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Review Article

Papain - An essential proteolytic plant enzyme

Jadhav Shreegita*, Pranjal Barbade, Akshay More and Swapnil Nirgude

Kasturi Shikshan Sanstha College of Pharmacy, Shikrapur, Pune Maharashtra-412409

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ABSTRACT

Papain is proyeolytic enzyme that catalyses the breakdown of a protein. It shows several biological functions in plants and animals. It use in many industry like food, leather,detergents, meat processing and medicinal application like blood coagulation, fibrinolysis and digestion process there are various process use for the extration of papain and purification also. Now a day the various formulation of papain is available in market in topical and other forms. Spoons of pulvevised papaya seeds mixed with hot water used in diabetes. The papain shows protelytic activity is determine by using various method and the previous studies reported activity in latex of papaya plant. Main objective of this study was to evaluated protease activity in the papaya nectar.

Key Words: Papain, Protelytic Enzyme, Papaya Isolation, Purification of Protein

Address for Correspondence: Jadhav Shreegita, Kasturi Shikshan Sanstha College of Pharmacy, Shikrapur, Pune Maharashtra-412208, India; E-mail: jadhavshrigeeta@gmail.com

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INTRODUCTION

21st century is the era of biotechnology is expanding its wings towards commercially valuable complicated biochemical processes. One of its large branches is enzyme technology that makes different industrial procedures confy, economical and perspicuous. Papain is one of the product of this technology has sundry applications in chemical and also food industries. Crude papayas are the principal source of papain enzyme. It carries proteolytic activity and involve to cysteine proteinase family. Active papain enzymes can be isolated and purified from the latex of green papaya fruits [1,2, 3, 4].

The proteolytic activity of papain has been more described into the literature, involving the degenaration of elastin and proteoglycans. According to Dr John and Whitman Ray Papain possesses a more powerful digestive action rover to pepsin and pancreatin. Recently Kinoshita in 2003 reported that Papain a large chemical compound extracted from latex of papaya used in detached industry for individualize industrial and pharmaceutical products. This review focuses primarily on two aspects. Firstly, on understanding morphological properties of papain enzyme, composition and its isolation and modification method of papain isolation from papaya, and secondly on how these modification are advanced enzyme quality and activity. This is the comprehensive review on papain that attempts to entire so many aspects of this economically very important enzyme that can prove valuable for professionals involved in both research and commerce. This study has been done to gather knowledge about papain enzyme and its isolation methods to build up this enzyme manufacturing industry in possibility for the new entrants.

Properties of Papain

Principal properties of Papain enzymes are mentioned here according to the published work of Abhijit and Gadeker in 2007[5].
Alternate names = Papaya peptidase I.
Specificity = Cleaves somewhat nonspecifically at exposed residues.
Source = Carica papaya latex.
Storage conditions =Store at 4°c
Molecular weight =23.000 Da
Inhibitors = Heavy metals. Carbonyls, NEM, p-Chloromercuro-benzoate.
Extinction coefficient = 76,630 cm-1 M-1
Isoelectric point: pH 9.6
Catalytic residues of the enzymes are [11,12]:
•Cysteine (C158)
• Histidine (H292)
•Asparagine (N308).
Papain: source and uses

Papain is the dried latex obtained from the papaya fruit (Carica papaya L). It has protease mostly used in the food processing applications mentioned above. Other important proteases ficin, gain from figs, and bromelain, which is obtained from pineapple. In addition to its pertinence to the food industry, papain are also used in the pharmaceutical industry for medicines, such as in preparation of vaccines and the treatment of hard skin. Papain also has veterinary applications such as in the de-worming of cattle, and is used in the tanning of leather, in the paper and adhesive industries, and in effluent disposition. Medical research uses papain for plastic surgery on cleft palates.[6]

