its milk clotting activity by using 20 % skim milk powdered solution. Papain activities in different purification steps were monitored by spectrophotometric method at 273 nm and protein contents were determined by Biuret method at 560 nm. The kinetic properties of papain enzyme (effects of pH, temperature and reacation time) were studied by spectrophotometric method.

Results and Discussion

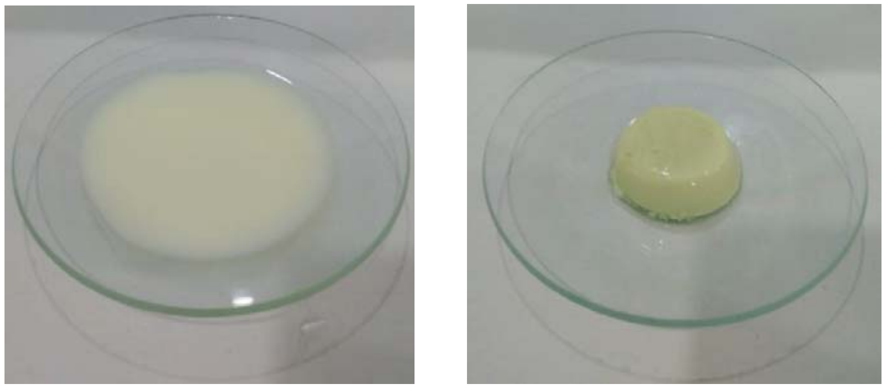
Papain (EC 3.4.22.2) is a proteolytic enzyme currently found in papaya. It breaks down proteins and is widely used in dozens of food processing applications (Welde and Worku, 2018). In this research, the papain enzyme was isolated from papaya peels sample obtained from the campus of Maubin University and it was partially purified by solid ammonium sulphate precipitation method. In this study, papain was extracted by using solid ammonium sulphate (20 % saturation followed by 70 % saturation). Milk clotting activity was qualitatively examined by using skim milk powdered solution. Figure 2 (a) shows 20 % skim milk powdered solution at 37 °C. Figure 2 (b) shows coagulated milk obtained by addition of papain in 20 % skim milk powdered solution at 37 °C for 3 hr. This experiment clearly shows milk clotting activity of enzyme. To determine the papain activity, spectrophotometric method was employed by using tyrosine as standard at 273 nm. Protein content was determined by Biuret method using Bovine Serum Albumin (BSA) as standard at 560 nm. Enzyme activity, protein content and specific activity of *Carica* papaya peels were determined in different purification steps.

In this present research, papain was purified 8.6 fold over crude extract and protein recovery was found to be 31.50 %. Specific activity of papain in crude extract was 0.0045 μmol min^{-1} mg^{-1} and increased to 0.0387 μmol min^{-1} mg^{-1} after 70 % ammonium sulphate precipitation (Table 1).

In this work, the partially purified papain was partially characterized by its reaction time, optimum pH and optimum temperature. For all enzymes, the reaction time is out of the relation to the function of pH. In this research, papain activity was determined at different reaction time ranging from 15 min to 180 min. It was found that the rate of reactions decreases linearly with time up to about 60 min so that the reaction time of 30 min was selected for the experiments (Table 2 and figure 3).

The optimum pH of papain was reported to be pH 7.0 (Chu Chi Ming *et al*., 2002), and pH 7.5 (Ding *et al.,* 2003). Optimum pH was determined by using phosphate buffer solution within the range of (6 – 8) with 0.2 intervals. Papain activity was gradually increased to pH 7.4 and decreased beyond pH 7.4. The optimum pH of papain was found at pH 7.4 (Table 3 and figure 4). The optimum pH 7.4 is an agreement with the literature values.

In this research work, papain activity was determined at different temperature ranging from 27 °C to 87 °C within 10 °C interval while the substrate medium (1% casein) was prepared and the optimum pH of 7.4 was fixed. Papain activity gradually increased from 27 °C to 57 °C and abruptly increased to 67 ºC and then it decreased. The optimum temperature was found at 67 ºC (Table 4 and figure 5). The optimum temperature of papain was reported to be 65 ºC - 80 ºC (Harton *et al.*, 2002). The optimum temperature was in agreement with reported values.



