REVIEW ARTICLE

Papain as a Multifunctional Biocatalyst: Advances in Pharmaceutical, Therapeutic and Biomedical Applications – A Review

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Abstract

Papain is a cysteine protease obtained from the latex of the *Carica papaya* L. and has been identified to have proteolytic activity and extensive industrial uses. More recent developments have underscored the importance of it as a multifunctional biocatalyst with a high potential in pharmaceutical, therapeutic and biomedical applications. Advances in extraction and purification methods have significantly enhanced the extraction, purity and processing efficiency of enzymes. Moreover, its functional stability and reusability, as well as resistance to unfavorable conditions, have been improved by stabilization strategies, including enzyme immobilization, cross-linking, and nano-encapsulations. These technologies have broadened the application range of papain, which includes antimicrobial and antibiofilm properties, wound healing, drug delivery systems, nanomedicine and cosmeceutical formulations. Overall, papain represents a versatile and eco-friendly enzyme with a wide role in industries, pharmaceutical and therapeutic fields.

Key words: Biocatalyst, biomedical applications, drug delivery systems, nanomedicine, pharmaceutical applications, proteolytic enzyme

INTRODUCTION

Papain is a cysteine protease enzyme derived from the latex of *Carica papaya L.,* and it is one of the most widely studied plant-derived enzymes, which has wide industrial and biomedical applications [Figure 1]. Although its protein-destroying has been the most prominent feature of its main activity, recent investigations have found a broad range of activity that goes beyond the traditional proteolysis. Various parts of *C. papaya* L., such as latex and peels, leaves and seeds, are used to extract papain.[1] Unripe fruit latex contains the highest level of papain and is the main commercially utilized source of papain.[2] The latex contains a complex of proteolytic enzymes such as papain, chymopapain, caricain and glycyl endopeptidases, all of which help the latex to break down proteins. There are different methods of extraction, purification and stabilization discussed in Table 1. The steps involved in the extraction and purification process of papain are shown in Figure 2.

Conventionally, papain has been widely utilized in the food industry for meat tenderization, brewing and protein hydrolysis, owing to its ability to convert proteins into peptides and amino acids, which also has its use in digestive supplements.[3] Papain has been used in the medical profession in the debridement of wounds and anti-inflammatory or digestive therapy. It is applied in cosmetic preparations as an exfoliating and skin-renewal agent, as well as in the textile and leather industries as a processing aid.[4]

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Moreover, new applications in drug delivery systems, biocatalysis and enzyme modification have greatly expanded its industrial and biomedical applications. Although a lot of studies have been conducted on papain, its potential as a versatile biomolecule is not fully exploited. This review will hence seek to thoroughly analyze the biochemical characteristics, emerging non-proteolytic roles, and recent technological developments involving papain, and the increased importance of papain as an all-round and sustainable biological product.

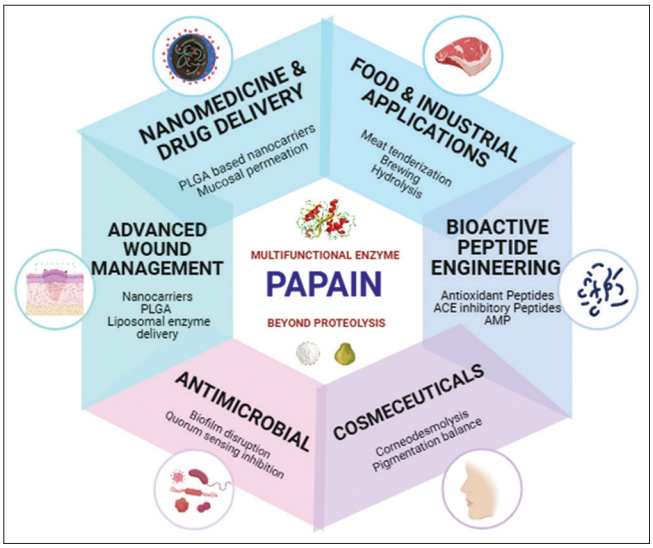

Figure 1: An illustration of papain demonstrates a broad and expanding range of applications

STRUCTURAL, BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF PAPAIN

Papain (EC 3.4.22.2), a single-chain globular protein, consist of 212 amino acids with a 23–25 kDa size and is stabilized by several disulfide bonds.[2,11,12] This structural rigidity, provided by these covalent linkages, allows the enzyme to resist partial denaturation and retain its catalytic structure in response to changes in physical and chemical conditions. The structural configuration of papain is illustrated in Figure 3.