Fig.1 sources of papain

Methods of collection and extraction

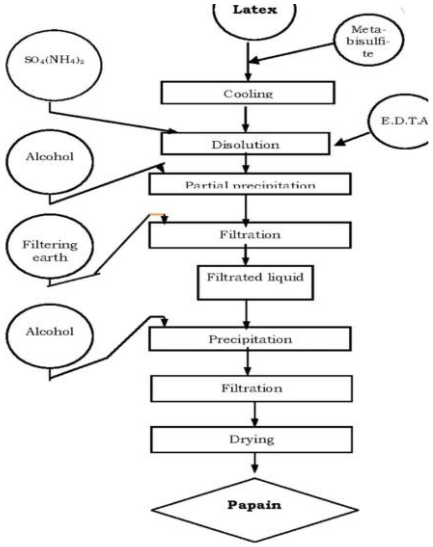

Fig.2 method of collection and extraction of papain
Papain is gain by cutting the skin of the unripe - but nearly mature - papaya before collecting and drying the latex which flows from the cuts. The fruit should be tapped some time during the morning (a period of high humidity). The small humidity levels, the lower the flow of latex.

Two or three perpendicular cuts (excepting the first cut, see below) 1-2mm deep is made, meeting at the base of the fruit. The incisions are made using a stainless steel razor blade set in the piece of rubber

attached to away stick. The blade should not put off more than about 2mm as cuts deeper than 2mm risk juices and starch from the fruit pulp adding with the latex, lowering quality.

Fruits should be tapped at intervals of about 4-7 days. For the first tapping, this is usually adequate to make only small cut. On subsequent tapings, the two or three cuts are distance between earlier ones. After about 4-6 minutes the flow of latex ceases. A dish is used to assemble the liquid; it is then scraped into a polythene-lined box with a close meet lid. The box should then be keep in the shade. Using a close fitting lid and storing the box in the shade ease the reactions which cause loss of enzyme activity.

Foreign matter such as dirt and insects can be kept out of the store latex. Any secreted latex adhering to the fruit can be carefully scraped off and put into the collecting box. Howsoever, dried latex cannot be mixed with recent latex, as this minimizes the quality.

When handling fresh latex, care should be taken to ensure that it does not come into contact with skin: it will cause burning. Neither should the latex come into contact with furious metals such as iron, copper or brass, as it causes discolouration and salve of activity. Pots, knives and spoons should only used if made from plastic or stainless steel. Fresh latex does not keep well and should be dried to below 5% moisture as soon as possible. This gives it a dry and easily breaks texture. After two or three months the fruits are ripe and should be removed out the tree. The ripe fruits are edible but have little sale value because of their scarred appearance. However, ripe green papaya skin does contain about 10% pectin (dry weight): the fruits can be processed to extract this.

Drying papaya latex: The method of drying is the main factor that appoints the final quality of papain. Various grades have been used since the enzyme has become an international raw material. Up until the mid-1950s, Sri Lankan papain dominated the market, three grades were known: 1 - fine white powder; 2 - white oven-dried crumb, and 3 - dark sun-dried crumb.

Until the 1970s there are two grades: 1 - first or maximum grade oven-dried papain in powder or crumb form (usually creamy white in colour); 2 - second or minimum grade sun dried brown papain in crumb form.

As a result of to date processing techniques, papain has been reclassified into three groups since 1970: 1 - crude papain – limiting from first grade white down to second-grade brown. 2 - crude papain in layer or powder form - sometimes referred to as semi chaste. 3 - spray-dried immature papain - in powder form, referred to as clean papain.

Sun-drying: Sun-drying is the lowest quality of product, as there is considerable damage of enzyme activity and the papain should be easily turn brown. In various countries, however, sun-drying is still the most general processing technique. The latex is simply spread on trays and left in the sun to dry.

Oven-drying: Papain driers can be of easy construction. In Sri Lanka they are generally simple outdoor stoves. They are typically about one metre high and made of mud or clay bricks. Drying times differ, but an about guide is 4-5 hours at a temperature of 35-40°C. Drying is complete when the latex is crumbly and no longer sticky. A good quality product is obtained if the latex is sieved before drying. The dried product can be kept in air-tight, light-proof containers (eg. Packed clay pots or metal cans) and keep in a cool place. Metal containers should be lined with polythene.