(a) **(b)**

Figure 2 Milk clotting activity of papain
(a) without papain enzyme
(b) with papain enzyme

Table 1 Enzyme Activity, Protein Content and Specific Activity of *Carica Papaya* L. Peels in Different Purification Steps

Fraction	Enzyme Activity (μmol min^{-1} mL^{-1})	Protein Content (mg/mL)	Specific Activity (μmol min^{-1} mg^{-1})	Protein Recovery (%)	Degree of Purity (fold)
Crude	0.0402	9.0199	0.0045	100	1
20 % $(NH_4)_2SO_4$	0.0781	7.1325	0.0109	79.08	2.42
70% $(NH_4)_2SO_4$ (ppt)	0.1099	2.8411	0.0387	31.50	8.60

Table 2 Velocity of Papain-catalyzed Reaction at Various Reaction Times

No	Time (min)	Velocity x 10^{-1} (10^{-1} mM min^{-1})
1	15	1.0869
2	30	0.5957
3	60	0.3641
4	90	0.2713
5	120	0.2226
6	150	0.1871
7	180	0.1566

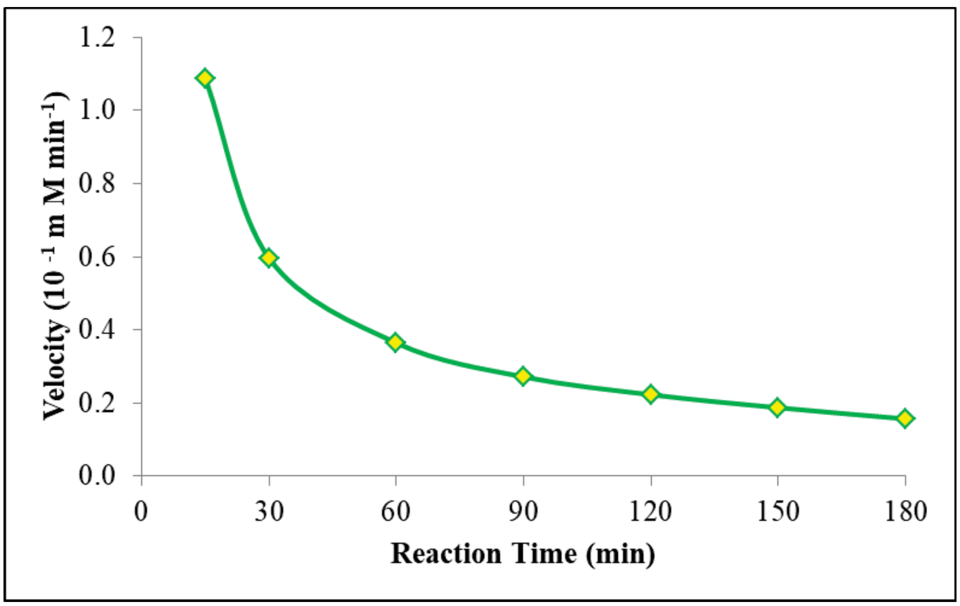


Figure 3 Plot of velocity of papain enzyme-catalyzed reaction as a function of reaction time

Table 3 Relation between Proteolytic Activity of Papain Enzyme-catalyzed Reaction and pH of Buffer Solution

No	pH	Activity (10^{-1} µmol min^{-1} mL^{-1})
1	6.0	0.9793
2	6.2	0.9855
3	6.4	1.0214
4	6.6	1.0338
5	6.8	1.0603
6	7.0	1.0806
7	7.2	1.0915
8	7.4	1.1508
9	7.6	1.0681
10	7.8	1.0353
11	8.0	0.9559

