Review on Papain Extraction, Processing And Its Application

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Abstract- *Papain, a cysteine protease from Carica papaya, is extensively applied in industrial, pharmaceutical, cosmetic, and biomedical applications. This review considers current and ancient techniques of papain extraction, purification, stabilization, and immobilization, and reviews its diverse applications with recent findings. Challenges, reproducibility, scaling, and sustainability issues are debated, as well as opportunities for better methods and novel applications*

Keywords- Papain, Proteolytic enzyme, Cysteine protease, Enzyme extraction, Purification techniques

I. INTRODUCTION

Papain is an enzyme contained within the latex, fruit, leaves, and other tissues of *Carica papaya*. It is found to be of commercial importance owing to its broad substrate specificity (able to cleave in a number of proteins) and robustness — being active under a range of pH and temperatures. These past few years, as the ease of extraction and purification, immobilization, and more complex uses, have advanced, more sophisticated applications have gained traction.

II. SOURCES AND LATEX COLLECTION

Large-scale commercial papain comes from latex of immature, green papaya fruits. Latex may also be recovered from stems and leaves in a few operations. Incisions are shallowly made on unripe fruit skin in common collection methods, and exuded latex is collected in cold containers holding weak buffering/stabilizing agents (e.g., cysteine or ascorbic acid) to maintain thiol groups and restrict proteolysis by endogenous inhibitors. Rapid cooling and acidification/alkalinization (as appropriate for downstream plans) are frequently employed to stabilize protein and prevent microbial growth.

III. CRUDE EXTRACTION METHODS

1 DIRECT BUFFER EXTRACTION

Crude latex or homogenate plant tissue is suspended in suitable buffer (usually phosphate, citrate or Tris) with a reducing agent to keep the active thiol (e.g., cysteine, dithiothreitol in the laboratory) and protease inhibitors to prevent autolysis where necessary. Solid debris are eliminated by centrifugation and/or filtration. This is the default procedure for laboratory-scale extraction and for downstream purification feeds.

2 MECHANICAL DISRUPTION AND HOMOGENIZATION

Mechanical grinding after which buffer extraction is employed in the case of leaves and peels (alternative sources). Mechanical methods may be coupled with cold conditions and protease stabilizers to maintain activity.

3 ASSISTED EXTRACTION (SONICATION, ENZYME-ASSISTED EXTRACTION, ULTRASOUND)

Ultrasonication and enzyme-assisted extraction have been found to enhance yield and reduce extraction time. These methods have to be optimized meticulously since over energy input can denature papain.

IV. CLARIFICATION AND CONCENTRATION

Following crude extraction, the slurry is microfiltered and low-speed centrifuged in order to eliminate particulates. Ultrafiltration is widely used to concentrate enzyme and exchange buffer (diafiltration), allowing removal of small impurities and salts while concentrating protein before precipitation or chromatography. Membrane concentration is also capable of buffer exchange to conditions suitable for the subsequent purification step.

V. PURIFICATION STRATEGIES

1.SALT OUT METHOD.

Historically we see that which is used for the fractionation of papain from other proteins is the ammonium sulfateprecipitation (single or two step) and sodium chloride. For the two step ammonium sulfate precipitation (different saturation ranges) it is a very economical large scale method

to remove most impurities before the final polish. Also which conditions (pH, ionic strength) you use will play a large role in the yield and residual activity.

2.AQUEOUS TWO PHASE SYSTEMS (ATPS).

ATPS (for example PEG/salt or PEG/phosphate systems) we see put to use in papain purification which we present as an industrial scale and mild partitioning method that also does the job of concentration and partial purification. ATSPs may be also be fine tuned via design of experiments approaches and also can be put in the at same time as in situ immobilization or cross linked aggregates for use in the next step.ATPS often provides better recovery and scalability than multi-step salt precipitations.

3.CHROMATOGRAPHY (ION EXCHANGE, GEL FILTRATION, AFFINITY)

Ion-exchange chromatography (DEAE, Q, SP resins based on pH) with size-exclusion (Sephadex/G-series) or affinity steps, for high-purity preparations, gives highly purified papain. Affinity ligands directed to the active site or general protease-binding matrices can be highly specific but increase the cost. Chromatography is ideal for research-grade or pharmaceutical-grade papain.

4 CRYSTALLIZATION AND POLISHING

Papain can be crystallized to very pure levels; crystallization once supplied the "reference" material for biochemical description. Crystallization is not typically applied for production of commodity enzymes but still has relevance for the production of purified enzyme for structural and kinetic studies.