Spray-drying: Not possible at small-scale because remarkable investment in appliance is necessary. However, it is viable to buy spray-dried papain for the small-scale processing of foods. Spray-dried papain is top level of enzyme activity than other types of papain and this is totally soluble in water. Extreme care must be taken when handling this form of papain: it can cause allergies and emphysema if breathe in. For this reason, spray-dried papain is often encapsulated in a gelatine coat.

Enzyme activity: Whether papain is to be feat commercially for an export market and for local food industry use, it is very important to be able to determine the level of enzyme activity, a process known as assaying. it can be carried out by the National Standards office. Papain is used to hydrolyse (or break down) proteins. Therefore, assays to measure papain activity are based on measuring a product of the hydrolysis. There are two main methods of carrying out assays:

Method 1: This method relies on the ability of papain to conglomerate milk. It is a low-cost method but is time consuming. Furthermore, the absence of a standardised method of finding the clotting point, combined with variations in the milk powder used, can introduce errors.

Method 2: The second method is based on the science of light absorption, or absorptiometry. This technique measures the amount of radiation (or 'colour' of light) absorbed by a chemical solution. It is known, for example, that a yellow-coloured solution absorbs blue light. (Blue is the

complementary colour to yellow). More concentration of yellow in the solution, the more blue light is absorbed. This is a useful technique because the resulting products of some chemical reactions are coloured. The more intense the colour, the more concentrated the resulting product. So, by shining the relevant complementary colour through the sample liquid, the amount of light absorbed can be related to the concentration of product. Not all 'colours' (or radiations of light) are visible to the human eye. The technique used when the 'colours' extend beyond the visible spectrum is known as spectrophotometry, and the instrument used is called a spectrophotometer.

In this second method of determining the amount of activity of papain, a known amount of the enzyme is mixed with a fixed amount of casein (the protein found in milk). The reaction is passed to continue for 60 minutes at 40°C. then this time the reaction is stopped by mixing a strong acid. The resulting product of the reaction is tyrosine, which is known to absorb ultraviolet light (invisible to the human eye). The solutions involving the tyrosine are prepared for analysis by using the spectrophotometer. The amount of ultra-violet light absorbed by the solution can be paragon to the number of tyrosine units engander by the papain sample. Hence, the greater this number, the greater activity of the papain sample. [6]

Production and Practical Action of papain

A papain sample is prepared by mixing a known weight of papain in a familiar volume of acetic acid solution. This is then mix to a definite amount of milk, which is build by dissolving a recognise weight of milk powder in a recognise volume of water, warmed to 30°C in a water bath. The contents are thoroughly mixed and then observed till the first signs of clotting – the formation of lumps is detected. The length of time from when the papain is added to the milk until clotting began is recorded.

The experiment is repeated using different familiar amounts of papain solutions. The varying amounts of papain sample used should give a range of clotting times, into 60 and 300 seconds for optimum results. The activity of the papain samples are then calculated by plotting a graph, finding the time taken to clot milk at an limitless concentration of papain and then using that value in a formula to calculate the activity. In order to introduce a measure of standardisation, the amount of milk can be fixed at a known concentration. This is complete by reacting a known concentration of high-grade papain with the milk. The concentration of milk powder solution to be adjusted in order to obtain the desired clotting time under fixed reaction conditions. The 'activity of pure papain' at this known amount of milk can then be measured. Testing the sample papain under the same reaction conditions and same (known) amount of milk will then give an activity relative to the aseptic papain.

Determination of proteolytic activity

This was measured according to Arnon (1970) using casein as a substrate. The reaction mixure(5.0 mL) contained aliquots (0.1-0.8 mL) of diluted papaya pulp (0.6 mg/mL); 0.2 mL of operative solution (0.02 M sodium EDTA, 0.005 M cysteine, pH 8.0); 0.1-0.8 mL 0.05 M Tris-HCl buffer, pH 8.0; and 1mL of 1% casein in the single Trish buffer solution. Following incubation (37^{O}C, 10 min), 3 mL 5% v/v trichloroacetic acid (TCA) was mixed to stop the reaction. A tube was stored at room temperature 40 min to precipitate non-hydrolyzed casein, which was concentrated by centrifugation (10000 g, 10 min). Absorbance of clear supernatant had measured at 280 nm. The reading was corrected for a blank, containing to the same reactants except that the sample was mixed after addition of TCA.

