

## **Extraction and Characterization of Protease From Senesced Leaves of Papaya (*Carica Papaya*) and It's Application**

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### **Abstract**

Natural senescence is associated with several fold increase in activity of protease and is characterized by the degradation and loss of proteins therefore plant proteases have been a common studied interestingly. Protease from senesced leaves of the papaya plant (*Carica Papaya*) was partially purified by ammonium sulfate precipitation. It showed 15 fold purification. Optimum protease activity was found at alkaline range 9 and 60°C. Mn<sup>2+</sup> ion had the least effect on the activity of the enzyme. While Cu<sup>2+</sup> showed the maximum inhibitory effect on the protease activity. The enzyme was then used in further application in the detergent industry. We reported that the enzyme remained active and can be used to destain protein stains (blood, leaf extract, flower pigment and turmeric) from prestained cloth. The easy availability of the senesced leaves of this common plant makes it a cheaper enzyme source and potential additive in detergents.

**Keywords:** Senesced leaves, Protease, Purification, Casein, papaya

### **1. Introduction**

Protease is the most valuable commercial enzyme and it covers the 60% of total enzyme market. Plants, animals and microbes, viruses, and archea are the main sources for protease production (*Rao et al., 1998*). Alkaline proteases hold a great potential for application in the detergent and leather industries (*Kumar and Takagi, 1999; Oberoi et al., 2001*) because of the increasing tendency to develop environmentally friendly and sustainable technologies. The preferred sources of proteases are microbes because of their rapid growth, and the ease with which they can be genetically manipulated to generate new enzymes with altered properties. All detergent proteases currently used in the market are serine proteases use serine

alcohol produced by *Bacillus* strains (Harwood and Cranenburgh, 2008). Different types of proteases perform same reaction but they have different catalytic mechanism. Applications of fungal alkaline proteases are advantageous because of the ease of downstream processing to prepare a microbe-free enzyme at low cost production (Tsomides and Goldberg, 1969). Protease enzyme is known as proteolytic enzyme or proteinase enzyme. Protease enzyme helps to degrade the long chain of proteins into small chains of proteins and also evolved multiple times. Senescing leaves show dominance of proteolytic enzymes belongs to four major classes, which are common in different like mammals, plants and microbes, viruses and archea. Senescence induced protein breakdown has been reported in many plant systems, and it result in presence of transportable nitrogen (*Buchanan-Wollaston and Ainsworth, 1997*).

## 2. Materials and Methods

### 2.1 Plant material

Senesced leaves of *Carica Papaya* growing in and around the ITS Paramedical College, Muradnagar, Uttar Pradesh India were collected in forenoon and weighed after washing and rinsing with distilled water.

### 2.2 Determination of protease activity

Protein was estimated by Bradford method and protease activity was assayed with casein, albumin and gelatin as substrate, absorbance was taken at 660 nm. Protease activity was assayed by a modified method of Tsuchida et al. (Tsuchida et al., 1986) by using casein as substrate. One protease unit is defined as the amount of enzyme that releases 1  $\mu\text{g}$  of tyrosine per ml per minute under the above assay conditions.

### 2.3 Effect of pH on enzyme activity

The protease activity of the purified enzyme was measured at different pH values (3.6 – 11). The pH was adjusted using the following buffers (0.2 M) of acetate (pH 2.0-4.0), phosphate (pH 5.0-7.0), Tris-HCl (pH 8.0), and glycine-NaOH (pH 9.0-12.0). Reaction mixtures were incubated at 45°C for 30 min and the activity of the enzyme was measured.

### 2.4 Effect of temperature on enzyme activity and stability

The activity of the enzyme was determined by incubating the reaction mixture at different temperatures ranging from 30, 40, 50, 60 and 70°C were studied. The activity of the enzyme was measured.

### 2.5 Effect of various metal ions on protease activity

The effects of metal ions (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Cu}^{2+}$  [10 mM]) were investigated by adding them to the reaction mixture and pre-incubated for 30 min at 45°C pH 10.0. The activity of the enzyme was measured.

### 2.6 Compatibility with detergents

The compatibility of protease with local laundry detergents was studied in the

presence of 10 mM CoCl<sub>2</sub>. Detergents used were ; Surf, Surf Excel, Tide, Rin (Hindustan Lever Ltd, India); and Ariel (Procter and Gamble, India). The detergents were diluted in distilled water (0.7% w/v) and incubated with protease for 30 min at 60°C, and the protease activity was determined. This was then compared with the control samples without detergents.