5.FORMULATION, STABILIZATION AND DRYING

Purified papain is typically formulated with stabilizers (sugars, polyols) and drying aids for storage. Two main drying methods are spray drying and lyophilization (freeze-drying). Spray drying is cost-effective for large scale but risks activity loss from heat; lyophilization better preserves activity at higher cost. Many manufacturers produce spray-dried papain from stabilized feed (e.g., with maltodextrin) to balance cost and stability.

VI. PHYSICOCHEMICAL CHARACTERIZATION AND ACTIVITY ASSAYS

Papain activity is typically measured by proteolytic assays on casein or azocasein substrates, and through small synthetic chromogenic substrates for quantitation (reporting specific activity units). SDS-PAGE and zymography (gelatin/casein zymograms) are employed for determination of molecular weight and activity bands. Mass spectrometry and N-terminal sequencing are utilized for detailed characterization. Stability (pH/temperature profile), kinetic constants (Km, Vmax), and inhibitor sensitivity (e.g., E-64, cystatins) are also routinely reported.

VII. PROCESS DIFFICULTIES AND QUALITY ISSUES

1. Autolysis and thiol oxidation decrease yield — reducing agents and rapid processing counteract this.
2. Endogenous inhibitors and other proteases present in latex make purification difficult; some inhibitors copurify and need to be removed.
3. Scale-up compromises: lower cost bulk fractionation (salting, ATPS) versus chromatographic polishing cost of high purity.
4. Regulatory/quality: medical or pharmaceutical applications demand validated purification, reproducible assay for potency, and profiling of impurities.

VIII. ENZYME YIELD AND QUALITY AFFECTING FACTORS

Numerous factors have been identified from the literature to affect the amount of papain one can extract how active it is, and its stability

1. Source tissue: Latex is more concentrated, although leaves and other tissues could also be used (e.g., leaf extracts lower activity/purity).
2. Harvesting method / maturity: Immature/unripe fruit latex produces better enzyme content.
3. Drying / preservation process: Spray drying, freeze drying, oven drying at regulated temperatures retain activity more than unregulated sun drying.
4. pH, temperature, reducing environment: Papain as a cysteine protease requires reducing environment (to maintain thiol activity), moderate temperature (heat degrades), pH in the optimum range.
5. Purification approach and immobilization: Processes such as ATPS + immobilization facilitate retention of activity, improvement in stability, possibility of reuse.

IX. APPLICATIONS WOUND HEALING / BIOMEDICAL DRESSINGS

A systematic review ("Use and effectiveness of papain in the wound healing process: a systematic review") examined papain in wounds (diverse etiologies) between

1987-2010. It concluded that papain is effective and relatively safe, although burning/pain reports exist.

1.IMMOBILIZATION OF PAPAIN IN CHITOSAN MEMBRANES

Chitosan membranes carrying 2.5-5% papain exhibited enhanced enzymatic activity and slow-release profile. Suggested asbioactive dressings for wounds of the skin.

2.HYDROGEL PREPARATIONS

Hydrogels containing papain + urea, tested in a double-blind randomized trial in lower limb ulcers. The hydrogel with 10% papain contained ~100% protein-proteolytic.

3.CLINICAL TRIAL IN VENOUS ULCERS

2% papain gel vs 2% carboxymethyl cellulose in chronic venous ulcer healing within 12 weeks. Reduction of lesion area was significantly higher in papain gel, especially week 5-12, accumulation of epithelial tissue.

INDUSTRIAL / FOOD/ OTHER APPLICATIONS

Investigation of extraction, functional characteristics, industrial applications: A 2024 review by Manna et al. ("Exploring the extraction, functional properties, and industrial applications of papain from Carica papaya") discusses novel extraction methods, along with anti-bacterial, antioxidant ,anti-obesity activity, and meat tenderization, etc. Oxidized bacterial cellulose membranes immobilized with papain for dressings: Formulated for dressings of chronic wounds; the dressing exhibits debridement activity without hindering collagen formation in fibroblasts.

X. CONCLUSION

An optimized latex collection, mild extraction with thiol-protecting conditions, economic bulk fractionation (e.g., ammonium sulfate or ATPS), and subsequent polishing step (chromatography or selective crystallization) give a versatile platform for producing papain of desired purity and activity. New integration of ATPS, membrane concentration, and immobilization holds prospects for more sustainable and scalable production schemes. And also we talk about the applications.

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