The structure of papain is three-dimensional and comprises two different domains, which are separated by a deep cleft that is home to the active site. Two of these conserved residues include cysteine-25 and histidine-159, which characterize papain as a cysteine protease.[12,13] The enzyme structure also contains the alpha-helices, 2- sheets and a hydrophobic core, which provides structural stability as well as polar surface

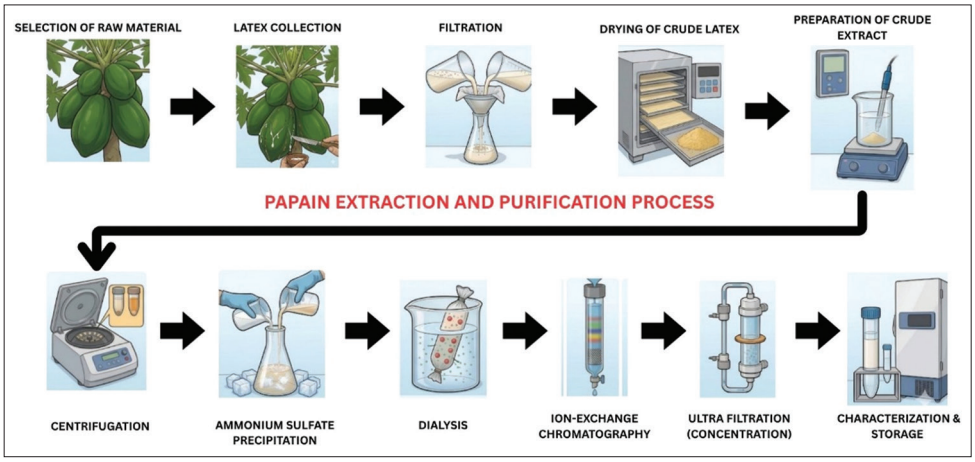

Figure 2: Papain extraction and purification process

Table 1: Methods for extraction, purification and stabilization of papain

Process stage	Method	Key outcome	References
Extraction	Grinding in a buffer solution	Yields a crude extract containing papain	[5]
Enhancement of extraction	Ultrasound-assisted technique	Increases the amount of enzyme recovered	[6]
Initial purification	Ammonium sulfate precipitation	Produces a more concentrated papain fraction	[7]
Desalting step	Dialysis	Effectively removes salt from the sample	[1]
Refined purification	Ion-exchange chromatography	Results in higher purity of the target enzyme	[2]
Purification by size	Gel filtration chromatography	Delivers fractions with high enzyme purity	[8]
Alternative purification method	Three-phase partitioning	Enables efficient recovery of the enzyme	[8]
Another alternative approach	Aqueous two-phase system	Improves the purification efficiency	[9]
Stabilization	Addition of antioxidants	Maintains up to 90% of enzymatic activity	[10]

residues that facilitate the dynamic interactions at the active site.[12] Such stability and flexibility enable papain to be effective in both aqueous and multiphase systems.

