**Study on industrial applications of papain: A succinct review**

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**Abstract.** Papain is an enzyme having proteolytic function with cysteine protease activity obtain from papaya having green color and is acquired from latex. It is classified in superfamily. Papain has function essential of proteolysis widely for peptides of amino acid in all living beings. Present review shed light on general properties, structuring features and biological importance of papain, especially its industrial applications for different purposes, with the help of technological advancement; it is now used to treat lethal diseases and additionally scientists are taking benefit of it for drug designing so there are high demands for its export. Papain uncommon properties have been making it more appreciable commercially. Papaya is present or grows in all tropical areas as there are no seasonal limitations for its growth and papain is resistant over wide range of Ph and temperature, so it has high demand for export and there is good prospect of papain marketing. Farmers at lower levels could use this enzyme as high source of income. Through recombinant DNA technology, engineering of proteins and procedures of immobilization papain could be ideal for biotechnological and industrial applications. Researchers could explore more potent applications by understanding detail mechanism and functions of papain. Then there will be novel papain function that would fulfill the massive need for betterment of life.

1. **Introduction**

Papain is an enzyme having proteolytic function with cysteine protease activity from endolytic plant. Source of papain is papaya having green color. Papain is acquired from skin cutting of immature papaya from where fluid (latex) comes which is assembled and gets dried. Purification including solublization and extracting active papain is necessary to remove undesirable products. The activity of
papain depends on green color of fruit. This enzyme is classified in superfamily. Papain has essential role in many important functions of all living beings [1]. Papain has proteolysis function widely for peptides of short chain, proteins, esterified amino acid and links of amide and is functional broadly in medication and in food material [2]. It specifically splits positively charge amino acids peptide linkage mainly lysine, arginine and phenylalanine residues [3]. Papain globular protein having molecular weight 23406 Dalton and 212 building blocks of proteins with 4 bridges of disulphide. There are 2 structural domains in three dimensional structure with cleft which comprises the active site in different positions of histidine, cysteine and aspartate [1, 4]. Papain hydrolase of cysteine and stable at high temperatures and active at many conditions [5]. Ideal PH for activity of papain is 3-9 [6, 7]. The protein folding and confirmation is influenced by hydrophobicity of papain with outer hydrophillic core interact with water and inner hydrophobic core stabilizing tertiary structure. Break down of protein is due to the catalytic activity of papain [8]. The functioning of papain is due to cysteine_25 and His_159 portion in triad active site attacking the carbon of carbonyl in peptide backbone having free portion of amino terminal [9]. Meat fibers are break down by it in active powder form and use for meat tenderizer. In preparations of cell culturing, papain is used for dissociation of cells. In different enzymatic preparations it is used for cleaning to remove infected cells or tissues. In tooth pastes papain is used for whitening purposes however it is diluted readily by saliva so require long time use for good results. For the treatment of decay of tooth papain is used in gel name papacaries. It impedes the effect of drug test for urine and act as detox. Antibody breakdown by papain gives 3 products which does not undergo precipitation or agglutination etc [10]. Papain from Carcia Papaya is a moist herbaceous plant and has stems which are supported by itself and it is present or grow in all tropical areas as there are no seasonal limitations for its growth. Papain comprises of enzymes like 1). Peptidase converts protein into polypeptides and dipeptide 2). Rennin such as coagulating enzyme acting on milk casein 3). Amylolytic enzyme converting carbohydrates into monosaccharaides. Papain is widely used in different industries like food, Pharmaceuticals, Breweries, leather, detergent, fishes, and meat and for different purposes. There are high demands for its export [11].

2. Related Works
A lot of exploration has been done on papain due to its beneficial and advantageous properties. Biotechnological advancement has produced helpful advancement for papain improvement. The importance of papain in different industries is discussed under.

