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# EXTRACTION, PURIFICATION, CHARACTERIZATION AND METAL ION CONCENTRATION OF PAPAIN FROM *CARICA PAPAYA* L.

**Joslin D., Gowthami R., Jenitta Emima Packiyam E., Rama Bhat P.*, Jayadev K. and Shrinidhi Shetty**

PG Department of Biotechnology, Alva's College, Moodbidri- 574 227, Karnataka, India

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***Correspondence for Author**
**Dr. Rama Bhat P.**
PG Department of Biotechnology, Alva's College, Moodbidri- 574 227, Karnataka, India.

## ABSTRACT

*Carica papaya* are rich in papain which shows extensive proteolytic activity towards proteins, short- chain peptides, amino acid, esters and amide links and is applied extensively in the fields of food and medicine. In the present study it was the goal to investigate the papain enzyme production and to test the enzyme activity under different conditions like pH, temperature and different metal ion concentration. The solution of papain exhibited an absorption maximum at a wavelength of 280nm. The enzyme activity of purified papain was of 0.3 U/ml by dialysis membrane and in case of ion exchange chromatography it was 0.24U/ml. The rate of enzyme catalysed reactions generally increases with temperature. In the present study the optimum enzyme activity was seen at the temperature of $65^0$C and enzyme has shown its maximum activity at pH 6.5, above and below this pH, the activity declines. Enzyme activity was found more with $Ca^{2+}$ and $K^+$ of the metal ions used.

**KEY WORDS:** *Carica papaya*, Metal ions, Dialysis, Ion-exchange chromatography.

## INTRODUCTION

Papain (EC 3.4.22.2) is an endolytic plant cysteine protease enzyme which is isolated from papaya (*C. papaya* L.) latex. Papain is obtained by cutting the skin of the unripe papaya and then collecting the latex which flows from the cut. The greener the fruit, more active is the papain. Papain enzyme belongs to the papain super family, as a proteolytic enzyme, papain is of crucial importance in many vital biological processes in all living organisms[1]. Papain acts as a debris-removing agent, with no harmful effect on sound tissues because of the enzyme's

specificity, acting only on the tissues, which lack the antitripsine plasmatic antiprotease that inhibits proteolysis in healthy tissues[2]. To make industrially useable state, these enzymes to be extracted and purified from green papaya fruits[3]. It is crucial to isolate papain in active crystalline state from fresh latex. Recently, this enzyme is being used for pharmaceutical and medical purposes. Therefore, the present research work was aimed to evaluate the potential papain production from native *C. papaya*.

## MATERIALS AND METHODS

### Sample collection and extraction

Fresh unripe papaya was collected from the local agricultural farm near Moodbidri, Karnataka. After proper washing and surface sterilization, four to six longitudinal incisions were made with a stainless steel knife reaching the base of the fruit. The latex exuded from the cut and it was collected in a vessel. The latex contains the enzyme and it was used for further partial purification steps.

### Purification of the crude enzyme by two step salt precipitation

The procedure used was modified from that reported by Kimmel and Smith (1954) [4]. The latex was mixed with 40 mM cysteine at ratio 3:1. The pH of the suspension was adjusted to 5.6 using 6M HCl and then stirred for 15 minutes at $40^0$C. The mixture was filtered using a Whatman No.1 filter paper. The pH of the filtrate was adjusted to 9.0 using 6M NaOH. A few proteins precipitate at this pH. This along with other insoluble materials was removed by centrifugation at 2500 rpm for 30 minutes. The clear supernatant was taken and added 2.24g of ammonium sulphate to get 40% saturation. The supernatant was discarded and the precipitate was washed once with 10 ml of 40% saturated ammonium solution and finally collected.

The precipitate obtained was dissolved in 15ml of 0.02M cysteine solution. Precipitate pairs in the solution by the slow addition of 1.5g of solid NaCl. The addition of salt should be in steps and should take at least 30 minutes. The precipitate collected was centrifuged at 2500 rpm for 30 minutes. To the suspension 10 ml of 0.0001M cysteine was added and kept at room temperature for about 30 minutes during which it develops a crystalline sheen. Keeping at $40^0$C over night the crystals of papain were formed.

**Dialysis:** The pretreated dialysis bag was used for the dialysis of the sample obtained after chromatography. The sample was dialyzed against 1mM EDTA at $40^0$C with three successive changes of 24 hours each.

**Purity analysis by ion-exchange chromatography**

Aliquots of proteins dissolved in 0.4M sodium acetate buffer, pH 5.0 were applied onto a DEAE cellulose column equilibrated with the same buffer. The material was eluted using a discontinuous gradient of sodium acetate buffer, pH 5. The collected purified sample was used for further steps.