Papain displays catalytic activity in a large pH range (3.0–9.0) and is generally active at a pH range of 4.5–7.5.[2,12,14] It is also characterized by a high degree of thermal stability, having the highest activity at 50–60°C and being able to maintain its functions at temperatures as high as 70–80°C under controlled conditions.[2,13,14] Despite the strength of papain as an enzyme, its free form in solution has a number of limitations that limit its use in long-term or continuous processes. It also becomes inactive when the pH levels are too high or when the temperature is higher than the optimal range of its functional activity.[13] To address these issues, several immobilization and encapsulation methods have been devised, which deal with the trade-off between a high catalytic efficiency and the vulnerability to self-degradation. Moreover, polymer or nanostructured carrier encapsulation is another approach that offers extra protection against oxidation and environmental stress factors, preserving the accessibility of substrates and functional activity.[7,14] To increase the use of papain in other areas other than the proteolysis, formulation and stabilization methods should be employed to improve the weaknesses of the enzyme, including poor stability in pH, low heat stability, autolysis, and inconsistency. The major formulation approaches and stabilization strategies that are employed to improve the papain functionality are illustrated in Figure 4.

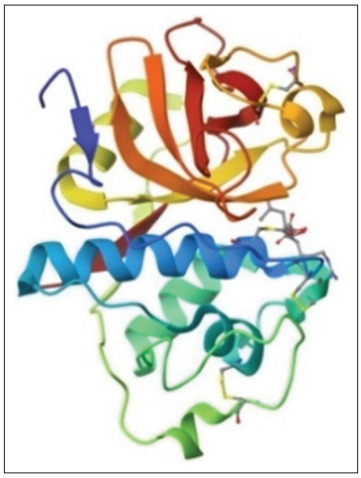

Figure 3: Three-dimensional structure of papain enzyme showing its folded protein domains and active site configuration (Source: Protein Data Bank)

PAPAIN IN ADVANCED WOUND CARE AND TISSUE DEBRIDEMENT (CLINICAL AND BIOMEDICAL APPLICATIONS)

The recent resurgence of interest in papain as a bioactive in contemporary wound care is due to the selectivity shown in the necrotic tissue removal coupled with simultaneous control of inflammation, microbial burden mitigation and tissue regeneration enhancement. Chronic and complicated wounds, e.g. venous ulcers, diabetic foot ulcers, pressure ulcers and infected surgical wounds, are commonly held at the inflammatory stage by the accumulation of necrotic tissue and continued colonization by bacteria. To solve this challenge, papain-based therapies are effective in enabling wound bed preparation without injuring viable tissue, thus making it possible to proceed to other stages of healing. On a molecular scale, papain breaks down denatured proteins, fibrin deposits and cell debris. The selectivity toward the damaged tissue is evidenced by the existence of endogenous antiproteases in healthy cells, which shield the intact structure against the action of enzymes.[15,16] Due to its biocompatibility, high catalytic activity and structural stability, papain can be

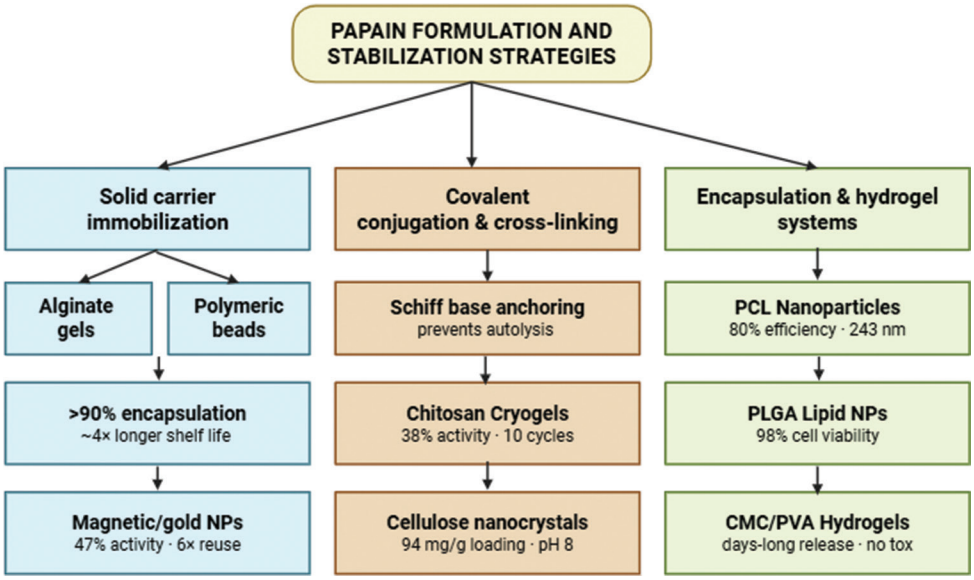

Figure 4: Papain formulation approaches and stabilization strategies for enhanced activity and shelf life

used safely in various stages of wound healing. Cysteine activation does not inactivate the active thiol group and increases the ability to remove necrotic tissue, preserving the activity of macrophages and promoting cell growth.[17]