The standard curve had prepared under the same conditions as the sample using a reaction mix (6.2 mL) where commercial papain (0.1- 2.0 mL, 0.2mg/mL in 0.05M Tris-HCl buffer, pH 8.0) was utilized. The same procedure, previously detailed for the sample, was followed to measure the absorbance of the commercial papain. Enzymatic activity in samples was calculated by interpolation of the standard curve. The commercial papain utilized had an activity of 0.9 units/mg solid. One unit of enzyme activity are defined as "the activity which gives rise to an increase of one unit of absorbance at 280 nm per minute digestion" (Arnon, 1970). Protein content of the samples was appointing using purbling serum albumin as standard by the Bradford system (1976). Cysteine, casein, and papain were buying from Sigma Chemical Company (St. Louis, MO). The EDTA was got from Fisher Scientific, Inc. (Pittsburgh, PA), and Tris buffer from AMRESCO, Inc. (Solon, OH). Proteolytic enzymatic activity was also determined in the latex of unripe papaya, in the seeds and skin + flesh of ripe papaya. Since preliminary results showed no significant differences in the activity of the skin + flesh paragon to the pulp, further research was not performed on the skin + flesh.[7-16]

Optimal Hydrogen Ion Concentration for the Papain Action

In as much as most ferments see are fixed range of acidity or alkalinity in that they exhibit their maximal activity, it seemed outlandish that papain should as sterio in the literature, acting equally well in acid or alkaline solution. To pitch more light on this point a series of experiments are undertaken to

determine at which hydrogen ion concentrations papain are most active proteolytic.The data store lower the typical of the results obtained in different experiments so the conclusion seems justified that papain, in common with other ferments, has an optimal hydrogen ion concentration, in this case approximately 10m5 N. In all cases, the indicator method was used and the results are therefore not more accurate than a half a unit in the pH2. In the present of proteins the indicator results are not fully to be relied upon except for comparative purposes. The absolute hydrogen ion concentrations of the sundry solutions used not is given with certainty. A 2 per cent solution of gelatin in water is treating with HCl and NaOH so that the solutions when tested with suitable indicators showed that they were of the hydrogen ion concentration desired. A 0.5 per cent solution of purified papain was divided into three parts and adjusted to 10-3, and lo? N. 25 cc. portions of the various gelatin solutions was measured out and treated with 5 cc. of the papain solution of the same range of acidity. Duplicate blanks were set up with the papain and the gelatin and triplex mixtures were made containing the protein and ferment. Proteolytic Action of Papain a sundry Hydrogen Ion Concentrations.[17]

Effect of papaya on human digestive system

The purpose of this research was to investigate the digestive properties of papaya fruit, either due to proteolytic activity or fiber content or a combination of both, and to evaluate the impact of processing on these characteristics. Papain activity in papaya fruit was evaluated through the different maturation stages, since it was previously unknown as to whether papain was active in the pulp. Active proteolytic enzymes were present in the pulp of papaya fruit throughout the different ripeness stages. Ripe papaya had the greatest enzyme activity (11.9 U/g pulp). The specific activity (U/mg protein) was higher in unripe papaya (11.3 U/ mg protein). Processing had a detrimental impact on proteolytic activity (loss of 60 % activity). To confirm enzymatic activity, an in vitro experiment was used to evaluate the action of proteolytic enzymes on meat bolus displacement. The meat bolus study confirmed the proteolytic activity presence through different ripeness stages of papaya fruit. Unfortunately, this method has some limitations, and thus, it was not possible to make general inferences based on this method; it was helpful to confirm the presence of active enzyme in papaya fruit with potential for assisting human digestion. [18-19]