### 2.7 Removal of different stain

Firstly we select the white cloth and cut square piece of this cloth .Then we select the four type stain flower extract, leaves extract, turmeric and blood stain. Put the one drop of each stain on the white cloth and observe the action of protease .We select the three type stained cloth one is control, one is treated with the crude protease extract and another one is treated with purified protease extract. Dip these clothes into the distill water in the separate test tubes and incubate this in water bath at 60°C for 30 min. Compare these with control which is not incubated.

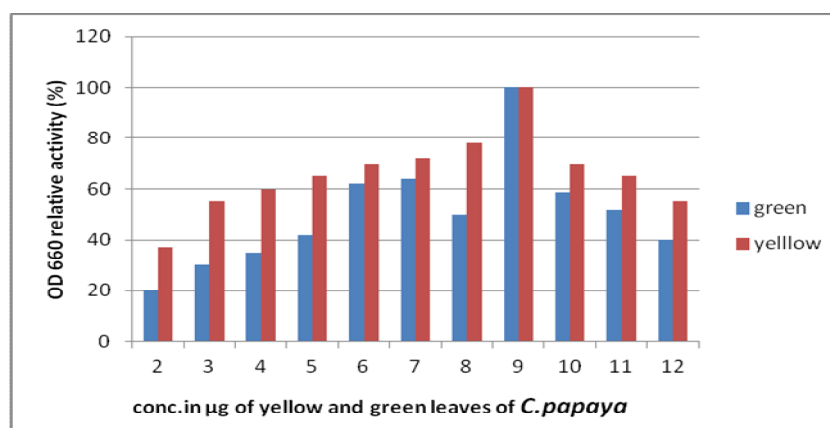
## 3. Results and Discussion

Natural senescence is associated with several fold increase in activity of protease and is characterized by the degradation and loss of proteins therefore proteases have been a common target of study (Dungey and Davies, 1982; Yoshida and Minamikawa, 1996). Internal factors such as plant hormones, reproduction and cellular differentiation influence senescence (Noodén and Leopold, 1988; Smart, 1994; Thomas and Stoddart, 1980). Degradation of macromolecules is one of the major events that occur during leaf senescence, the process also involves the synthesis of RNA and proteins *de novo* and degradation is remobilized to young and expanding organs of the plants (Buchanan-Wollaston, 1997).

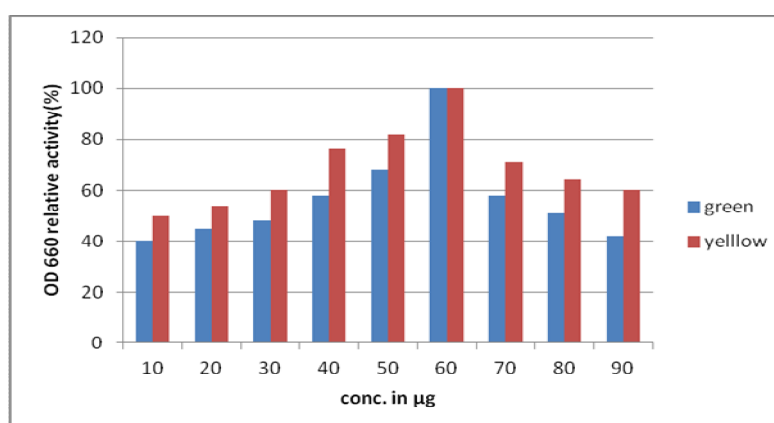
The crude enzyme preparation isolated from senesced leaves of *Carica Papaya* showed more protease activity when compared with the crude enzyme preparation from green leaves using casein as substrate. Activity of the enzyme was determined at different pH ranging from 2-12. The culture exhibited maximum enzyme activity at pH 9. Protease activity was found to be stable in the alkaline range starting from the pH 8-11 at 45°C (Fig 3.1). The thermal stability of the enzyme was also tested at different temperatures 10 °C -90 °C and the optimum temperature recorded was 60°C. The enzyme showed the stability at 50 °C -80 °C (Fig 3.2). The protease enzyme activity was determined at different metal ions such as Cu<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Na<sup>2+</sup> and Ca<sup>2+</sup>. The protease enzyme was recorded high in these metal ions Mn<sup>2+</sup>, Co<sup>2+</sup>, Mg<sup>2+</sup> and the mixture is incubated at 45°C for 30 min. In this study we report that Mn<sup>2+</sup> (10mM) strongly increases the enzyme activity (Fig 3.3). These results suggest that concerned metal ions apparently protected the enzyme against thermal denaturation and played a vital role in maintaining the active conformation of the enzyme at high temperatures (Pan and Lin, 1991; Steele et al., 1992; Donaghy and Mckay, 1993). This is possible because of the activation by the metal ions. Besides pH, a good detergent protease is expected to be stable in the presence of commercial detergents. The enzyme fraction that showed protease activity showed excellent stability and compatibility in the presence of locally available detergents (Tide, Surf

