

DISSERTATION

on

“Isolation and Purification of Papain enzyme from Papaya”



Dissertation Submitted to

Hon. Director, MGM University

Institute of Bioscience and Technology,
Chh. Sambhajinagar in partial fulfillment of the
requirement for the degree of

**BACHELOR OF SCIENCE IN
BIOTECHNOLOGY**

Submitted by

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Course Title: Mini Project

Project Guide

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-2022-23-

DECLARATION BY THE CANDIDATE

We hereby declare that the thesis entitled “ **Isolation and purification of papain enzyme from papaya**”, submitted by us is based on the actual work carried out by us under the guidance and supervision of **Mr. Kiran Pawar**. The extent of information derived from the existing literature have been duly cited and referenced. The existing research work or its any part is not submitted anywhere else for the award of any degree or diploma.

We also hereby declare that no sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is cited and duly referenced. There is no plagiarism; the work presented is original and own work of the researcher. No ideas, process, results or words of others have been presented as researcher's own work.

Place: Chh. Sambhajinagar

Date:

Signature of the Candidate

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2.Vedant Joshi (MUBBT202437)



Institute of Bioscience and Technology

CERTIFICATE-I

This is to certify that the **Mini project** entitled, “**Isolation and Purification of papain enzyme from papaya**” submitted by **Vaishnavi Khuje (MUBBT202436), Vedant Joshi (MUBBT202437)** in partial fulfillment of the requirements for the award of the degree of **BACHELOR OF SCIENCE (Hons.)** in the subject of **(Biotechnology)** for the academic year **2023-24** submitted to the MGM University, IBT, Chh Sambhajinagar. This is a record of bonafide research work carried out by Vaishnavi Khuje and Vedant Joshi under my guidance and supervision. The thesis or its any part has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Chh. Sambhajinagar

Date:

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Institute of Bioscience and Technology

CERTIFICATE-II

This is to certify that the **Mini project** entitled, “**Isolation and purification of papain enzyme from papaya**” submitted by **Vaishnavi Khuje (MUBBT202436)**, **Vedant Joshi (MUBBT202437)** in partial fulfillment of the requirements for the award of the degree of **BACHELOR OF SCIENCE (Hons.)** in the subject of(**Biotechnology**) to the MGM University, IBT, Chh. Sambhajinagar has been approved by the Research Advisory Committee after viva voce examination of the student in collaboration with the External Examiner

External

Project guide

Project Incharge

Director

PLAGIARISM CLEARANCE CERTIFICATE

This is to certify that thesis entitled, “**Isolation and purification of papain enzyme from papaya**” submitted by Vaishnavi Khuje (MUBBT202436), Vedant Joshi (MUBBT202437) has been properly examined. The percentage of uniqueness found in the thesis is 100%.

No sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is duly cited and referenced. The work presented is original and own work of the researcher (i.e.there is no plagiarism).No ideas, process ,result or words of other have been presented as researcher's own work.

Signature

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Signature

(Mr Kiran Pawar)

PLAGIARISM SCAN REPORT

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METHODOLOGY

1. Isolation of papain enzyme from papaya

Different varieties of papaya were taken. Green Berry, Mountain papaya and Hawaiian varieties were taken. The peels were kept for drying in sunlight and hot air oven. After drying the peels were crushed and powder was obtained. The powder was filtered and then it was mixed with distilled water. Eppendorf tubes were filled with the powder and then it was centrifuged at 9000 rpm for 12 minutes. After centrifugation two layers were obtained supernatant and pellet. The tubes were stored in refrigerator

2. Confirmation test for presence of papain enzyme

Raw milk sample was taken and then the supernatant was added to it and then it was kept for boiling. The milk started to coagulate that indicated the presence of papain enzyme. It was a positive test for the confirmation of papain enzyme in papaya.

3. OD at 280nm

Optical density of papain was recorded at 280nm. All the three varieties of papaya were taken and their absorbance was observed at 280nm on spectrophotometer.

4. Purification of papain enzyme

For the purification of papain enzyme 70% ethanol was used. Chilled 70% ethanol was added to the eppendorf tube and it was centrifuged at 9000rpm for 14 minutes. After centrifugation the enzyme was obtained in purified form.

