Extraction of Papain from Latex of *Carica papaya* L. (papaya) Fruits

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DOI: https://doi.org/10.51584/IJRIAS.2025.1005000107

Received: 28 May 2025; Accepted: 06 June 2025; Published: 19 June 2025

ABSTRACT

Papain is an enzyme of significant biological and economic importance. Papain shows extensive proteolytic activity towards proteins, amino acid esters and amide links and its applications in the fields of food and medicine are ever emerging. In the present study, we extracted the papain from latex of papaya fruit (*Carica papaya* L.). Presence of papain in papaya latex was confirmed by the coagulation of milk. The protein content latex extract of papain was found to be 51.4 µg/ml.

Keywords: Extraction, Papaya fruit, latex, papain.

INTRODUCTION

The papaya (*Carica papaya* L.) is one of the most popular and widely grown fruit crops in India. It is a fast-growing, short-lived, tropical tree, cultivated for its fruit, papain, pectin, and antibacterial substances (Niklas and Marler, 2007). It belongs to the family Caricaceae and genus Carica. The pulp of the fruit is the edible portion whereas skin, seeds and peel are used as waste materials. Each part of the papaya has a rich source of nutritional value such as protein, fat, fiber, carbohydrates, minerals, vitamin C, thiamine, riboflavin, niacin, carotene and amino acids (Adiaha, 2021). Papaya is commonly consumed as a dessert or processed food in the form of jam, puree, or wine and green fruits are used as cooked vegetables (Ahmed *et al*., 2002).

Papain (E.C. 3.4.22.2) also called Papaya proteinase I (PPI), is a proteolytic enzyme with molecular mass of 23.4 k Da, and a 212 amino acids polypeptide chain (Margarita *et. al*., 2011). The commercial importance of papain is mainly due to its strong proteolytic activity against a broad range of protein substrates, and because it is active across a broad range of operational conditions.

Papain is obtained from the skin of the unripe papaya and then collected and drying the latex which flows from the fruit. The green the fruit, more active is the papain. Papain enzyme belongs to the papain superfamily, as a proteolytic enzyme, papain is of crucial importance in many vital biological processes in all living organisms (Tsuge *et al*., 1999). Papain 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 (Uhlig, 1998). It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine (Menard *et al*., 1990)

Papain enzyme plays an important role in medicine. Such as used to treat sports injuries, other causes of trauma and allergies. The mechanism of biochemical removal of caries involves cleavage of polypeptide chains and hydrolysis of collagen cross linkages. These cross-linkages give stability to the collagen fibrils, which become weaker and thus more prone to be removed when exposed to the papain gel (Beeley *et.al*. 2000). Papain is used as a clarifying agent and meat tenderizer in food industry and also used in reducing dyspepsia, other digestive disorders and disturbances of the gastrointestinal tract (Huet *et. al*., 2006).

MATERIALS AND METHODS

Material

The locally grown un-ripe fruits of *Carica papaya* were collected. The papaya fruits were washed with water to remove dirt and soil. The collected plant fruits identification was made with the help of standard floras (Naik., 1979, Naik., 1998., Yadav and Sirdesai, 2002).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Latex extraction:

Fresh latex was obtained from developing green fruits directly picked from trees. Three or four vertical incisions were made in the fruits with a sharp stainless steel instrument to a depth of 2 to 3 mm. The latex that surfaces after incision lasts only 1 to 2 minutes and then rapidly coagulates and can be collected into a glass container. After extraction, the latex was immediately used for the purification of papain in its native state or stored at -8 °C for further use (Monti *et. al.*, 2000).

Isolation of Papain from latex:

The latex from papaya fruit was collected, dried and ground at room temperature using mortar and pestle to form a homogenous mixture. 200 ml of 0.04M cysteine solution was added and continued grinding and the suspension was filtered using filter paper. The pH of the suspension was found to be between pH 5 to 7. The extraction methods was carried out at 0-4^{0}C. The pH of the filtrate was adjusted to 9.0 by 1N sodium hydroxide and the solution was centrifuged at 3500 rpm for 20min. The supernatant was collected and 40% ammonium sulphate was added and kept at 4^{0}C for 20min. The precipitate formed is removed by centrifugation at 3500rpm and the supernatant was discarded. The collected precipitate was washed with 40% ammonium sulphate solution. The precipitate was dissolved in 0.02ml of cysteine (pH-7). To the above solution 15g of sodium chloride was added and stirred continuously for 20min and incubated for 15min at 4 ^{0}C. The precipitate was collected by centrifugation at 2500rpm for 20min. The precipitate was suspended in 0.02M cysteine (pH 6.5) at room temperature and stored at 4^{0}C (Bala and Padma, 2019).

Identification of papain:

Three drops of papain extract was added to 10ml of 20% powdered skim milk and the pH was adjusted to 5.5 using acetic acid and it was incubated at 37^{0}C (Vishal *et. al*., 2013;)

RESULT AND DISCUSSION

Isolation of latex from *C. papaya*: Fresh latex was collected from locally grown *C. papaya*. Initially, four to six longitudinal incisions were made on the unripe fruit using a stainless steel knife. The exuded latex was allowed to run down the fruit and drip into collecting device. Following collection, the latex was transferred to a plastic bottle and stored at -8 °C for further use.

In the present study, extraction of papain enzyme was carried out from latex of un-ripe fruits of *Carica papaya.*

Fig1: Collection of latex

Presence of papain in papaya latex was confirmed by the coagulation of milk (Fig.2). The protein content latex extract of papain was found to be 51.4 µg/ml. As papain is a protease of broad

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

Specificity and no specific synthetic substrate is available, casein was used as a substrate to determine the total protease activity present in the latex.

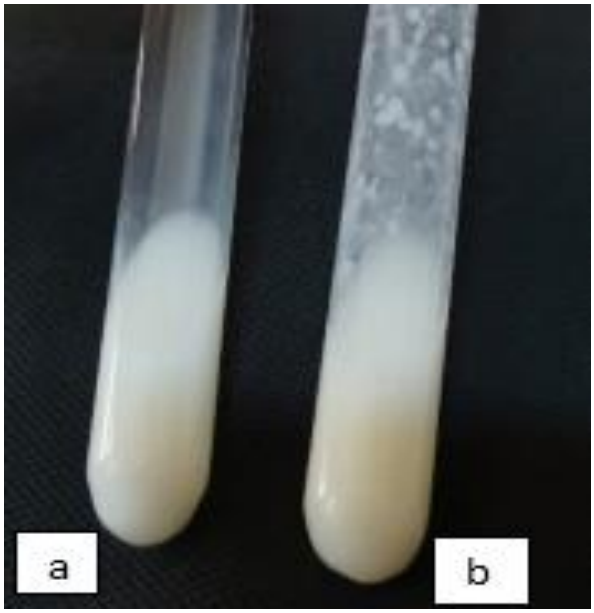

Fig.2. Identification of papain from latex. a: Normal milk, b: Latex treated

ACKNOWLEDGEMENT

Authors are grateful to Principal Dr. MD. Shaikh Babar, Dnyanopasak College, Parbhani for encouragement and providing of necessary facilities. The authors are also thankful to resource person for kindly sharing their valuable knowledge with us during field work.

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ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

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