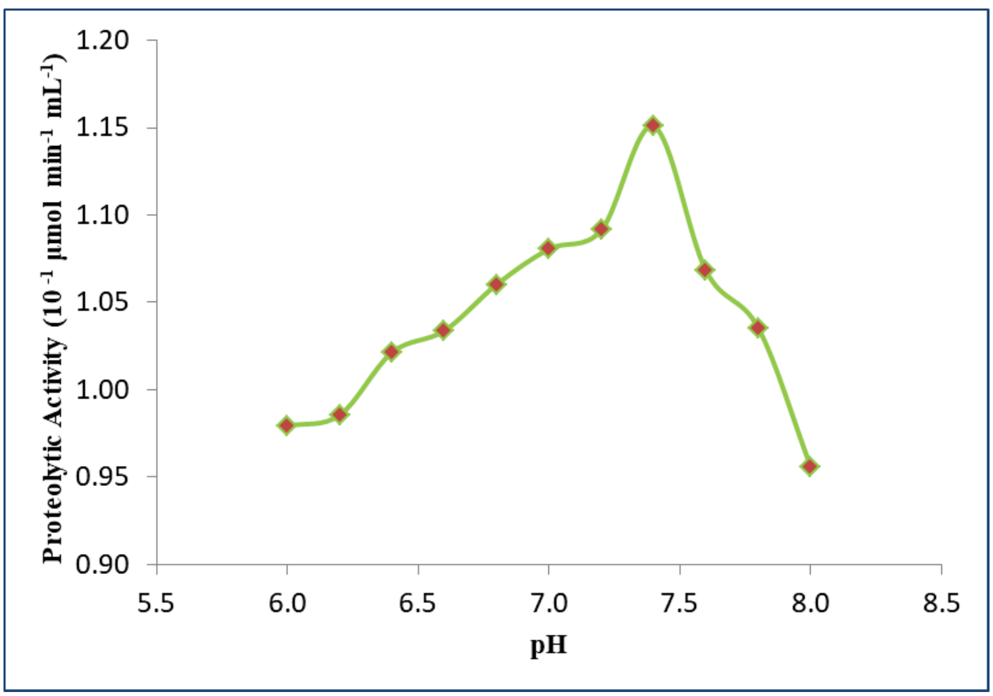


Figure 4 Plot of proteolytic activity as a function of pH of solution

Table 4 Relation between Proteolytic Activity of Papain Enzyme-catalyzed Reaction and of the Solution at pH 7.4

No	Temperature (°C)	Activity (10^{-1} µmol min^{-1} mL^{-1})
1	27	1.1336
2	37	1.4424
3	47	1.6778
4	57	1.8416
5	67	1.9850
6	77	1.6014
7	87	1.5952

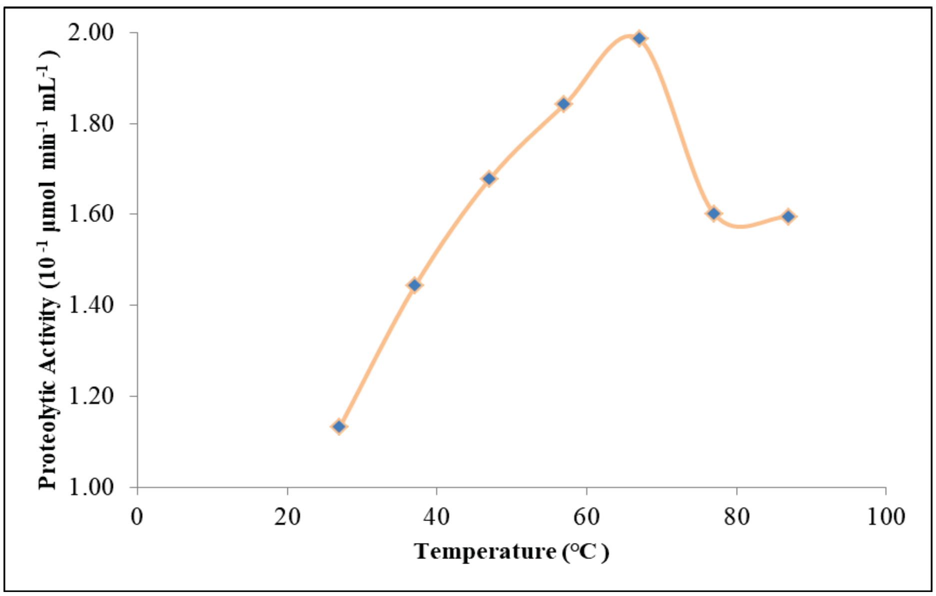


Figure 5 Plot of proteolytic activity as a function of temperature of solution at pH 7.4

Conclusion

Papain (EC 3.4.22.2) from papaya peels (*Carica papaya* L.) was collected in the compound of Maubin University. In this research, partially purified papain enzyme was isolated from matured green papaya peels by ammonium sulphate precipitation (20 % - 70 %) method. The partially purified papain was qualitatively examined by skim milk powdered test for its milk clotting activity. Papain activity was determined by spectrophotometric method using tyrosine as standard and casein as substrate at 273 nm. Protein content was determined by Biuret method using Bovine Serum Albumin (BSA) as standard at 560 nm. Papain was purified 8.6 fold over crude extract and protein recovery was found to be 31.50 %. Specific activity of papain in crude extract was 0.0045 µmol min^{-1} mg^{-1} and increased to 0.0387 µmol min^{-1} mg^{-1} after 70 % ammonium sulphate precipitation. The optimum pH of the papain - catalyzed reaction was determined by using different pH values (6 - 8) and the maximum papain activity was found at 7.4. The optimum temperature of the papain - catalyzed reaction was conducted at different temperature in the range of (27 °C - 87 °C) and the highest papain activity was observed at 67 °C. Nowadays, fermented fish products are very popular all over the world. Many fermented fish products are prepared in different parts of the world and the method of processing depends upon various factors, such as availability of raw materials, consumer's preference and the climatic conditions of the region. To preserve or improve the quality of fish fermentation, microbial enzymes or proteolytic enzymes is added to convert fish to sauces, pastes, soup stocks and protein concentrates. In the present work papain from *Carica papaya* peels will be applied for the preparation of fish sauce.