The most common clinical preparations of papain are topical gels and hydrogels. Papain preparations with 2–10% papain in a 5% urea-based carboxypolymethylene hydrogel are physically stable, retain proteolytic activity and have appropriate rheological characteristics to be used in the clinic.[16] Increased levels of papain are effective in the treatment of venous and leg ulcers through slough removal, stimulation of the formation of granulation tissue and increased re-epithelialization.[15,16] Papain-functionalized BC dressings showed full inhibitory activity on Gram-positive and Gram-negative bacteria. Functionalization using succinic acid also added amide groups, which enhanced enzyme retention and biocompatible interactions with fibroblasts, along with glutaraldehyde cross-linking, further improved enzyme loading and antibacterial activity.[18,19] Chitosan–papain nanoparticles, characterised by high porosity, swelling capacity and fluid absorption, are particularly suitable for highly exuding wounds, enabling controlled enzyme release under acidic conditions typical of chronic wounds.[20] Elastic liposomes have demonstrated improved delivery efficiency, and in hypertrophic scar models, they have been associated with improved tissue architecture, reduced collagen deposition and decreased fibrotic signaling through the transforming growth factor-beta 1/Smad and nuclear factor kappa-light-chain-enhancer of activated B cells pathways.[21]

Papain-loaded liposomal systems integrated with natural latex membranes and red-light phototherapy achieved nearly 100% wound healing in diabetic mouse models, accompanied by improved tissue organization.[22] In addition, biomaterials such as $CaSO_4$–alginate, poly (vinyl alcohol)/alginate and oxidized bacterial cellulose have been reported to preserve papain activity, thermal stability and functional longevity under chronic wound conditions.[23-25] Incorporation of papain into hydrogels based on chitosan, chito-oligosaccharides, or poly (γ-glutamic acid) has been shown to enhance moisture retention, support fibroblast activity, stimulate angiogenesis and promote collagen deposition, resulting in improved tissue regeneration with reduced scarring.[26] Furthermore, the application of quality by design principles has enabled the optimization of formulation parameters such as viscosity, spreadability and adhesion, thereby improving product consistency, shelf life and clinical applicability.[27] Clinical and veterinary studies have reported dose-dependent improvements in infected and necrotic wounds, including accelerated granulation, reduced healing time and improved scar quality across various wound types.[28] In general, the existing evidence confirms papain as a compound, which can be utilized in various ways as a therapeutic enzyme in the treatment of complex wounds. Its versatile applications in selective debridement, antimicrobial effects, inflammation and tissue remodeling are far beyond its traditional proteolytic activities. The fact that papain can be successfully incorporated into gels, biopolymer-based dressings, nanocarriers and combination therapies sheds light on its increased relevance in higher wound healing technologies.

ROLE OF PAPAIN IN COSMETICS AND COSMECEUTICALS

Papain is a cysteine protease that has long been known to have properties of protein hydrolysis and skin exfoliation. Its cosmetic potential is much more than these traditional functions. Papain has an effect on the extracellular matrix, promotes the functionality of the skin barrier, and provides a multifunctional platform of innovative and sustainable cosmetic formulations. Papain has been demonstrated to modulate collagen turnover in dermatological tissue remodeling through a rise in matrix metalloproteinase-1 (MMP-1) and a fall in tissue inhibitor of metalloproteinase-1 (TIMP-1) to shift the TIMP-1/MMP-1 balance towards fibrotic tissue degradation.[29] The therapeutic potentials of this mechanism in the treatment of hypertrophic scars, keloids, and other fibrotic skin disorders are highlighted. Furthermore, papain helps in keeping the tissue pH optimal, thereby providing an environment favorable to enzymatic action in the body, as well as tissue repair by supporting the skin barrier. Papain-mediated exfoliation in cosmetics is a close replica of the real process of skin desquamation. It selectively breaks down orneodesmosomes, which allows exfoliation to be gentle with no harm to the regenerative ability of the stratum corneum.[30] The parameters like viscosity, the type of emulsifiers used, and rheological characteristics are critical parameters that can influence the behavior of enzymes and overall performance of the formulation.[31]