2.1. Food Industry
Around the world papain is more often studied and most commonly used protease in food industry. Papain is used to tenderize meat protein especially of myofibril and connective tissue. Different papain concentrations (0.25, .05, .075, .1percent) were used to treat hen leg cut at 5 or 3% levels for improvement of meat tenderness. Papain with concentration of 0.25% at %level of 3 show significant tender meat more than 0% group of control. Injection multiple and technique of forking with infusion plus was used to cut meat so this study revealed that papain with 0.25% forking with adoption plus infusion procedure was used to tenderize and to improve functioning properties for meat of spent hen cuts for effective use [12]. Papain has vital role as agent of clarifying in processes of food industry. As it break downs protein so it can digest it and play important role in controlling dyspepsia and other disorders of digestive system and GI tract [13]. It was proposed that papain could improve meltability and stretchability of Nabulsi cheese use in different pastries, pizza and kunafa by acting on cross linking of casein. Papain treated Nabulsi cheese showed well developed fibrous structures, showing good results and excellent storage in the use of pizza and pastries inducing meltability or stretchability due to high salt so it was proved effective method. Papain was used in gums which are chewing as tenderizer [14].
2.2. Health Care Industry
Papain supports system of immunity for treating tumor as it controls and modify leukocytes in response of immune reaction. Swelling and redness at joints and prostate is reduced [15]. Papain from unripe papaya showed 90 percent activity. In 2012 Hitesch et al examined this enzyme action through methods of formation of lumps and absorptiometry. Obtained enzyme degraded levetiracetam and granisteron compounds of Hcl drug having dangerous effects on system of cells. Analysis through spectrophotometry and Tlc of drugs was checked showing efficient results [16]. Papain also has property of functioning against fungi. In 2010 Chukwuemeka and Anthonia reported that papaya reduce fungal infection which causes Rot in papaya when it was investigated that different fungi obtained from species of rhizopus, aspergillus and mucous. Inhibition zone of reducing mycelium was seen and statistically it was examined that shelf life of papaya was increased using papaya [17]. Papain reduced the allergy of indigestion like (stomach related) syndrome of gut which is leaked, decreased hydrochloric acid and (intestinal related) reduce ability to tolerate gelatin. It was proved to be significant as analgesic and antiflammation role for allergies of pain in head and tooth pain having no side reactions [18]. It has vital role in skin and wound care and Hawaii and Tahitti made use of it from concentrated part of papain from papaya and applied for treatment on skin wounds, burning, bed sores, irritation, rashes and was proved to be successful. In 2012 Detrich used the Papain to treat sports injury and athletic foot and 3-8 days was the recovery timing after using papain protease [19].

2.3. Pharmaceutical and Medicine Industry
It was studied that papain can relief patients of dyspepsia who are unable to digest gliadin which papain protect damages of cells due to presence of radicals freely. It is used in preservation of food because it lowers the bacterial attacks and oxidation spoilage. It is also proved that in yogurt there is good survival of bacteria which are probiotic due to property of oxidation [20]. Papain gel was used to give away caries due to break down of polypeptide chain and collagen cross-linkage. It is also used for tooth removing purposes [21, 22, 23].

2.4. Detergent Industry
In 2016 Sangheeta and Abram proposed that Papain could be used as detergent by removing debris. Chemically modifying methods are used to alter enzyme determinants functionally. Papain was modified chemically by anhydrides of succinic, maleic and citraconic acid which than interacted with amine group of lysine in papain causing the change in charge from + to – so the ph also varied to alkalinity and its temperature stability increases. Immobilization by good entrapment in polysaccharides gel also increases the stability of papain which was modified so resulted in high retention power when added in detergents [24].

2.5. Cosmetic Industry
Cosmetic industry is paying attention towards the use of enzymes to improve the beauty of skin avoiding skin problems. Different skin problems are trying to resolve. In 2012 Sim et al deduced that papain was combined with Schizophyllum commune soluble biopolymer. Conjugations enhanced the stability and storage in cosmetics oil and surfactants. Stratefied corneum layer of skin was exfoliated more effectively by using papain than lactic acids which is widely used agent for exfoliation [25].

2.6. Drug Design
Papain showed similar patterns of folding near active site which are useful for designing of drugs. In 2015 Gayosso Garcia et al solved the coordinate method of x-ray at 1.8A and papain was reported as useful model for understanding cathepsin l inhibitors mechanism, antioxidant characteristic used in treating medical problems. Cathepsin l contains many amino acids for proper papain binding having excellent resolution model structure which is helpful for designing good inhibitors of cathepsin k [26].
Papain was reported to be used for designing inhibitor for cathepsin k and catalyze amino acid oligomerization [27].

