Enzymatic Activity and Its Biomedical Importance of Papain: A Review

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ABSTRACT

Papain has been classified in the cysteine proteases that processes the protein more broadly than pancreatic protease compounds. Papain mostly comprises a single peptide chain with a sulfhydryl gathering and three sulfide spans. Papain has been isolated from the papaya latex that assembled from the dried unripen papaya. The movement of protein relied upon the unripen papaya natural product. Fundamentally, papain has been settled by disulfide connects and collapsed around these scaffolds. 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 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. In the present review, its biological importance, properties and structural features that are important to an understanding of their biological function are presented. Its potential for production and market opportunities are also discussed.

KEYWORDS: papain, structure, hydrophobic, Proteolytic enzyme, cysteine protease.

Highlights

- Papaya is rich in terms of phytochemicals, thus used in numerous pharmacological activities.
- Papaya fruit used to produce a large number of processed foods.
- Fruit peels showed several applications in cosmetics, wastewater treatment, as animal feed, and as a binder in ceramics.
- Papaya plants have also been explored for green synthesis of NPs.

INTRODUCTION

Papaya, a mildly sweet, melon-like tropical fruit belonging to the family Caricacae, is a native of tropical America. After the Spaniards took the fruit to Luzon Island in the Philippines in the mid sixteenth century, it reached Malacca shortly afterward, and then India. It has been widely grown throughout tropical and subtropical regions such as Australia; Hawaii, Florida, Texas, California, and Puerto Rico in the USA; Peru; Venezuela; various parts of Central and South Africa; and Bangladesh, Pakistan, and India. The Australians call it ‘pawpaw’ while the Venezuelans call it ‘lechosa.’ Fruit size may vary from less than 0.5 kg to 3 kg. A climacteric fruit, it is mainly consumed fresh when ripened after harvest. Ripening is judged by the approximate percentage of yellowness on its skin, and more accurately by measuring its total soluble solids (TSS) contents with a refractometer. Export-grade papayas grown in Hawaii should have a minimum of 11.5% TSS. Firmness, as gaged by touch or by a texture-measuring device, is another way of judging ripening. The firmness is related to the biochemical changes in three fractions of pectins in papayas, and is not a very accurate index of ripeness. Green papayas can also be consumed as a salad or in soups, both of which are quite popular in South-east Asia[1].

Papain is an endolytic plant cysteine protease enzyme which is isolated from papaya (*Carica papaya* L.) latex. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut. The greener the fruit, more active is the papain. It is a small and somewhat strange-looking fruit tree native to Central America. The bole is soft wooded, beautifully carved with conspicuous leaf scars, and laticiferous. The bark is papery, light brown, and almost glossy. The leaves are 30–60 cm long, glabrous, palmatifid, and palmatinerved[2]. The petiole is about 30 cm long, fleshy, and thin. The flowers are light yellow, slightly fragrant, and generally dioecious. The male flowers are arranged in long dropping panicles. The female flowers are arranged in short clusters. In female flowers, the ovary is single-locular, the stigma is sessile, five-lobed, and lacerated. The fruits are succulent, indehiscent, single-celled, ovoid to oblong, greenish-yellow, smooth, and 15 cm×30 cm berries. The flesh is red, juicy, and delicious. The seeds are numerous, black, and taste like capper[3].

Scientific classification

Kingdom	: Plantae
Clade	: Tracheophytes
Clade	: Angiosperms
Clade	: Eudicots
Clade	: Rosids
Order	: Brassicales
Family	: Caricaceae
Genus	: Carica
Species	: C. papaya

Properties, Structure and Features of Papain

The protein is stabilized by three disulfide bridges in which the molecule is folded along these bridges creating a strong interaction among the side chains which contributes to the stability of the enzyme. Its three-dimensional structure consists of two distinct structural domains with a cleft between them[4]. This cleft contains the active site, which contains a catalytic diad that has been likened to the catalytic triad of chymotrypsin. The catalytic diad is made up of the amino acids-cysteine-25 and histidine-159. Aspartate-158 was thought to play a role analogous to the role of aspartate in the serine protease catalytic triad, but that has since then been disproved[5]. Papain molecule has an all-α domain and an antparallel β-sheet domain. Papain apart from being most studied plant cysteine proteases, further researches in understanding the specificity, the structural the effect brought by inhibitors, low pH, metal ions and fluorinated alcohols has been identified as of critical importance[6].

