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# Studies on Enzymatic Action, Manufacturing Process, and Purification of Papain Enzymes

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***Abstract— Papain is a common enzyme obtained from the green papaya (Pawpaw) fruit. It is cysteine protease present in Papaya, are essentially synthesized as inactive proenzymes with N- terminal propeptide regions.The activation process involves removal of propeptide regions. Papain are used in many Industrial purposes, along with pharmaceuticals, food, leather, detergents and fish processing etc. The enzyme Papain was isolated from the unripe papaya fruit and obtained by milky latex under suitable temperature and humidity. Papain was then manufactured by the process involving the harvesting and drying of latex. And, around 150 tonnes per year of Papain are produced in India. The study of Papain proteolytic action were done by the method of hydrolysis of protein with the use of casein substrate and other method " Absorptiometry". Then the Papain was purified by Gel filtration method with the use of sephadex G-75, Non denaturing Page method and SDS Page method, stained by Coomassie Blue R 250, and Brilliant blue G colloidal concentrated.***

***Keywords: papain, cysteine protease, proteolytic action, sephadex G-75, SDS Page***

## I. INTRODUCTION

Papain is a proteolytic enzyme from the cysteine proteinase family.Fig1 Papain is manufactured from the latex of raw papaya fruits as Papaya. Papaya is a versatile plant having no.of uses and enzymatic properties. Papaya fruits contains 2 proteolytic enzymes: Papain and Chymopapain[1]. Dry powder made from the latex of raw papaya is commonly known as crude papain. The term papain was first introduces by Wurtz and Brochut[2][3].Papaya is believed to be a native to tropical America,perhaps in southern Mexico and neighbouring Central America. Papain is used in many Industrial purposes. Some of theendusersarebreweries,pharmaceuticals,food,leather ,detergents,meat and fish processing etc.[3] Papain used in combating dyspepsin and other digestive disorder used in the manufacturer of pharmaceuticals preparation like Liquid Papain and liver tonics[1].Papain consists of a single polypepetide chain with 3 disulfide bridges and sulphydryl group necessary for activity of the enzyme. Papain molecular weight is 23,406 Da, PH 6.0-7.0, Temperature optimum activity is 65◦C.Papain exhibits broad specificity, cleaving peptide bonds of basic amino acid,leucine or glycine. It also hydrolyses ester and amino acid[4][5]. The shape of Papaya Plant resembles palms leaves with large monoaxial palms. Mature papaya leaves are palmate with deep lobes,supported by smooth,hollow petioles. Ripe Papaya fruit resembles melons and are rich in vitamin A and Vitamin C. The Papain showed the proteolytic activity by absorptiometry method and the assay method measured basing on the hydrolysis of protein by the clotting of milk. The papain has been isolated by cutting the skin of unripe papaya fruit and then collecting and drying the latex,flows out from the incision from the fruit. The method of manufacturing process involves the collection of latex, cleaning and sieving,mixing and drying , and packing of extracted Papain. The papain was then purified by gel filtration method, non denaturing Page and SDS Page.

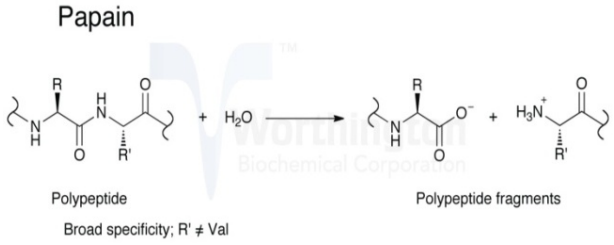


Fig 1. Chemical Structure of Papain

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## II. ENZYMATIC ACTION

### PROTEOLYTIC ACTIVITY

Papain proteolytic activity was measured by the method called "Assay"[6].This is a low cost method but also time consuming. Papain hydrolyses proteins and these assays are measured basing on the ability of the papain to clot the milk.In this method, known amount of Papain sample ( Dissolve a known weight of Papain in a known volume of acetic acid)was taken and were added to the known amount of milk ( Dissolve a known weight of milk powder in known volume of water). Then, this has been kept for some time in a water bath at the temperature of 30°C Solution. After cooling it, thoroughly the mixture was mixed and were observed until the formation of lumps were detected. The time taken to reach this stage,from when the Papain was added to the milk, was recorded.And the experiment was repeated again by using different known amounts of Papain solutions, and the different amount of Papain sample used have the clotting times between the range of 60 and 300 seconds to know the optimum results. For introducing a measuring standardization, the amount of milk can be fixed at a certain concentration, and this was done by reacting a known concentration of high grade Papain with the milk. The concentration of milk was adjusted to the desried clotting time under the suitable reaction condition. Now, the activity of the Papain can be calculated at this known amount of milk. The graph was plotted for its calculation,and finding the time taken to clot milk at an infinite concentration of Papain.