**Determination of protein content**

The protein content in the samples during purification was determined by Lowry's method[5].

**Determination of enzyme activity**

The reaction mixture contained 50mM cysteine, 20mM Tris-HCl and 1 ml of enzyme solution buffer maintained at pH 8.0. The mixture was incubated at $37^0$C for 5 minutes before starting the reaction by adding 1ml of 1% casein solution. After 10 minutes the reaction was stopped by adding 3 ml of 5% Trichloroacetic acid (TCA) and then cooled for 1 hour. Absorbance was measured at 275nm.

**Determination of optimum temperature**

The optimum temperature for enzyme activity was determined by incubating the sample at different temperature ranges. The samples were incubated at $0^0$C, $37^0$C, $45^0$C, $65^0$C, and $85^0$C and the enzyme activity determined in each case.

**Determination of optimum pH**

The optimum pH for enzyme activity was determined by taking Tris-HCl at different pH range in the reaction mixture. The buffer of pH 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were taken and the enzyme activity was determined in each case.

**Effect of metal ions on the enzyme activity**

Effect of Metals ions on enzyme activity was determined ($Ba^{2+}$, $K^+$, $Mg^{2+}$,$Ca^{2+}$ ,$Fe^{2+}$, $Na^+$) at different concentrations (50 µl, 100 µl and 150 µl). Enzyme was treated with these metal ions, and make up the volume equal by adding distilled water then it was preincubated for 10 minutes at $37^0$C. The reaction was stopped by adding 3ml of 5% Trichloroacetic acid (TCA), and the enzyme activity was determined.

## RESULT

### Characterization of enzyme

### Determination of protein content

The estimated protein content of the crude papain was found to be 32.4mg/ml.

**Determination of enzyme activity:** The rate of hydrolysis of papain is determined by measuring the released cysteine. One unit releases 1µmol of cysteine/minute at 37$^0$C and pH 8.0 under the specified conditions. Micromoles of cysteine released were determined using cysteine standard curve (Fig. 1). The enzyme activity of enzyme was determined.

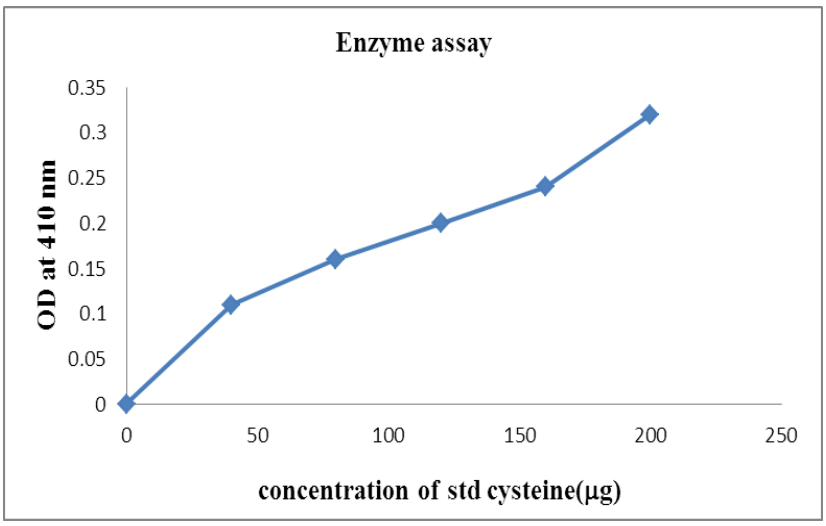


**Fig.1: Standard graph for cysteine**

The optimum temperature was found to be 65$^0$C for the sample. The maximum activity (4.56U/ml) showed at this temperature. At temperature 0$^0$C the activity was 1.14U/ml. After A decrease in the enzyme activity was observed after 85$^0$C (Table 1).

**Table 1: Enzyme activity of papain at different temperatures Effect of temperature on enzyme activity**

| Temperature ($^0$C) | Enzyme activity (U/g) |
|---|---|
| 0 | 1.14 |
| 37 | 3.42 |
| 45 | 4.33 |
| 65 | 4.56 |
| 85 | 4.5 |
| 100 | 4.1 |

**Effect of pH on enzyme activity:** The optimum pH was found to be 6.5 for the sample. In this pH the enzyme showed maximum activity (91.2U/ml). The minimum activity was at pH

5(31.92U/ml). At pH 5.5 it showed the activity of 82.08U/ml but when the pH was at 7 the activity was 84.36U/ml (Table 2).