Hydrophobicity of Papain

It is often useful to examine the relative hydrophobicity or hydrophilicity values of the amino acids in a protein sequence. Since hydrophobic residues tend to be more buried in the interior of the molecule and hydrophilic residues are more exposed to solvent, a profile of these values can indicate the overall folding pattern. The hydrophobic interactions have the major influence in protein conformation and the most hydrophobic of the amino acid side chains are those of alanine, Valine, leucine, methionine and Isoleucine which vary in degrees of hydrophobic. The graph indicates that carbon content is maintained at 31.45% of carbon all along the sequence. Some regions along the sequences have values above 31.45%, these are considered to be higher hydrophobic regions as it has previously been reported when using carbon content distribution profile. Thus, the overall hydrophobicity of papain enzyme being maintained at 31.45% of carbon all along the sequence contribute to stability of protein as previous been reported that stable and ordered proteins maintain 31.45% of carbon all along the sequence[7].

NO.	Categories	Phytoconstituents	Plant Part(s)
1	Enzymes	Papain, chymopapain A and B, endopeptidase papain III and IV glutamine cyclotransferase, peptidase A and B and lysozymes.	Unripe fruit (Latex)
2	Carotenoids	β carotene, crytoxanthin, violaxanthin, zeaxanthin.	Fruits
3	Alkaloid & Enzyme	Carposide, and an enzyme myrosin.	Roots
4	Glucosinolates	Benzyl isothiocynate, benzylthiourea, β-sitosterol, papaya oil, caricin and an enzyme myrosin.	Seeds
5	Minerals	Calcium, potassium, magnesium, iron, copper, zinc.	Shoots and Leaves
6	Monoterpenoids	4-terpineol, linalool, linalool oxide.	Fruits
7	Flavoniods	Quercetin, myricetin, kaempferol.	Shoots
8	Alkaloids	Carpinine, carpaine, pseudocarpine, vitamin C and E, choline, carposide.	Leasves and Heartwood
9	Vitamins	Thiamine, riboflavin, niacin, ascorbic acid, α-tocopherol.	Shoots and Leaves
10	Carbohydrates	Glucose, sucrose, and fructose.	Fruits

Figure 1. Phytoconstituents present in Papaya.

Antidiabetic Activity

Diabetes is a chronic disease, predominantly due to the insulin resistance or insulin insufficiency phenomenon, which leads to elevation of blood glucose level, a condition known as hyperglycemia. Uncontrolled diabetes can lead to various macro and microvascular complications in which ultimately affect the quality of life of diabetes patients. It has been shown that oxidative stress plays an important role in diabetes and its progression. The papaya plant is a member of the small family of plants, Caricaceae[8]. Chemical components identified as antihyperglycaemic and antihyperlipidaemic agents along with mechanisms of action in the various cellular, animals and human/clinical models are also outlined. Beyond direct antidiabetic effect, the growing trend of FFP in dietary supplement application and mechanism of papaya as wound healing and antihypertensive agent are further discussed[9].

Antiparasitic Activity

Administration of a water suspension of latex (2 g/kg–8 g/kg) reduced by 55.5–84.5% the number of Heligmosomoides polygyrus nematodes in Balb/c mice. The plant produces the piperidine alkaloid carpaine which is antiamoebal and antiplasmodial effects. The latex is strongly proteolytic and must never be ingested. It has been used by Malays to poison their enemies[10].