Mechanism and Biological Importance

The mechanism in which the function of papain is madeaccessible is through the cysteine-25 portion of the triad in the active site that attacks the carbonyl carbon in the backbone of the peptide chain freeing the amino ultimate portion. As this occur throughout the peptide chains of the protein, the protein separate apart. The mechanism by which they break peptide bonds involves deprotonation of Cys-25 by His-159. Asparagine-175 helps to orient the imidazole ring of His-159 to allow that deprotonation occurs. Although virgate apart within the chain, these three amino acids are in close proximity due to the folding structure. It is though these three amino acids working together in the active site that supply this enzyme with its unique functions. Cys-25 then performs a nucleophilic onset on the carbonyl carbon of the peptide backbone (Menard et al., 1990; Tsuge et al., 1999). In the active site of papain, Cys -25 and His -159 are thought to be catalytically competant as a thiolate-imidazolium ion pair. Papain can be efficiently inhibited by peptidyl or non-peptidyl N-nitrosoanilines (Guo et al., 1996; 1998). The inactivation are due to the formation of a stable S-NO bond in the active site (Snitroso-Cys25) of papain (Xian et al., 2000).[20-22]

FUTURE SCOPE

Proteases which constitute a broad class of industrially most significant enzymes has involved in diverse physiological and cellular processes. Since proteases are physiologically needed, they occur ubiquitously in animals, plants, and microbes. However, microbes is a goldmine of proteases and record the preferred source of enzymes in view of their rapid growth, limited interval required for cultivation, and ready facilitate to genetic manipulation. Microbial proteases has extensively used in the food, dairy and detergent industries since ancient times. There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly open out fatal diseases such as cancer, malaria, and AIDS.

The applications of proteases in industry and therapeutics have increase rapidly in the last two decades. Novel protein engineering strategies and techniques will continue to spread the commercial protease markets. Encouragingly, the recent success of apoptotic caspase activation with engineered low molecule-activated proteases represents a new way to specifically control human protease activity for clinical applications. In addition, taking advantage of the proteolytic activities of proteases in diseased tissues may also offer upto date strategy for site-specific drug targeting and tumor imaging

The advent of techniques for rapid sequencing of cloned DNA has yielded an volcanic increase in protease sequence information. Analysis of sequences for acidic, alkaline, and neutral proteases are supply new insights into the evolutionary relationships of proteases. In contravation of the systematic application of recombinant technology

and protein engineering to alter the properties of proteases, it cannot possible to obtain microbial proteases that are ideal for their biotechnological applications. Industrial applications of proteases have posed several problems and challenges for their further reform. The biodiversity represents a golden resource for biotechnological innovations and plays an important role in the search for reform strains of microorganisms used in the industry.

A new trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or very acidic or alkaline habitats. The proteases isolated from extremophilic organisms are happy to minimize some of the unnatural properties of the enzymes that are desirable for their commercial applications. Exploitation of biodiversity to feed microorganisms that engader proteases well suited for their diverse applications is considered to be one of the most promising future alternatives. Introduction of extremophilic proteases into industrial processes is hindered by the difficulties encountered in growing the extremophiles as laboratory cultures. Revolutionary robotic approaches such as DNA irritated are being developed to rationalize the use of enzymes from extremophiles. The existing knowledge about the structure function relationship of proteases, coupled with gene-shuffling system, promises a fair scope of success, into the adjacent future, in evolving proteases that were not made in nature and that would pertinent the requirements of the multitude of protease applications.

A century after the pioneering work of Louis Pasteur, the science of microbiology has stretch out its pinnacle. In a relatively low time, modern biotechnology has grown dramatically from a laboratory interest to a commercial activity. Advances in microbiology and biotechnology are produced a favorable niche for the growth of proteases and will continue to facilitate their applications to provide a sustainable environment for mankind and to improve the quality of human life. With the improvement of synthetic biology, relating design, crystallography, and screening technologies, we can anticipate that the future of protease will be a multi-disciplinary task with polydramatic successes to arrive.