**Table 2: Enzyme activity of papain at different pH**

| pH | Enzyme activity ( U/g) |
|---|---|
| 5 | 31.92 |
| 5.5 | 82.08 |
| 6 | 86.64 |
| 6.5 | 91.2 |
| 7 | 84.36 |
| 7.5 | 79.8 |
| 8 | 68.4 |

**Enzyme purification:** Crude papain extract which was extracted from latex was further purified using dialysis and DEAE-column chromatography (Table 3).

**Table: 3 Enzyme activity after different purification steps**.

| Sample | Concentration of protein(mg) | Enzyme activity(U/ml) |
|---|---|---|
| Crude extract | 0.15 | 4.5 |
| After dialysis | 0.1 | 3 |
| After ion exchange chromatography | 0.8 | 2.4 |

**Effect of metal ions on the enzyme activity:** According to the graph, $Ca^{2+}$ enhanced the enzyme activity at higher concentration of 150µl. $K^{+}$ and $Mg^{2+}$ enhanced the enzyme activity at the concentration of 100µl followed by $Ba^{2+}$, $Fe^{2+}$ and $Na^{+}$ (Fig. 2).

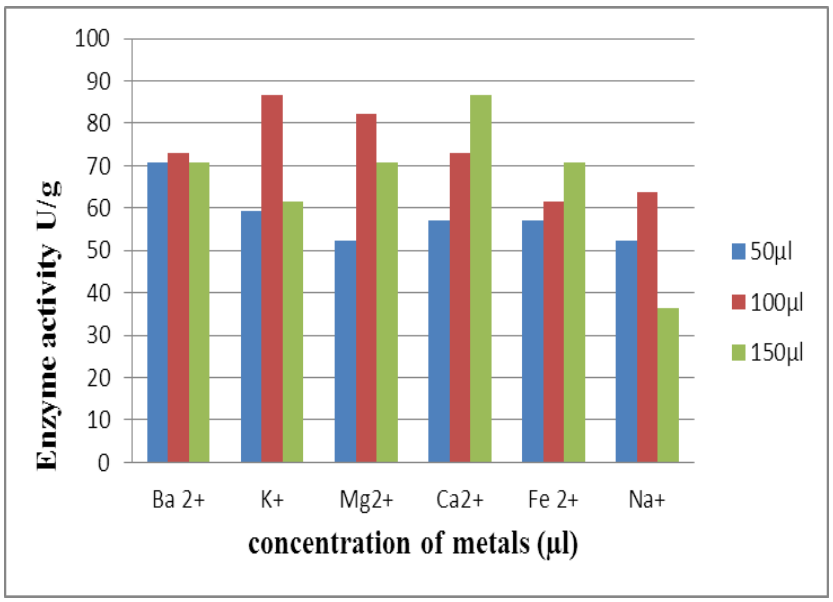


**Fig. 2: Effect of metal ions on the enzyme activity**

## DISCUSSION

The unique structure of papain gives its functionality that helps to understand how this proteolytic enzyme works and it's useful for a variety of purposes. The present work revealed the structural features of enzyme, the biological importance and processes in which papain participates and its potential for production market opportunities. Trivedi et al. (2013)[5]. studied The extraction process of papain in the latex of papaya fruit (*Carica papaya* L.), analysis of pepsin and papain were carried out in earlier some studies [6,7]. The variables studied in the extraction of papain were: latex: alcohol ratio (1:2.1and 1:3) and drying method (vacuum and refractance window). In the present study papain enzyme responses were obtained in terms of enzymatic activity and yield of the extraction process. The solution of papain exhibited an absorption maximum at wavelength between 270 to 280nm. The enzyme activity of purified papain was of 0.3 U/ml by dialysis membrane and in case of ion exchange chromatography it was 0.24U/ml. Biswaji et al. (2013) [3] explained different well established methods for isolation and purification of crystalline papain . ATPS and Sephadex G-75 based methods followed by several drying procedure are widely used for papain isolation and purification. In the present work purification by two step salt precipitation method was followed were the crystals of papain was formed. Ming et al. (2002)[8] reported that papain enzyme was shown to be more active at neutral pH, whereas the enzyme activity was also active at pH 6[7] . In the present study the optimum enzyme activity was seen at the temperature of $65^0$C and enzyme as shown its maximum activity at pH 6.5, above and below this pH, the activity declined. Ming et al. (2002) [8] made a investigation on the effect of ionic and non ionic surfactant on papain activity and its was reported that the concentration does not impair the activity of papain. On the other hand in the present work enzyme activity was found more with $K^+$ and $Ca^{2+}$ metal ions.

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