The enzymatic activity is also based on the another method called as " Absorptiometry". This analytical technique was measured on the amount of radiation absorbed by chemical solution. For eg. Yellow coloured solution will absorb blue light. Greater the concentration in yellow colour, the more is the absorption of blue light.The more is the colour, greater is the concentration. Therefore, by shining the complement colour through the sample liquid the amount of absorbed light can be related to the concentration of product.The technique used when the "colours" beyond the visible spectrum, is called as spectrophotometry and the instrument called as spectrophotometer.

In this method, the activity of the Papain was observed by mixing the known amount of Papain sample to the known amount of casein. And the reaction was taken for 60 mins at 40°C. After 60mins the reaction was stopped by the addition of an acid. The product of the reaction, was known as tryosine, known to absorb ultra- violet light. The tyrosine was added in the solutions for analysis using the spectrophotometer. In the spectrophotometer, the result was calculated basing on the amount of ultra-violet light absorbed by the solutionand the no of tyrosine units produced by Papain sample. Therefore, if the greater is the number of tyrosine units, then greater is the activity of Papain. Fig 2

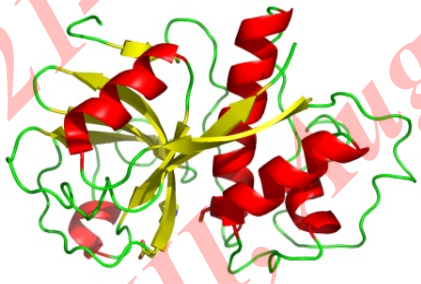

Fig.2.Papain Structure

## III. MANUFACTURING PROCESS

*Isolation of Papain:*

The Papain was obtained by the milky latex, which was collected and dried by making 3 vertical incisions in the lower part of unripe papaya fruit. This process was done during the morning on misty/cloudy days and the best seasonal time was with warm temperature and high humidity. The latex was then kept under processing, so as to avoid the losing its enzymatic activity[7].

*Production of Papain:*

In India, the production of papain is 150 tonnes per year[8]. The source of the papain production depends only on milky latex. The process of the manufacturing includes the harvesting and drying of the latex. The Papain was obtained by making 3 vertical cuts 1-2 mm deep at the base of the unripe papaya fruit

with the use of stainless steel razor blade. Fruits are to be tapped at the intervals of 4-7 days. Around 5 mins, the latex flow ceases.The latex was collected in the stainless steel trays, while the latex coagulated at the surface of the fruit were scraped and transferred in the polythene box with a close fitting lid.The box was kept under the shade so as to reduce the reactions, leading to the loss of enzymatic activity.The milky latex obtained was passed through the 50 mesh sieves to remove the dirt and insects. The dried latex and the milky latex should not be mixed together as this lowers the quality.After collection of the latex, latex was mixed with Potassium meta-bisulphite (Kms) ($K_2S_2O_5$) and dried in a vaccum shielded drier at the temperature of about 55°C for 4-5 hrs. Now, the dried product was kept in the air tight box in the cool dry place,in the form of flakes, because the powder decreases the stablility during the cold storage.Then, the dried flakes were powdered and diluted with lactose powder to obtain the BPC grade Papain. To avoid the loss of enzymatic activity of papain, metal containers or boxes were not used,only plastic boxes were preferred to pack the crude papain flakes. So, the ready papain was finally packed into the air tight polythene bags.Papain should be stored at the temperature of 20°C, and its shelf life is 5-6 months with the proper handling and storage. Then the proteolytic activity of papain was tested in the laboratory[9].Fig 3.