ACKNOWLEDGEMENT

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
D/W	Distill Water
<i>et al</i>	And other
Fig	Figure
L	Liter
Gm	Gram
OD	Optical Density
RPM	Revolution per minute

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ABSTRACT

Title of the thesis: **Isolation and purification of papain enzyme from papaya**

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Degree to be awarded: **Bachelor of Science (Hons) in Biotechnology**

Papaya (*Carica papaya*) is widely grown in tropical and subtropical lowland regions. The papaya is a herbaceous perennial 2–10 m in height. Deep, well drained sandy loam soil is ideal for cultivation of papaya. The possible health benefits of consuming papaya include a reduced risk of [heart disease](#), [diabetes](#), [cancer](#), aiding in digestion, improving blood glucose control in people with diabetes, lowering [blood pressure](#), and improving wound healing. Papain is a proteolytic enzyme extracted from the raw fruit of the papaya plant. Proteolytic enzymes help break proteins down into smaller protein fragments called peptides and amino acids. Papain supplements are used to improve gastrointestinal dysfunction and common digestive issues like bloating and constipation. Papain has been [shown to](#) reduce inflammation in patients with asthma, arthritis and other inflammatory conditions.

Three different varieties of papaya were taken. Green berry, Hawaiian and mountain papaya varieties were taken. The papain enzyme was obtained from the peels of papaya which were kept for drying in oven and in sunlight. Confirmation test was done using casein. Optical density was measured at 280nm. Purification of enzyme was done using 70% ethanol. The highest absorbance was observed in mountain papaya 4.179 and the lowest absorbance was observed in Hawaiian dried in oven 3.857. The highest purity was observed in green berry papaya i.e 70% and lowest purity was observed in mountain papaya i.e 62%.

CHAPTER-I

INTRODUCTION

Enzymes which are found in plant and its product are called plant protease. Enzyme may formed simultaneously with proteins, as for maintaining balance between protein formation and denaturation. There are many plant parts from which enzyme can be extracted. But mainly, the activity of a particular enzyme is found in latex producing plant.(Salas et al.,2018).

Papain is one of plant enzyme, extracted from papaya fruit, as it is found in larger quantity. It is globular protein with 212 amino acid and weight of 23 kDa. Papain is cysteine protease and comes with high stability at wide range of condition even at high temperature.(Amri et al.,2012).

Papain has many medicinal properties such as curing allergies, healing of sport injuries Amri et al.,2012), wound debridement (Dhivya et al., 2018), degradation of toxicity or adverse effect of drug (Patel et al., 2012), meat tenderization (Islam et al., 2013), antibacterial, etc. Extraction of papain from plant is necessary due to wide application in medicinal uses, pharmaceutical, food industries, etc. As papain has many scientific applications, it needs to be extracted in small quantity for research work or large quantity for industrial purpose.

Papaya effectively treats and improves all types of digestive and abdominal disorders. It is a medicine for dyspepsia, hyperacidity, dysentery and constipation. Papaya helps in the digestion of proteins as it is a rich source of proteolytic enzymes. Even papain-a digestive enzyme found in papaya is extracted, dried as a powder and used as an aid in digestion. Ripe fruit consumed regularly helps in habitual constipation.(Aravind et al., 2013).

Twenty first century is the era of biotechnology has spread its wings towards commercially valuable complicated biochemical processes. One of its major branches is enzyme technology that makes different industrial procedures convenient, economical and simple. 'Papain' is one of the product of this technology has diverse applications in chemical and food industries. Unripe papayas are the principal source of papain enzyme (Brocklehurst K. et al., 1981). It carries proteolytic activity and belongs to cysteine proteinase family.

Hawaiian papayas are a grouping of multiple varieties of papayas that are typically smaller in size and average up to one pound in weight. The oblong to pear-shaped fruits have smooth, semi-thick, and inedible skin that matures from green to shades of yellow-green when ripe, softening slightly in texture. Underneath the surface, the flesh ranges in color from bright yellow, orange to pink, depending on the variety, and is tender, aqueous, and smooth, encasing a central cavity filled with oval, black-brown seeds. The seeds are covered in a gelatinous coating and are edible, bearing a sharp, pepper-like flavour with a crunchy consistency. Hawaiian papayas are aromatic and have a mild, sweet, and fruity taste with delicate notes of peaches and melon.

OBJECTIVES

1. Isolation of papain enzyme from papaya
2. Confirmation test for presence of papain
3. Purification of papain enzyme from papaya

CHAPTER II

REVIEW OF LITERATURE

Amri and Mamboya (2012) studied that Papain is a plant proteolytic enzyme for the cysteine proteinase family cysteine protease enzyme in which enormous progress has been made to understand its functions. Papain is found naturally in papaya (*Carica papaya L.*) manufactured from the latex of raw papaya fruits. The enzyme is able to break down organic molecules made of amino acids, known as polypeptides and thus plays a crucial role in diverse biological processes in physiological and pathological states, drug designs, industrial uses such as meat tenderizers and pharmaceutical preparations. The unique structure of papain gives it the functionality that helps elucidate how proteolytic enzymes work and also makes it valuable for a variety of purposes.