Acknowledgements

I would like to express my special thanks of gratitude to Dr Aung Kyaw, Acting Rector, Maubin University, for his permission to write this research paper. I would like to express my sincere thanks to Dr Hnin Hnin Than, Professor and Head, Department of Chemistry, Maubin University, who gave me the opportunity to do this research.

References

Aravind, G., B. Debjit, S. Duraivel and G. Harish. 2013. "Traditional and Medicinal Uses of *Carica Papaya*". *J. Med. Plants Studies.*, **1**(1), 7-15

Azarkan, M., R. Wintjens, Y. Looze and D. Baeyens-Volant. 2004. "Detection of Three Wound-Induced Proteins in Papaya Latex". *Phytochem.*, **65**, 525-534

Bari, L., P. Hassan, N. Absar, M. E. Haque, M. I. I. E. Khuda, M. M. Pervin, S. Khatun and M. I. Hossain. 2006. "Nutritional Analysis of Two Local Varieties of Papaya (*Carica papaya* L.) at Different Maturation Stages". Pak. *J. Biol. Scie.,* **9**(1), 137-140

Binod, P., P. Palkhiwala, R. Gikaiwari, K. M. Nampoothiri, A. Duggal, K. Dey, and A. Pandey. 2013. "Industrial Enzymes Present Status and Future Perspectives for India". *J. Scie. & Ind. Res.,* **72**, 271-286

Chu Chi Ming, A. B., K. Duduku and H. Tie Sing. 2002. "Effects of Ionic and Non-ionic Surfactants on Papain Activity". *Borneo Science*, **12,** 71-77

Ding, L., Z. Yao, T. Li, Q. Yue and J. Chai. 2003. "Study on Papain Immobilization on a Macroporous Polymer Carrier". *Turkish J. Chem*., **27**, 627-637

Harton, M., R. Ochs and E. Scrigeour. 2002. *Principle of Biochemistry*. New York: Prentice-Hall Inc., 568-570

Hewitt, H., S. Whittle, S. Lopez, E. Bailey and S. Weaver. 2000. "Tropical Uses of Papaya in Chronic Skin Ulcer Therapy in Jamaica". *J. W. Ind. Med.*, **49**(1), 32-33

Milind, P. and Gurditta. 2011. "Basketfull Benefits of Papaya". *Int. Res. J. Phar.*, **2** (7), 6-12

Nordin, N.D.B. 2010. *The Extraction of Papain form Papaya Leaves*. Malaysia: Biotechnology, Faculty of Chemical & Natural Resources Engineering University

Sanchez, O. F., A. M. Rodriguez, E. Silva and L. A. Caicedo. 2010. "Sucrose Biotransformation to Fructooligosaccharides by *Aspergillus* sp N74 Free Cells". *Food Bioprocess Tech.,* **3,** 662-73

Tripathi, S., J. Y. Suzuki, J. B. Carr, G. T. McQuate, S. A. Ferreira, R. M. Manshardt, K. Y. Pitz, M. M. Wall and D. Gonsalves. 2011. "Nutritional Composition of Rainbow Papaya, the First Commercialized Transgenic Fruit Crop". *J. Food. Composition & Analysis.,* **24**(2), 140-147

Trivedi, V., R. P. S. Rathore, P. R. Kamble, M. Goyal, N. Singh. 2013. "Pepsin, Papain and Hyaluronidase Enzyme Analysis: A Review". *Int. J. Res. Phar. & Scin.,* **3**(1), 01-18

Welde. Y. and A. Worku. 2018. "Identification and Extraction of Papain Enzyme from Papaya Leaf in Adigrat Towen, Northen Ethiopia". *J. Med. Plants Studies*., **6**(3), 127-130

Yogiraj, V., P.K. Goyal, C.S. Chauhan, A. Goyal, and B. Vyas. 2014. "*Carica* papaya *Linn*: An Overview". *Int. J. Herbal Med.*, **2(**5), 01-08