Papain improves the exhibits antioxidant properties and disrupts melanogenesis pathways, which leads to a more homogenous skin tone by removing pigmented keratinocytes and regulating the activity of tyrosinase.[32] Papain-containing cosmetics have been shown to have a better skin texture and luminosity, lesser irritation than acid-based preparations, and anti-inflammatory, antimicrobial, and antioxidant advantages. Developments in encapsulation methods have promoted their infiltration and stability, which promotes skin retention, therapeutic efficacy, wound healing, and dermal remodeling in the presence of a delivery system like liposomes and hydrogel.[33]

Papain has also been used as a part of starch-carboxymethyl cellulose films, in which it can maintain enzymatic activity and enhance mechanical strength and water solubility, which is why it can be used in peel-off masks and controlled-release cosmetic systems.[30] Collagen is very soluble in papain, and the α-helical content is preserved, which is used in anti-ageing and wound-healing preparations.[34] Leaf-derived papain has been successfully used to extract collagen out of waste

materials without interfering with the native triple-helical structure, which contributes to sustainable and eco-friendly methods of cosmetic ingredient sourcing.[35] Whitening gels containing papain are effective in the removal of surface dental stains and are less oxidative to tooth structures than peroxide-based preparations.[36] Moreover, it has been demonstrated that papain could be used in the extraction of virgin coconut oil to contribute to its quality and make it a better lipid fraction when used as a moisturizer in cosmetics.[37] Although papain has a greater potential as a cosmetic, its safety profile should be critically reviewed before it is used on a large-scale basis, since it has been linked to contact allergens with occupational sensitization, especially in people who are exposed to latex or papaya-based products.

ANTIMICROBIAL AND ANTIBIOFILM PROPERTIES

The mounting body of experimental data shows that papain has a strong antimicrobial and antibiofilm activity. In contrast to the traditional antibiotics, which usually act to interfere with a certain intracellular process, papain acts mainly by destabilizing the cell surface structure, extracellular polymers, quorum-sensing networks, and the overall biofilm structure.

The antimicrobial activity seems to be species-specific and conditional upon the experimental conditions because certain studies have shown no significant antimicrobial activity against the Gram-positive or Gram-negative bacterium with the help of agar diffusion assays, but antifungal activity against *Candida albicans* has been observed.[38] Papain hydrolysis of bovine and caprine milk proteins was observed to produce a significant antibacterial effect against diarrheagenic *Escherichia coli* and *Staphylococcus aureus*, and inhibition zones more than 19 mm were observed in goat milk hydrolysates.[39] Likewise, the papain-digested camel whey proteins produce low-molecular-weight peptide fractions with strong antimicrobial activity against Gram-positive and Gram-negative microorganisms. Electron microscopy analysis revealed severe disruption and leakage of the membranes in *E. coli,* whereas mass spectrometric analysis revealed the enrichment of bioactive peptides in the most active fractions.[40] Moreover, papain-hydrolyzed porcine liver and meat matrix proteins have demonstrated antimicrobial properties in refrigeration storage processes.[41,42] All these results indicate that the antimicrobial effects attributed to papain are often mediated by bioactive peptides produced during enzyme hydrolysis, but not by the intact enzyme.