Skin Aging Activity

Skin aging is characterised by extracellular matrix (ECM) degradation in which human skin naturally becomes drier, thinner, unevenly pigmented, and wrinkled, as a human being ages, due to the inevitable intrinsic aging factors. Extrinsic aging factors are avoidable, in which both factors may synergize and lead to premature skin aging. ROS is known to be the culprit of skin aging by contributing to oxidative stress and inflammation. Photoaging is a process that produces ROS, which eventually leads to augmented ECM turnover and degradation[11]. Although not fully deleterious to the cells, excessive ROS can oxidise skin proteins and lipids leading to roughen the skin by altering the function of the skin barrier and further stimulate wrinkle formation[12].

Wound Healing Activity

Wound healing is rather complex and well-coordinated with involvement of several stages of cellular responses, including inflammation, proliferation, and remodeling. The duration of each phase usually ranges from 1 to 4 days, 5 to 10 days, and 11 days onwards. Characterization for each phase includes presence of leukocytes, angiogenesis, protein synthesis and deposition, epithelialization, wound contraction, and scar formation. These processes can be altered by the presence of oxidative stress. The efficiency of the wound healing process decreases with advancing age. Oxidative stress can alter the speed of wound recovery as it depends on the amount of ROS present at the wound site. Although minimal ROS prevents infection, excessive ROS is known cytotoxic to fibroblasts and reduce flexibility of skin lipids. In addition, it also causes impairment to lipids, DNA, proteins, and cellular membranes, and subsequently, severely damages the tissue and promotes inflammation[13].

Cancer Activity

Cancer is a prevailing topic and there is no absolute cure to date for various types of cancers. ROS generation as a result of metabolic reactions in the mitochondria plays a role in both initiation and potentially elimination of cancers. With a low amount of ROS that is tolerable by the body cells, the progression of cancer could occur through either promoting genomic DNA alterations or DNA damage that alters the normal physiological signaling pathways[14]. For instance, mitogen-activated protein kinase (MAPK) activation, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) phosphorylation, and cyclin D1 expression are correlated to cancer progression and survival. In the normal healthy cells, a significantly high level of ROS can lead to cellular damage and eventually cell death. However, cancer cells generally have a higher resistance to oxidative stress than normal cells to allow for uncontrolled proliferation and to compensate for the survival of cancer cells during metastasis from their site of origin. However, increasing ROS to a specific threshold level, specifically for cancer cells is proven to attenuate cancer cell growth and progression[15-17].

Anti-inflammatory Activity

Papain, also known as papaya proteinase I, is a cysteine protease enzyme that is found in species of papaya, Carica papaya and Vasconcellea cundinamarcensis. The enzyme is found to be localized in the skin of papaya, and is collected from slashed unripe papayas as a crude latex. Papain is used in food, pharmaceutical, textile, and cosmetic industries. While it has been used for the treatment of inflammation and pain via topical administration, papain has also shown to have anthelmintic and tooth-whitening properties[18]. Present in over-the-counter mixture products consisting of different digestive enzymes, its active site contains a catalytic diad that plays a role in breaking peptide bonds. Papain is also used as an ingredient in various enzymatic debriding preparations[19].