2.7. Immobilization Property of Papain for Industrial Applications
In 2018 Sheng et al deduced that with the help of covalent bond papain was immobilized on nanoparticle prepared from fibroin of silk. Ph was 6 with time of hydrolyzing 1 hour and support ratio of 10 mg was the conditions for immobilization. Immobilized papain showed excellent activity than papain which is free. Proteolytic function was lost completely due to 8 operations continuously and papain was separated by magnetic field. Immobilized papain on nanoparticles showed greater storage and activity for use of industry and medicine [28]. In 2018 Atacan and Ozacar explored that nano particles which were nontoxic was synthesized thermally and was used for immobilization of papain as precursor of magnet than antibacterial property was examined. For enhancing property against bacteria Ag nanoparticle was used mixed with cu magnetic nanoparticle which were more active against gram + bacteria than gram - so the activity against bacteria was enhanced by immobilization [29].

2.8. Textile and Leather Industry
In textile industry there is significant role of papain in different processes. In 2013 Bushra and Tjul proved that papain was used in wool and silk dust removing before dye [30]. Soaking, dehairing, bating and tanning are different processes in leather industry. Conventionally chemicals like Na sulphide was used for treating leather causing environmental and effluent problems. Alternative method is the use of enzymes which replaced the conventional method and papain is now use in industries of leather in fur and hair removing from hide tanning to guarantee that leather dying is uniform [13].

2.9. Brewery Industry and Miscellaneous
Papain is used in beer formation as there is formation of precipitates due to storage of beer at cool temperature which is known as chill-Haze that contains proteins, so to control this papain works by breaking down these proteins [5]. Papain also has role in industrial applications other than mentioned above which includes role in; clotting of milk as rennet, obtaining oil from liver of tuna, conditioners of shampoos, rubber manufacturing, removing dust from soft lenses (optics) and photography [31].

3. Discussion
Present review deduced that Papain is inimitable enzyme class which poses extensive applications in different industries, which is supported by other studies explaining that it is an enzyme having proteolytic function with cysteine protease activity from endolytic plant [1]. Source of papain is papaya having green color and is acquired from latex, skin cutting of plant from where fluid comes which is assembled and get in dried form.

Papain as meat tenderizer showed reaction of allergy among pharmacy and factory workers, these reactions were systemic mediating antibody type IgE passed through route of gastrointestinal tract. Papain in products of drug and balancing salt solution cause loss of vision, fall in pressure of blood and elevated rate of heart [32]. Although industrial applications of papain have constituted numerous troubles and trails, but advancement in biotechnology figure out these issues. Now papain is widely used in different industries as it is covering steps of biocatalyst cycle to fulfill needs of successful applications in industries which includes food, Pharmaceuticals, Breweries, leather, detergent, and meat and for different purposes. With the help of technological advancement it is now used to treat lethal diseases so there are high demands for its export [11]. Additionally researchers are taking benefit of papain and now it is used for drug designing [26, 27]. Papains possess uncommon properties making it more appreciable commercially. Papaya producing papain’s is present or grow in all tropical areas as there are no seasonal limitations for its growth so it
has high demand for export and there is good prospect of marketing of papain and also farmers at lower levels could use this enzyme as high source of income. Although papain is widely studied protease of cysteine because of its numerous applications but there should be future understanding for specificity of structure, inhibitors effect, pH effect and thermodynamic effect and should be critically assessed. Through recombinant DNA technology, engineering of proteins and procedures of immobilization papain could genetically or chemically modified and will become ideal for biotechnological and industrial applications. Researchers could explore and derive more potent applications by understanding detail mechanism and functions of papain deeply. Then there will be novel papain function that would fulfill the massive need for betterment of life.