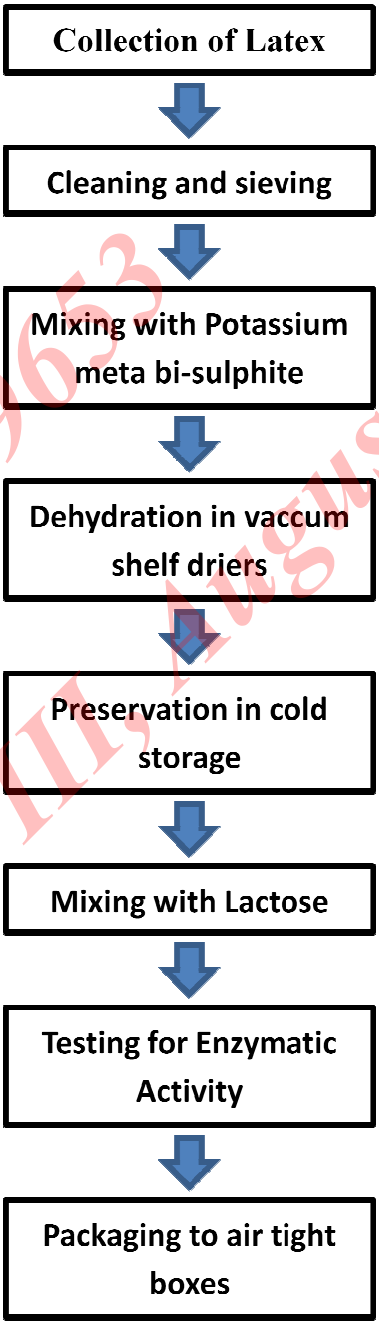


Fig 3. Manufacturing Process

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## IV. PURIFICATION OF PAPAIN

The Papain was purified by gel filtration and the molecular determination was performed by Non denaturing Page. In the gel filtration method[10], the molecules are separated according to their difference in sizes as they pass through the gel filtration medium which are packed in the column. The medium is a porous matrix, which are in the form of spherical particles.The medium is packed into a column, equilibrated with buffer which fills the pores of the matrix and the space between the particles. The liquid inside the pores is referred to as stationary phase and the liquid is in equilibrium with the liquid outside the particles, referred to as mobile phase. In the case of purification of papain enzyme by gel filtration, with the use of sephadex G-75. The concentrate was applied to a column of ( 100 * 2.5 cm) which was pre-equilibrated with 0.1M Sodium phosphate buffer and 1mM EDTA (PH 7.0) eluted with the same buffer. The flow rate was 20ml/hour at room temperature.The detection was carried out at 280nm. The column was calibrated with Ovalbumin( 43kDa), Cytochrome C (12.4kDa), Lysozyme (14.3 kDa), Bovine Serum albumin (67kDa).[11]

The molecular weight determination of papain enzyme was performed by Non- denaturing Page.In this method, 12 % Ployacrylamide gel,34 mM Beta- alanine buffer at the $P^H$ 4.3 and a constant 4mA current per tube was used[12]. In the SDS-Page method, 12 % acrylamide gel was used and the samples were prepared in Trisglycerol-b-mercaptoethanol and placed in boiling water for 1min [13]. Now, the gels for both the Non denaturing page and SDS Page were stained by Coomassie-Blue-R 250 and Brilliant Blue G colloidal concentrated by the method[14].

## V. APPLICATIONS

Papain enzyme has a wide range of applications in different industries. In pharmaceutical industry, Papain is used for pain and swelling as well as for fluid retention following trauma and surgery. Papain are used as a digestive aid and for treating parasitic worms,inflammation of the throat and pharynx, diarrhea, hay fever, and a skin condition called psoriases. They are also used with conventional treatments for tumors. People apply directly to the skin to the infected wounds,sores and ulcers. Papain acts as a debris-removing agent,with no harmful effects on sound tissues because of the enzymes specificity,acting only on the tissues,which lack the α1-antitripsine plasmatic antiprotease that inhibits proteolysis in healthy tissues [15] Papain has advantages for being used for chemomechanical dental carries removal since it does not interfere in the bond strength of restoration materials to dentin[16]. Papain are used significantly as analgesic and anti-inflammatory activity against symptoms of acute allergic sinusitis like headache and toothache pain without side effects[17]. Papain has been known to interfere with urine drug tests for cannbinoids. It is found in some drug detox products. One major use is the treatment of cutaneous ulcers including diabetic ulcers and pressure ulcers. 2 Papain based tropical drugs are Accuzyme and Panafil, which can be used to treat wounds like cutaneous ulcers. Medical research uses Papain for plastic surgery on cleft palate. Papain also has a veterinary aplications such as de-worming of cattle,and is used in thr tanning of leather,in the paper and adhesives industries, and in sewage disposal. In the food processing industry, the chill-proofing of Beer (ensuring that fine precipitates or haziness does not accur in the beer when it is cooled), tenderisation of meat, the production of dough for pizzas, and the production of batter for waffles and wafers. Papain has other applications, they are also used in pet food to reduce viscosity and increase palatibility, shrink proofing of wool,used as an ingredient in cleaning solutions for soft contact lenses, used as an indigestion medicine, can also be found as an ingredient in some tooth pastes or mints a teeth- whitener. Papain, made into a paste with water, also home remedy treatment for jellyfish,bee,yellow jacket, stings, mosquito bites and possibly sting ray wounds, breaking down the protein toxins in the venom. They are used in cell isolation procedure where it is more gentle than other proteases (ie. Cortical neurons, Retina, and smooth muscle).Traditionally, some of the plant extracts including papaya have beedn used to treat infections by gastrointestinal Nematodes that have been consequences on human and alos in livestock farming. Papain are also used for the red cell surface modification for antibody screening or identification, Fab preparation from Ig G and Ig antibodies, solubilization of integral membrane proteins,production of glycopeptides from purified proteoglycans[18].