Aravind et al., (2013) studied Papaya, botanical name (*Carica papaya*), is an lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties. *Carica papaya* can be used for treatment of a numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many, as a result *Carica papaya* can be regarded as a Neutraceutical.

Dhivya et al., (2018) investigated that Enzymatic debridement is the process of removal of dead tissue, accumulated on the wound surface by using an enzyme. An enzyme which is responsible for wound debridement is a proteolytic enzyme. Papain is a proteolytic enzyme that catalyzes the breakdown of a protein. It has wide application in the medical field such as blood coagulation, fibrinolysis and digestion process.

Paul et al., (2013) reported that Papaya (*Carica papaya*.) is one of the most popular and economically significant fruit highly prevalent in both the tropical and subtropical arena of the world such as Hawaii, South Pacific, South America, East Africa and South East Asia. Not only this item is consumed as fresh fruit or vegetable but also utilized as potential enzyme and nutrients supplement in food industry. Unripe green papaya as well as other part of papaya tree is concentrated with latex, which is composed of several valuable enzymes: papain, chymopapain, caricain and glycyndopeptidase. To make industrially useable state, these enzymes need to be extracted and purified from green papaya fruits. It is crucial to isolate papain in the native crystalline state from fresh latex.

CHAPTER III

MATERIALS AND METHODOLOGY

MATERIALS

Samples: Papaya (*Caricapapaya*)

Varieties: Green Berry Papaya and Hawaiian

Enzyme: papain

Chemicals: Sodium chloride (NaOH), Phosphate buffer, Ammonium Sulfate, 70% ethanol.

Glasswares & Accessories: Conical flasks, Beakers, Measuring Cylinder, Centrifuge tube, Cuvette

Instruments: Pan balance, Centrifuge, Refrigerator, Spectrophotometer.

METHODOLOGY

1. Isolation of papain enzyme from papaya

Different varieties of papaya were taken. Green Berry, Mountain papaya and Hawaiian varieties were taken. The peels were kept for drying in sunlight and hot air oven. After drying the peels were crushed and powder was obtained. The powder was filtered and then it was mixed with distilled water. Eppendorf tubes were filled with the powder and then it was centrifuged at 9000 rpm for 12 minutes. After centrifugation two layers were obtained supernatant and pellet. The tubes were stored in refrigerator

2. Confirmation test for presence of papain enzyme

Raw milk sample was taken and then the supernatant was added to it and then it was kept for boiling. The milk started to coagulate that indicated the presence of papain enzyme. It was a positive test for the confirmation of papain enzyme in papaya.

3. OD at 280nm

Optical density of papain was recorded at 280nm. All the three varieties of papaya were taken and their absorbance was observed at 280nm on spectrophotometer.

4. Purification of papain enzyme

For the purification of papain enzyme 70% ethanol was used. Chilled 70% ethanol was added to the eppendorf tube and it was centrifuged at 9000rpm for 14 minutes. After centrifugation the enzyme was obtained in purified form.

CHAPTER-IV

RESULT

1.Isolation of papain enzyme from papaya

Isolation of papain from papaya was done using different varieties of papaya. The peels were kept for drying in sunlight and in hot air oven. Peels were crushed and powder was obtained and then it was centrifuged and enzyme was obtained.

1. Confirmation test for presence of papain enzyme

The confirmation test for the presence of papain in papaya was done by using casein. Raw milk sample was taken and papain enzyme was added to milk and it was kept for boiling. The milk got coagulated indicating the presence of papain.

2. OD at 280nm

Optical density was recorded at 280nm using spectrophotometer. All three varieties of papaya were taken. Different absorbance was observed.