Current Status of the Structure of Papain: Cognizance of the complete amino acid sequence of papain is a compulsary prerequisite to an understanding of structure to function relationships and the mechanism of action of this proteolytic enzyme. Furthermore, the structure of papain is of added interest as a model of a proteolytic enzyme set out a requirement for a free sulfhydryl group. It can be recalled that papain shows those properties typical of a "sulfhydryl enzyme." 1 inquisition of the structure of papain was initiated by us a number of years ago and this effort has involved the co-operation of a number of investigators.

The amino acid structure of papain was derived from recent analyses performed on the Spinco amino acid analyzer. The total number of amino acids is near to 200 which is considerably greater than the 178 reported in 1954. 3 It will still be necessary to await the completion of the amino acid sequence in order to deduce the exact composition of this protein; the precision of measurement with a protein of this size places a degree of uncertainty on the accurate number of residues.

Papain contains a only one sulfhydryl group at the active center. 4 This group is partially"masked" in the crystalline enzyme but is present in stoichiometric amounts after activation with mercaptans4 ' 5 or sodium borohydride.5 The activity of papain readiness before activation is directly related to the reactive sulfhydryl content. 4 ' 5 The still existing sulphur residues are present in three disulfide bridges.

Previously to structural studies, the enzyme must be inoperative to prevent autolysis and the disulfide bridges cleaved and converted to a stable derivative to prevent disulfide interchange and to improve the susceptibility of the substrate to proteolysis. Performic acid oxidation was first used to change the sulphur residues to the stable cysteic acid residues,6 whereas in recent studies we employed reductive split of disulfide bonds with mercaptoethanol in urea and alkylation of the resulting sulfhydryl groups with iodoacetate.7 These papain derivatives served as substrates for the purpose of tryptic and chymotryptic hydrolyses. For identification of the precede containing the catalytically active sulfhydryl group and the establishment of the position of the disulfide bridges, we have used crystalline papain inoperative with C 14-labeled iodoacetate. The sulfhydryl group in the active enzyme was change into the inactive S-carboxymethyl derivative in 53 per cent yield.8

Fractionation of peptides was able by chromatography on ion exchange resins, either Dowex-50 or Dowex-l, or a intermix of the two. Further purification, when necessary, was carry out by chromatography or electrophoresis on paper, and by chromatography on dextran oils. The fineness of isolated peptides was found by a two-dimensional separation on paper with electrophoresis in one direction at pH 1.9 or 6.5, and chromatography in the another with butanol-acetic acid water or butanol-pyridine-acetic acid-

water .later on hydrolysis with 6 N HCI for 20 or 40 hr, each peptide was analyzed quantitatively by the method of Spackman, Stein, and Moore. 9 The stoichiometry, purity and outturn were determined from the analyses without correction. The outturn were variable and ranged from 5 to 70 per cent.

Applications of papain

- Papain digestions are proven useful for structural studies of enzymes and other proteins. Papain are used in red cell serology to small change the red cell surface to increase or destroy the reactivity of poly red cell antigens as an consequent to distributing, antibody screening, or antibody reorganization procedures.
- Papain has also been visual to be useful in platelet serology.
- Papains are also important in the enzymatic synthesis of amino acids, peptides, and other molecules.
- Fab antibody fragments has used in assay systems where the presence of the Fc region may cause problems. In these cases this is preferable to use only the antigen binding (Fab) portion of an antibody.
- It is also been used in the exreation of flavor and colour compound from plant.
- Proteases of aspergillus found application as digestive aids in gastro-intestinal disorders like dyspepsia.
- Papain and bromelain are used to edify to nutritional value of feeds.

Conclusion

An innovation aspect of the current study helps to determine the protease activity in the difference lactease of plant that could have possible positive effect on local economics in relation to garden composting, leather industries, food technology, modification of function and nutritional properties of protein controlled enzymatic hydrolysis. The new and innovation study help in formulation of papain in future aspects and use in various purpose.

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