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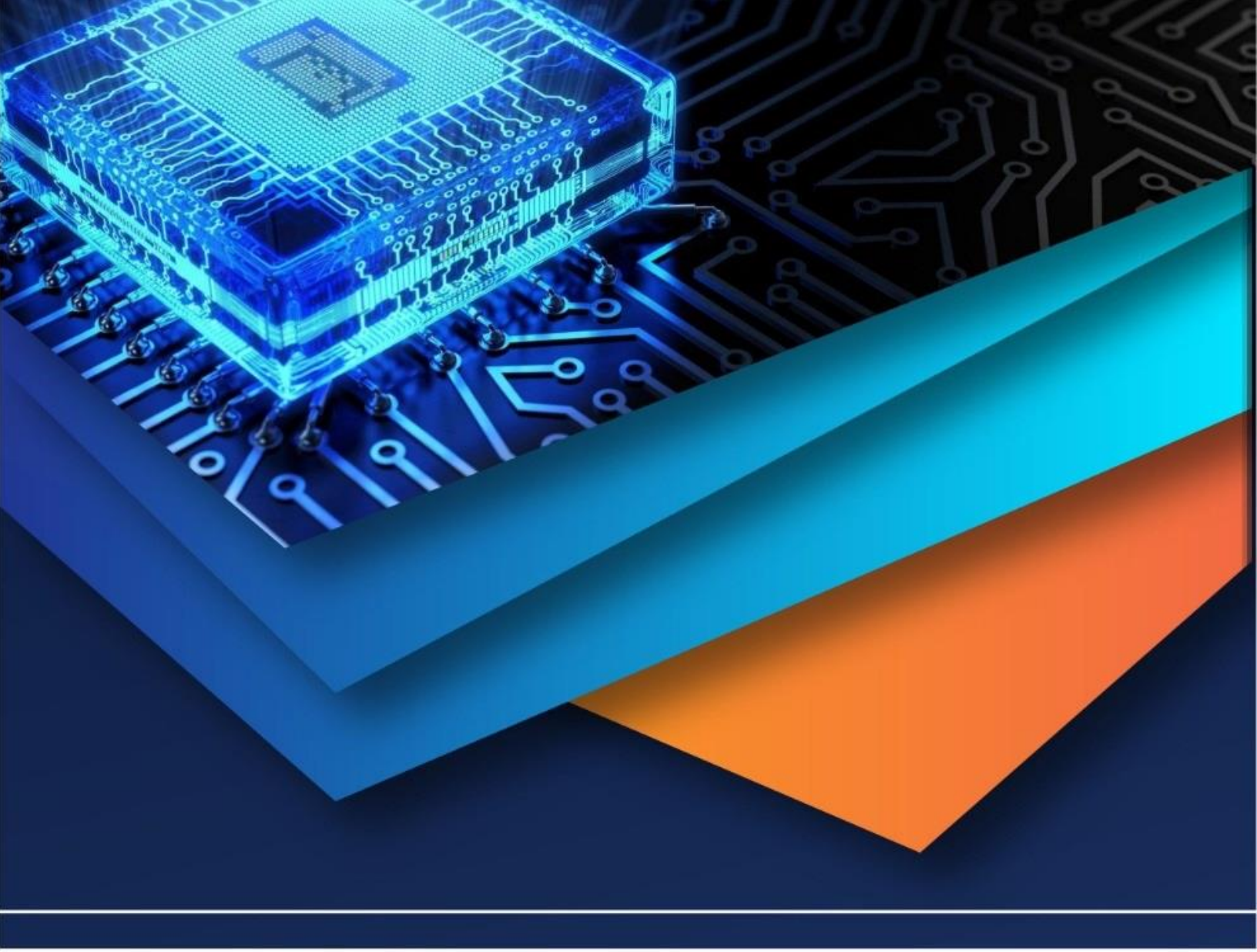

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