4.Purification of papain enzyme from papaya'

Papain enzyme was purified using 70% ethanol. Chilled ethanol was added to the papain enzyme and it was centrifuged at 9000 rpm for 14 minutes. After centrifugation purified papain enzyme was obtained.

$$\% \text{Purity} = \frac{\text{Mass of pure compound in sample}}{\text{total mass of impure sample}}$$

$$1. \text{Hawaiian papaya} = \frac{95 \times 100}{140}$$

$$\text{In oven sample} = 67.85\%$$

$$\text{Natural Sample} = \frac{90 \times 100}{140} = 64.28\%$$

$$2. \text{Mountain papaya} = \frac{87 \times 100}{140} = 62\%$$

$$3. \text{Green Berry papaya} = \frac{98 \times 100}{140} = 70\%$$

1.Isolation of papain enzyme from papaya

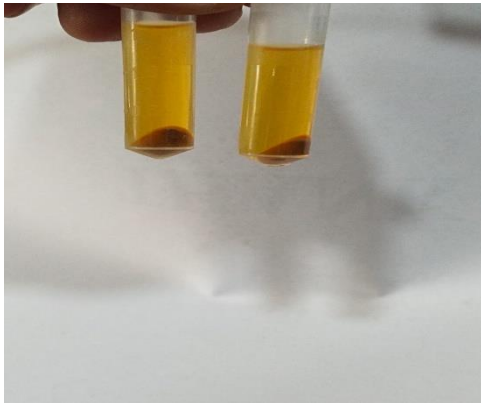


Fig No 1 Hawaiian papaya

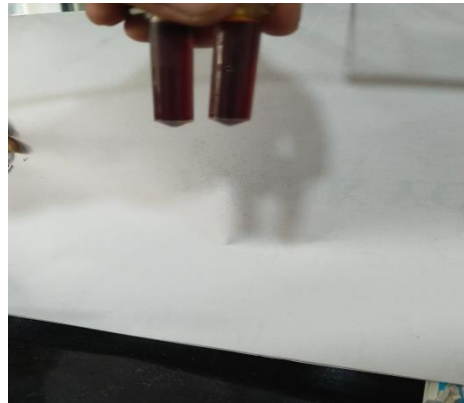


Fig No 2 Green Berry



Fig No 3 Papain extracted from different varieties of papaya sample

1. Confirmation test for presence of papain enzyme

Confirmation test for presence of papain was done by using casein.



Fig No 4 Milk kept for boiling when papain was added

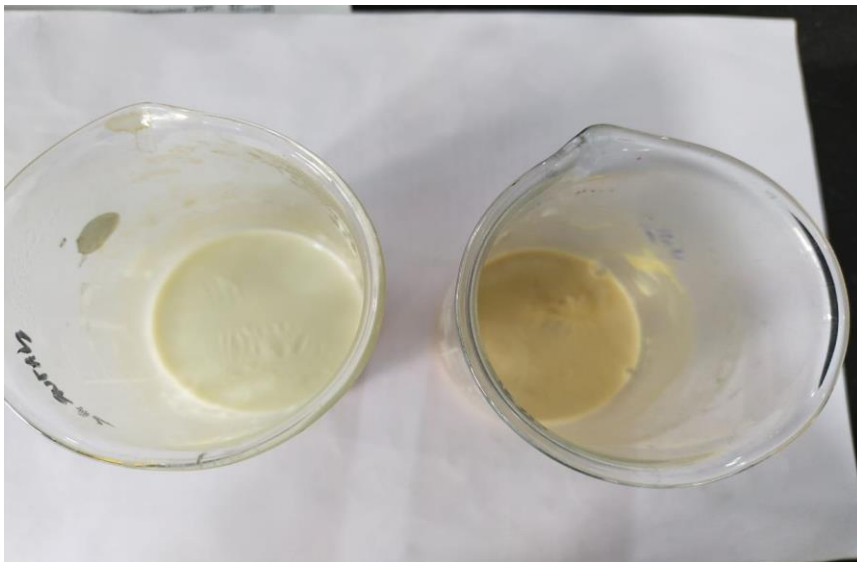


Fig no 5 Milk sample after it got coagulated indicating the presence of papain

3.OD at 280 nm

Different optical density were recorded for different varieties of papaya.

Table 1: OD for Green Berry

Sample(Green Berry)	OD at 280nm
1	4.349
2	3.856
3	3.872
4	4.122

$$\frac{4.349+3.856+3.872+4.122}{4}= 4.049$$

Table 2: OD for Hawaiian dried in oven

Sample (Hawaiian dried in oven)	OD at 280nm
1	3.87
2	4.080
3	3.937
4	3.807
5	3.865
6	4.218

$$\frac{3.87+4.080+3.397+3.807+3.865+4.218}{6}=3.857$$

Table 3: OD for Hawaiian dried in sunlight

Sample (Hawaiian dried in sunlight)	OD at 280nm
1	2.554
2	3.349
3	3.201
4	4.672
5	3.168

$$\frac{2.554+3.349+3.201+4.672+3.168}{5}=3.398$$

Table 4: OD for mountain papaya

Sample (Mountain papaya)	OD at 280 nm
1	3.456
2	4.654
3	4.567
4	4.564
5	3.654

$$\frac{3.456+4.654+4.567+4.564+3.654}{5}=4.179$$

The highest absorbance was observed in mountain papaya 4.179 and the lowest absorbance was observed in Hawaiian dried in oven 3.857

4.Purification of papain enzyme

Purification of papain enzyme was done by using 70% ethanol.