Excel, Ariel, and Rin) (Fig 3.4). The enzyme showed good compatibility with Hi Power Surf excel. Protease enzyme activity of stain removal was estimated with different stain such as flower extract stain, leaf extract, turmeric and blood (Fig 3.5). Then we compare the removal of stain with control, crude enzyme and partially purified form of protease enzyme.

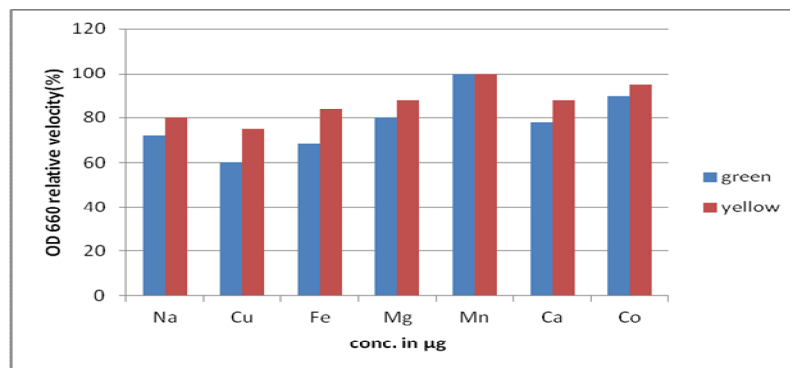
We have extracted protease from senesced leaves of *Carica Papaya*. The enzyme was thermostable at 60°C and retained activity in presence of detergents. The easy availability of the senesced leaves of this common plant makes it a cheaper enzyme source which is thermostable and makes it a good choice to be an additive in the cheaper detergents already available in the market. Protease's extracts from microorganism are now used in the detergent industries to improve the detergent quality. The application of this protease in food industry is being investigated. In future the inhibitor would be purified and compared with other protease inhibitors using bioinformatics tools and its other uses would be in pharmaceutical industry.



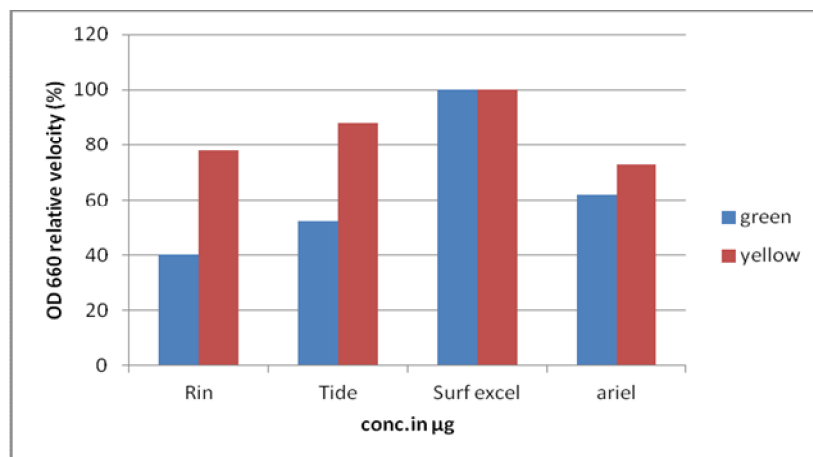
**Fig 3.1:** pH determination of green and yellow leaves of *C. papaya*



**Fig 3.2:** Effect of temperature (°C) on protease activity of green and yellow leaves of *C. papaya*



**Fig 3.3:** Effect of different metal ions on protease activity of green and yellow leaves of *C.papaya*



**Fig 3.4:** Effect of partial purified protease on different detergent of green and yellow leaves of *C.papaya*



**Fig 3.5:** Stain removal with crude and partially purified protease

L- leaves stain, B-blood stain, T-turmeric stain, F-flower stain, CR-crude enzyme, AP-acetone powder protease, C-crude protease.

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