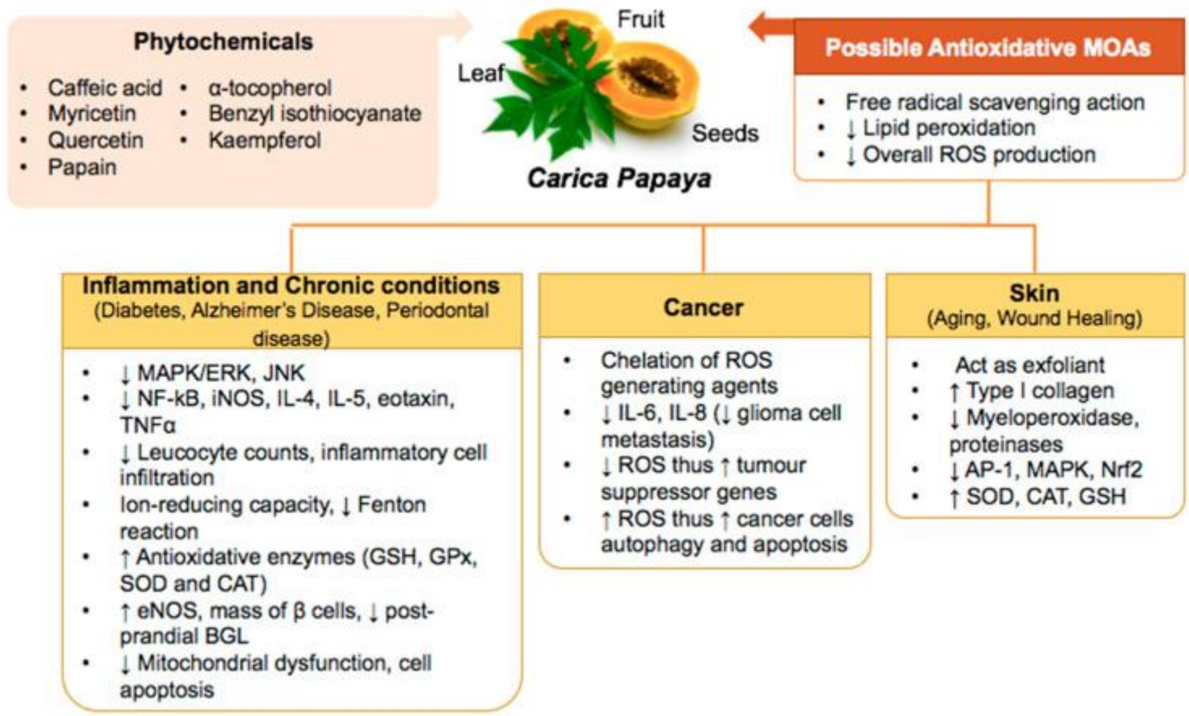

Figure 2. Pharmacological action different parts of Papaya

Papain in Medical Uses

Papain acts as a debris-removing agent, with no harmful effect on sound tissues because of the enzyme's specificity, acting only on the tissues, which lack the α1- antitripsine plasmatic antiprotease that inhibits proteolysis in healthy tissues. The mechanism of biochemical removal of caries involves cleavage of polypeptide chains and or hydrolysis of collagen cross- linkages[20]. 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. Papain-based gel has also been reported as a potential useful in biochemical excavation procedures for dentin. Papain has advantages for being used for chemo mechanical dental caries removal since it does not interfere in the bond strength of restorative materials to dentin. Papain enzyme has a long history of being used to treat sports injuries, other causes of trauma and allergies. Fortunately papain has a proven track record in managing all of these conditions with clinical evidence of significant benefits for use of papain protease enzyme in cases of sports injury[21].

Papain Uses in Drug Design

Papain shares many features with physiologically important mammalian cysteine proteases and show nearly identical folding patterns especially around the active site which has been useful for drug design[22]. The X-ray coordinate system for papain solved at 1.7 A resolutions is a representative example of the structure of a covalent ligand-bound cysteine protease complex particularly in the papain superfamily. Thus, papain is reported to be useful as an experimental model structure to understand the inhibition mechanism of newly developed specific inhibitors of cathepsin L, the papain superfamily and its an antioxidant properties can be useful in preventing certain types of illnesses. Since most of the amino acid residues that are involved in the binding to papain are conserved in cathepsin L, this publicly available high resolution structure has provided an excellent model for the successful design of highly active and specific cathepsin L inhibitors. Papain is also reported to be used as a surrogate enzyme in a drug design effort to obtain potent and selective inhibitors of cathepsin K, a new member of the papain superfamily of cysteine proteases that is selected and highly expressed in osteoclasts[23].