4. Conclusions
This review delivers glance about papain describing its general structure, mechanism of function, production and particularly industrial applications. Papain has vital role in food pharmaceutical, health care industry, detergent, cosmetic, textile and leather field. Papain is one of the most commonly use protease commercially and there is good prospect of marketing. Through technologies of recombinant DNA and immobilization, papain could be ideal for industrial applications. Researchers could explore more potent applications by understanding detail mechanism and functions of papain. Then there will be novel papain function that would fulfill the massive need for betterment of life.

References
[1] Tsuge H, Nishimura T, Tada Y, Asao T, Turk D, Turk V, Katunuma N 2012 Biochem. Biophys. Res. Commun. 266 411-416.
[2] Mamboya EA 2012 Am. J. Biochem. Biotech. 8 99-104.
[3] Menard R et al 2010 Biochemistry 29 6706-6713.
[4] Mitchel REJ, Chaiken IM, Emil LS 2011 J. Biol. Chem. 14 3485-3492.
[5] Cohen LW, Coghlan VM, Dihel LC 1986 Gene 2 219-227.
[6] Edwin F, Jagannadham MV 2000 Biochem. Biophys. Acta-Prot. Str. Mol. Enzymol. 2 69-82.
[7] Ghosh S 2005 Colloids Surf. A. 264 6-16.
[8] Madej T, Addess KJ, Fong JH, Geer LY, Geer RC, Lanczycky CJ 2011 Nucleic Acids Res. 40 461-464.
[9] Rajasekaran E, Vijayasarathy M 2011 Bioinformatics 5 455.
[10] Burrows DL, Nicolaides A, Rice PJ, Duffore M, Jhonson DA, Ferslew KE 2005 J. Anal. Toxicol. 29 275-295.
[11] Kamalkumar R, Amutha R, Muthulaksmi S, Mareeswari P, Rani WB 2007 Res. J. Agri. Biol. Sci. 3 447-449.
[12] Khanna N, Panda PC 2007 Indian J. Anim. Res. 41 55-58.
[13] Huet J, Bartik Y, Raussns V, Wintjens R, Boussard P 2006 Biochem. Biophys. Res. Commun. 341 620-626.
[14] Hejazin RK, El-Qudah M 2009 Am. J. Agri. Biol. Sci. 4 173-178.
[15] Krishna KL, Paridhavi M, Patel JA 2008 Biochemistry 32 123.
[16] Hitesh P, Manojbhai BN, Mayuri BA, Kiranben DV 2012 Int. J. Pharm.Biol. Sci. 2 113-115.
[17] Chukwuemeka O, Anthonia AB 2012 Afri. J. Agric. Res. 5 1531-1535.
[18] Mansfield LE, Ting S, Harvery RW, Too TJ 2010 Annals of Allergy 55 541-543.
[19] Diettrich RE 2012 Pa. Med. 68 35.
[20] Farahat AM, El- Bataway OI 2013 J. Dairy. Sci. 8 38-44.
[21] Piva E, Ogliari FA, Moraes RR, Cora F, Henn S, Sobrinho LC 2008 Braz. Oral. Res. 22 427.
[22] Beeley JA, Yip HK, Stevenson AG 2000 Br. Dent. J. 188 427.
[23] Lopes MC, Mascaranic RA, da Silva BM, Florio FM, Basting RT 2007 J. Dent. Child. 74 93-97.
[24] Sangeetha K, Abraham TE 2016 J. Mol. Catal. 38 171-177.
[25] Sim YC et al 2012 Biotech. Let. 22 137-140.
[26] Garcia SG, Tellez EMT, Agular GAG 2015 Am. J. Agr. Biol. Sci. 5 194-203.
[27] Schwab LW, Kloosterman WM, Konieczny J, Loos K 2012 *Polymers* **29** 710-740.
[28] Sheng W, Xi Y, Zhang L, Ye T, Zaho X 2018 *Int. J. Biol. Macromol*. **114** 143-148.
[29] Atacan K, Ozacar M 2018 *Int. J. Biol. Macromol*. **109** 720-731.
[30] Boshra V, Tajul AY 2013 *Health Environ J*. **4** 68-75.
[31] Anibijuwon II, Udeze AO 2009 *Ethnobot Leaflets* **7** 4.
[32] Mansfield LE, Bowers CH 2013 *J. Aller. Clin. Immunol*. **71** 371-374.