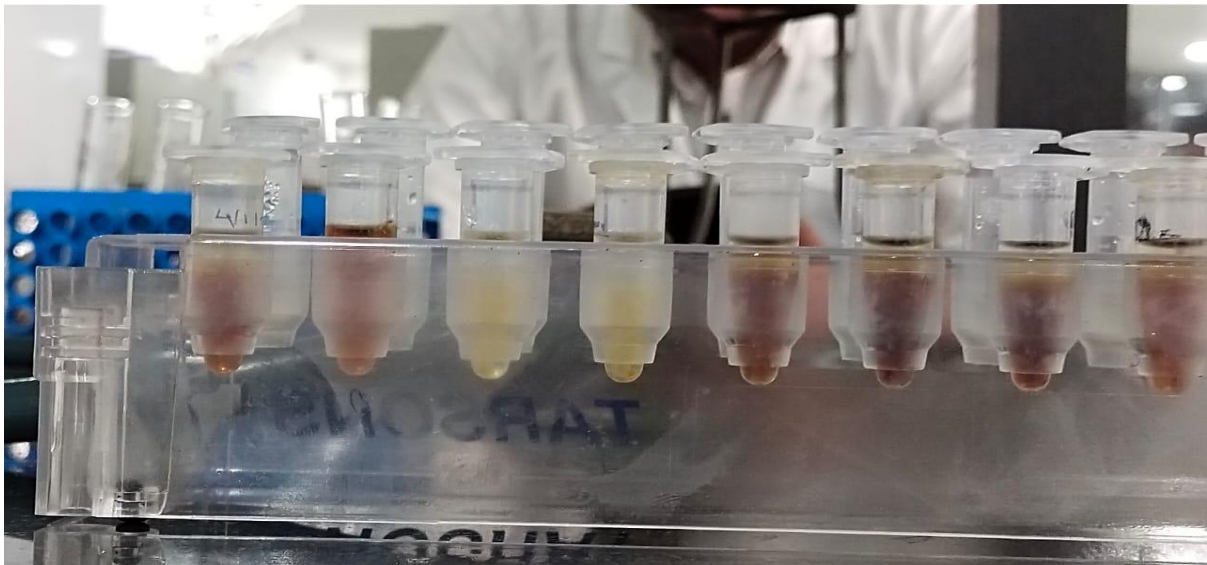


Fig No 6 Purified papain enzyme

Purity of papain enzyme

Varieties

1.Hawaiian papaya

In oven sample = 67.85%

Natural Sample = 64.28%

2. Mountain papaya= 62%

3. Green Berry papaya= 70%

The highest purity was observed in green berry papaya i.e 70% and lowest purity was observed in mountain papaya i.e 62%.

CHAPTER- V

DISCUSSION

Enzymes are proteins that help to increase the rate of reaction. Different enzymes are present in fruits. Papaya contains proteolytic enzyme called papaya. Papain is present in the papaya peels and latex of papaya. Isolation of papain enzyme was done from papaya peels.

Confirmation test for the presence of papain in papaya was done using casein. Purification of papain enzyme was done using 70% ethanol.

CHAPTER VI

CONCLUSION

Papain enzyme is present in papaya. It is found in papaya peels and latex of papaya. Papain is a proteolytic enzyme. Papain enzyme needs to be extracted from papaya peels and latex as it has various benefits. It digests protein in muscle fiber and connective tissue and it can be used for improving meat quality. Confirmation test was done for the presence of papain by casein test. Optical density was recorded at 280 nm. Purification of papain enzyme was done using 70% ethanol. The highest purity was observed in green berry papaya i.e 70% and lowest purity was observed in mountain papaya i.e 62%. The highest absorbance was observed in mountain papaya 4.179 and the lowest absorbance was observed in Hawaiian dried in oven 3.857. As the highest purity was observed in green berry papaya it can be used widely for various different uses.

FUTURE LINE OF WORK

Papain is a proteolytic enzyme which is found in papaya. It has various applications in different industries. Papain is used in meat tenderization in which it digests the protein and makes the meat soft. Papain enzyme is widely used in different industries like food, Pharmaceuticals, Breweries, leather, detergent, fishes and meat for more different purposes

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