Studies have demonstrated that papain does not significantly affect planktonic multidrug-resistant *Klebsiella pneumoniae*, but effectively inhibits biofilm formation and disrupts established biofilms in a dose-dependent manner.[43] Scanning electron microscopy and mechanistic analyses further revealed that papain selectively cleaves extracellular polymeric substances (EPS)-associated proteins without exerting direct bactericidal effects, thereby increasing bacterial susceptibility to antibiotics.[44,45] Demonstrated that papain covalently attached to polymeric surfaces retained over 90% of its activity after prolonged storage and effectively removed biofilms of *S. aureus* and *Acinetobacter* spp., with a significant reduction in EPS Protein and carbohydrate content. In addition, papain-modified packaging films achieved a reduction in microbial load on chilled meat. Similarly,[46] reported that papain immobilized on chitosan maintained enzymatic activity with enhanced thermal stability and exhibited strong antibiofilm activity against *S. aureus* and *Staphylococcus epidermidis*, functioning primarily as a biofilm-disrupting rather than bactericidal system.

Papain immobilized on Ag/$CuFe_2O_4$ magnetic nanoparticles[47] and papain-based nanoflowers applied to food-contact surfaces[48] have demonstrated high biofilm degradation efficiency. This anti-virulence mechanism limits selective pressure for resistance development.[49] Moreover, papain exhibits a dual role as both a bioactive agent and a structural template for antimicrobial design, as highlighted in recent studies.[50] Although much of the current evidence is derived from *in vitro* and short-term investigations, the collective findings strongly support the classification of papain as a multifunctional modulator of microbial behavior, particularly in biofilm disruption and resistance management.

FOOD AND INDUSTRIAL APPLICATIONS

Papain has extensive substrate specificity and functional versatility that have made it a popular biotechnological reagent in food and industry applications. The wide range of applications has been discussed in Table 2, which highlights the functional diversity of papain across both food and other industrial sectors.

ROLE OF PAPAIN IN DRUG DELIVERY AND NANOMEDICINE

Papain has become a versatile bioactive molecule, which has applicability in biotechnology and nanotechnology. In these areas, papain has been used in a sophisticated delivery system which takes advantage of its catalytic selectivity, biocompatibility, and structural flexibility. The modern methods include encapsulation, immobilization, surface modification, and integration into nanoscale carriers to increase stability, overcome biological barriers, and improve therapeutic precision. Functional longevity and environmental stress resistance are effectively incorporated into polymeric matrices by the incorporation of papain. The enzyme was immobilized to have better stability in a broad pH and temperature range, and still showed catalytic activity during

Table 2: Food and industrial applications of papain

S. No.	Food and industrial applications of papain	References
1.	Meat tenderization through degradation of myofibrillar proteins and connective tissues (collagen), improving tenderness, juiciness, and texture under optimal conditions (pH 5.5–6.0; 50–60°C)	[51]
2.	Modification of plant proteins (soy milk), increasing hydrolysis, antioxidant activity, and ACE inhibitory activity, while reducing antigenic components such as Kunitz trypsin inhibitor and Bowman–Birk inhibitor	[52]
3	Conversion of animal by-products (chicken skin proteins) into value-added functional ingredients with improved antioxidant properties and amino acid composition	[53]
4.	Enhancement of the melting and stretching properties in dairy products such as cheese	[50]
5.	Modification of gluten structure in cereals, improving dough performance and reducing immunoreactive gliadin levels	[4,54,55]
6.	Improvement of emulsification by hydrolyzing egg-yolk lecithin, enhancing hydrophilic–lipophilic balance, reducing surface tension, and increasing zeta potential	[56]
7.	Development of functional foods and nutraceuticals via generation of ACE inhibitory peptides, antioxidant fragments, and hypoallergenic hydrolysates	[52,57]
8.	Use in active packaging systems where papain-derived peptides provide antioxidant and antimicrobial properties for shelf-life extension	[58]
9.	Use in brewing to hydrolyze haze-forming proteins, improving beverage clarity and shelf stability	[4]
10.	Application in aquaculture feed to enhance nutrient absorption, growth performance, and immune responses (e.g., grass carp)	[59]
11.	Papain-assisted extraction of collagen from poultry by-products, producing biomaterials with high emulsifying capacity and water retention	[60]

repeated use cycles.[61] Likewise, microparticle systems based on chitosan have proven to be able to improve the catalytic stability of papain, especially when added with ascorbic acid, which stabilizes reactive thiol groups.[62] Encapsulation in poly(ε-caprolactone) nanoparticles has likewise been noted to enhance structural integrity and decrease cytotoxicity, which also justifies the appropriateness of papain in biomedical and nanotechnological applications.[63]