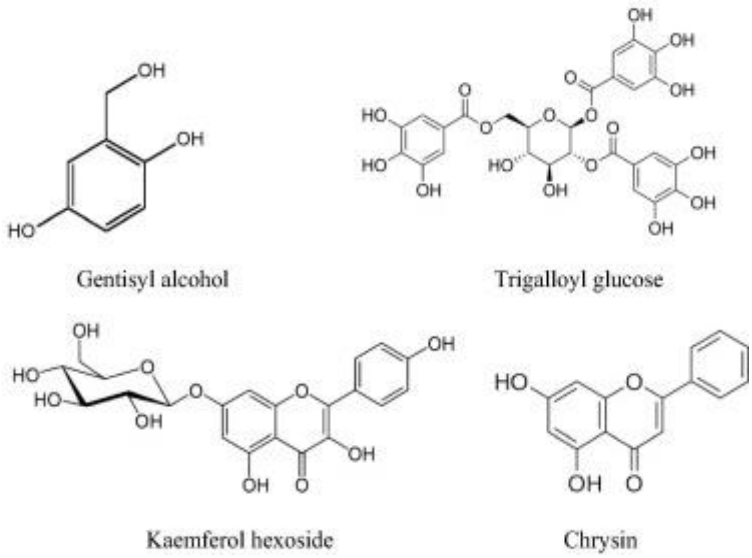

Figure 3. Phytochemicals present in different parts of Papaya.

Industrial Uses and Pharmaceutical Preparations

Papain is used in meat tenderizers; the major meat proteins responsible for tenderness are the myofibrillar proteins and the connective tissue proteins. Protease enzymes are used to modify these proteins and papain has been extensively used as a common ingredient in the brewery and in the meat and meat processing[24]. Papain importance as tenderizers in the food industry is similar to collagenases, which have application in the fur and hide tanning to ensure uniform dying of leather[25]. Papain also can act as a clarifying agent in many food industry processes. As a protein digestant, papain is used in combating dyspepsia and other digestive disorders and disturbances of the gastrointestinal tract. Papain has for quite a long time been used in pharmaceutical preparations of diverse food manufacturing applications as the production of high quality kunafa and other popular local sweets and pastries. Papain has been reported to improve meltability and stretchability of Nabulsi cheese with outstanding fibrous structure enhancing superiority in the application in kunafa, pizza and pastries . Also as pharmaceutical products in gel based a proteolytic cisteine enzyme, papain presents antifungal, antibacterial and anti-inflammatory properties[26-30].

Potential for Production and Market Opportunities

Papain enzyme is extracted from Carica papaya which is a tropical and a herbaceous succulent plant that possess self supporting stems which grows in all tropical countries and many sub-tropical regions of the world. Moreover, there is no limitation due to seasonality as the papaya is available almost round the year. Consequently, there is a need to facilitate the entrepreneurs in understanding the potential of papaya production and the importance of setting up a unit of papain[31-32]. A well managed papaya production has recorded higher papain yield of 8.17 g per fruit and highest papain of 686.29 g per plant in a period of 6 months. Papain is used in many industries such as breweries, pharmaceuticals, food, leather, detergents, meat and fish processing for a variety of processes. Therefore, the end use segments are many in signifying that papain has high export demand. Since there are good prospects for papain market, the papaya production and extraction of papain can be a high source of income even for small farmers[33-35].

CONCLUSION

Papain has been classified in the cysteine proteases that processes the protein more broadly than pancreatic protease compounds. Papain mostly comprises a single peptide chain with a sulfhydryl gathering and three sulfide spans. Papain has been isolated from the papaya latex that assembled from the dried unripen papaya. The movement of protein relied upon the unripen papaya natural product. Fundamentally, papain has been settled by disulfide connects and collapsed around these scaffolds. Papain is an enzyme found in the white fluid (latex) that occurs in raw papaya fruit. It is a protease, meaning it breaks down proteins. Papain has revealed to be an enzymatic protein of significant biological and economic importance. It is through the unique structure of papain that provides functionality and helps explain how this proteolytic enzyme works and also makes it valuable for a variety of purposes. Further researches on papain enzyme in understand the specificity, the structural the effect brought various thermodynamic pathways is of critical importance. Papain is found naturally in papaya which is a versatile plant having number of uses and enzymatic properties. Since the papaya grows in a wide range of climate, papaya production for extraction of papain can be a source of earning a high income to farmers.

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