Papain-loaded solid lipid nanoparticles have a uniform size distribution, controlled release, and anticancer and immunomodulatory properties compared to the free enzyme. The role of papain-functionalized nanocarriers in improving tumor targeting, inducing apoptosis by generating reactive oxygen species (ROS) and through caspase pathways and improving anticancer efficacy by controlling drug release is demonstrated in Figure 5. The lipid matrix maintains the activity of enzymes in acidic environments and improves interactions with immune cells, activating lymphocytes and stimulating the production of cytokines.[64] In a similar way, nanostructured lipid carriers with a statistically optimized geometry have shown a high loading capacity and stability in simulated gastric conditions.[65] Hybrid systems made of cores of poly(lactic-co-glycolic acid) polymer with phosphatidylcholine lipid shells have also demonstrated high encapsulation efficiency and release of antibacterial.[66] In addition to encapsulation, papain has also been conjugated to the surface of nanocarriers to regulate biological barriers. Papain-modified self-emulsifying drug delivery systems are reported to improve oral absorption of poorly soluble drugs by temporarily disrupting the mucus structures.[67,68] The addition of papain-palmitate conjugates into lipid nano emulsions also aids in the transportation through the intestinal mucus and reduces cellular damage.[69] Furthermore, paracellular drug delivery has been achieved by cyclodextrin-based complexation, which does not disrupt epithelial integrity.[70] Papain has also found its way into stimuli-responsive nano systems to target cancer therapy. Redox-reactive papain-decorated micelles have shown better tumor penetration and drug delivery in response to intracellular levels of glutathione.[71]

Papain, as a reducing and stabilizing agent, has been reported to improve the anticancer effect of other agents like 5-fluorouracil. Moreover, the papain-functionalized selenium nanoparticles have caused apoptosis in liver cancer models through caspase-mediated mechanisms.[72] Technetium-99m-labelled papain nanoparticles have demonstrated potential in tumor targeting in the sphere of diagnostic imaging.[73]

Antimicrobial systems made of chitosan, papain, and gold nanoparticles have been shown to have antibacterial and antibiofilm effects by disrupting extracellular polymeric material and the production of ROS.[74] Natural photo-crosslinking methods to achieve long-term antibiofilm performance in packaging materials have also been attained.[45] Moreover, the immunological response of papain-loaded nanoparticles to systemic delivery is not sufficiently characterised. Increased lymphocyte activation and cytokine release in papain-loaded solid lipid nanoparticles have been seen to be advantageous in the aspect of anticancer,[64] but these immunostimulatory responses should be carefully considered. Another critical

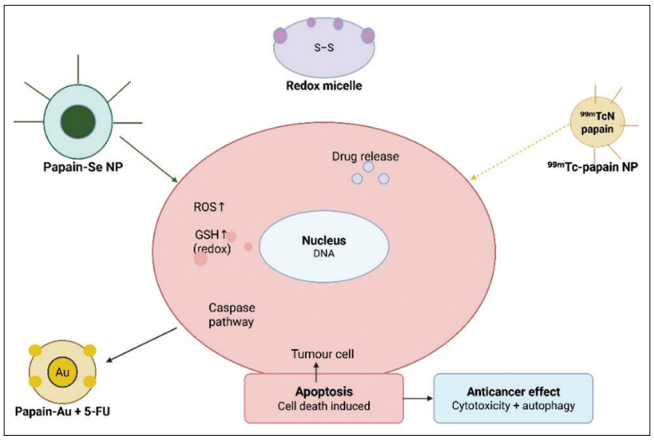

Figure 5: Papain-functionalized nanocarriers for targeted cancer therapy and controlled drug release

challenge that has not been given a lot of attention is stability in biological fluids. When ingested into the bloodstream or gastrointestinal tract, nanocarriers are quickly coated with serum or mucosal proteins to create a protein corona that may mask targeting ligands on the surface, change cellular uptake pathways, and can radically alter release kinetics relative to behavior in buffer systems.[75] *In vitro* stability experiments in simulated gastric and intestinal fluids are valuable, but cannot be used to replace tests in real biological systems under dynamic flow conditions and physiologically relevant protein concentrations.[65] The absence of extensive toxicological information regarding papain-based nanomedicine systems is a weakness. *In vitro* biocompatibility tests do not suffice to satisfy preclinical requirements by regulatory authorities. These gaps outline an obvious and viable research agenda in the area of systematic *in vivo* pharmacokinetic studies on rodent models, followed by comprehensive immunological safety studies on immunocompromised animals, and finally, formal toxicological studies under Good Laboratory Practice conditions.

CONCLUSION

Papain has become a very versatile biomolecule that has a substantial practical implication in various fields. Its biochemical properties, such as cysteine-dependent catalytic activity, wide substrate specificity, and activity at various environmental conditions, are the basis behind its well-established industrial uses. Although traditionally applied in food processing, meat tenderization, and pharmaceuticals, recent developments have broadened applications to biofilm disruption, wound healing, production of bioactive peptides, and integration into sophisticated drug delivery systems. Its flexibility to meet the demands of modern science can be seen in its use in nanomaterials, in biomaterial engineering, and cosmetic formulations. Autolysis limitations, instability, and unreusability of native papain have been successfully overcome using formulation and stabilization strategies. Immobilization, encapsulation, and nanoparticle incorporation have also been used to enhance its stability, longevity of functional activity, and controlled and targeted delivery, which has led to better predictability and precision as a bioactive agent. In addition, using papain derived from papaya, such as peels, seeds, and leaves, this process promotes the concepts of sustainability and circular economy because it reduces the amount of waste and is aligned with the technological methods of environmental responsibility. Collectively, these developments position papain as a model example of how a traditionally known enzyme can be transformed into a multifunctional tool through interdisciplinary innovation. Its evolution from a simple digestive enzyme to a broadly applicable bioactive molecule highlights its growing importance in both research and industrial applications.

CURRENT RESEARCH GAPS AND FUTURE PROSPECTS

Recent literature on papain demonstrates that there are many critical gaps in the current literature, such as the absence of mass *in vivo* studies and clinical trials to confirm its effectiveness and safety, inadequate toxicological and immunological characterization despite the established allergenicity, absence of standard units of activity and formulation direction, and little knowledge of protein corona effects in nanomedicine systems. Furthermore, its non-proteolytic activities (antimicrobial and antibiofilm) have not been sufficiently elucidated concerning their precise molecular mechanisms. Large-scale industrial optimization of processes and economic viability of complex extraction and purification procedures, as well as little research into the concept of genetic and protein engineering to increase enzyme specificity and stability, also exist. None are well established with respect to long-term stability, storage behavior, and environmental interactions, whereas there is limited clinical evidence in wound healing based on small-scale studies with limited randomised trials. Those gaps will give a clear orientation to further studies in which novel technologies, including stimulus-responsive nanocarriers, smart hydrogels, and engineered biomaterials, may allow the effective and efficient control of papain activity at the spatiotemporal level to enhance therapeutic efficacy and reduce off-target activities. Development of engineered papain variants of increased specificity, stability, and reduced autolysis has the potential to be achieved through advances in site-directed mutagenesis and computational protein modeling. Other antimicrobial resistance properties of papain include biofilm disruption and quorum-sensing inhibition, which need corroboration *in vivo* to be clinically translated. Finally, to close the space between the laboratory-scale novelty and reality, stringent safety assessments, standardised formulation protocols, and the unified regulatory standards will be required and will allow papain to become a multifunctional, sustainable, and clinically applicable biomolecule in the next generation of biotechnological and biomedical applications.

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