

A Review on Encapsulation and Immobilization Technologies for Controlled Enzyme Release in Meat Tenderization

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Abstract: Although enzymatic tenderization has proven to be one of the best methods for enhancing the quality of tough meat cuts, its industrial use is still restricted because of issues including unchecked proteolysis, the formation of off flavours, and uneven textural results. Technologies for encapsulation and immobilization present a viable way to manage enzyme activity, control diffusion, boost stability, and increase consistency while tenderizing meat. Despite clear benefits, there are still significant technological obstacles, such as a lack of food-grade carriers, a poor comprehension of the release kinetics within meat, a lack of comparative studies with free enzymes, and a lack of validated models explaining the interactions between enzymes and meat. Current developments in encapsulation and immobilization methods for proteolytic enzymes used in meat tenderization are critically assessed in this review. In order to optimize enzyme delivery systems that improve meat texture, sensory qualities, safety, and industrial scalability, it is critical to fill in the knowledge gaps regarding the science underlying carrier materials, controlled release mechanisms, and the impact of encapsulation and immobilization on enzymatic activity, specificity, and physicochemical properties of meat. The review concludes by outlining future research directions necessary to promote the use of encapsulated and immobilized enzymes in contemporary meat tenderization.

Keywords: Encapsulation, Enzyme, Immobilization, Meat, Tenderization.

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I. INTRODUCTION

Exogenous proteolytic enzymes, such as microbial and plant proteases (such as ficin, papain, and bromelain), have been shown to be an efficient way to improve meat tenderness, especially for lower-grade or collagen-rich cuts.¹ However, conventional techniques like marination, injection, and surface treatments that depend on the use of free enzymes have serious disadvantages. These include quick and unregulated proteolysis, the possibility of over-tenderization leading to a mushy or "mealy" texture, unequal penetration into meat, increased cooking losses, and detrimental impacts on juiciness and flavour.²

The pharmaceutical and food processing industries frequently use encapsulation and immobilization technologies, especially for the delivery of nutraceuticals and functional foods. However, their application in meat processing, such as the treatment of meat cuts and evaluation of texture, flavour, and consumer acceptability, is notably limited, indicating a significant translational gap between theoretical concepts and practical implementation.^{3,4} These technologies have been successfully used to regulate the activity of enzymatic tenderization processes and improve their industrial viability.⁵

This review explores methods for immobilizing and encapsulating proteolytic enzymes used in meat tenderization. It identifies major technological and knowledge limitations that now restrict their use in industrial settings and describes key mechanisms and materials involved in these processes.

II. EXISTING CHALLENGES AND RESEARCH GAPS

However, there are currently several important scientific and technology limitations that prevent the use of encapsulated or immobilized enzymes in meat systems. Key problems include:

Enzyme release kinetics in meat are poorly understood, which is a major problem. The diffusion and release of encapsulated enzymes within thick, heterogeneous, and organized meat tissue under practical processing conditions such as marinating, tumbling, injecting, and sous-vide cooking are not well documented. There is a knowledge gap that has to be filled in order to maximize the use of enzymes in meat tenderization because no regulated release curves tailored to meat applications have been published.^{1,6}

Few experimental research have been done on the encapsulation of enzymes to prevent over-tenderization in meat.⁵ Additionally, there aren't many comparative investigations of free, encapsulated, and immobilized enzymes in real meat matrices. The majority of the literature

now in publication discusses free enzymes, and although some research has been done on encapsulated protease microcapsules, there has been no assessment of immobilized enzyme reactors for meat tenderization in conditions that mimic industrial settings.⁵

Diffusion restrictions and steric hindrance are the main ways that encapsulation impacts enzyme activity and stability. Nevertheless, there aren't many published research that compare the precise peptide profiles and cleavage sites of free and encapsulated enzymes in meat. Rather than offering precise *in situ* cleavage site mapping within a meat matrix, the majority of research concentrates on the preservation of total enzyme activity or the bioactivity of peptides produced after digestion.⁷

There are significant gaps in the practical usage of immobilized enzymes for large scale meat tenderization, despite the potential financial and environmental benefits of immobilization. These deficiencies are especially related to the effectiveness, reusability, and influence on meat quality results in processing settings.⁸

The interactions between enzymes carrier and meat are not well predicted by any computational or mathematical models that have been validated. Existing diffusion or kinetic models, which are intended for homogenous liquids, are inapplicable in the context of meat due to its structural complexity, which includes muscle fibres, connective tissue, and changing water content.⁹

The use of encapsulated enzymes in meat products is not yet governed by any defined safety or regulatory framework. Several elements of the use of encapsulated enzymes, including appropriate carrier materials, the enzymes' residual activity after cooking, labelling requirements, and possible allergic or migratory issues, have not been formally addressed by food regulators. Only free enzymes are covered by current laws and safety standards.¹⁰

III. CURRENT STATUS OF PROTEOLYTIC ENZYMES IN MEAT TENDERIZATION

Plant-derived proteases, including bromelain, ficin, and papain, have been the focus of meat tenderization research. However, newer proteases like actinin, cucumisin and zingibain, are being explored for their potentially milder and more controllable tenderizing effects. Additionally, microbial enzymes are becoming increasingly popular due to their cost-effectiveness, scalability for commercial use, and favorable functional properties in food processing.¹¹

Because free enzyme systems are sensitive to different processing conditions, including salt, pH, marinating, tumbling, and heat, they can produce unpredictable texture

and flavour results. This inconsistency puts their industrial application at risk and creates difficulties.¹

IV. ENCAPSULATION

Encapsulation is a process that involves the entrapment of enzymes within a carrier material. This technique serves to protect the enzymes from direct contact with substrates, thereby transforming the incorporation of enzymes into a system that allows for controlled release. For many applications where careful control of enzyme activity is required, this regulated release is beneficial.¹²

V. ENCAPSULATION MATERIALS USED FOR ENZYME DELIVERY IN MEAT SYSTEMS

Materials used for encapsulation must be stable, biodegradable, food-grade, and compatible with meat matrices.¹³ Common categories include:

➤ *Polysaccharide-Based Materials*

Alginate, carrageenan, starch derivatives, gum Arabic, and cellulose derivatives are examples of polysaccharide-based compounds that have important benefits such as moderate processing and efficient gelation. However, they also have drawbacks, such as inconsistent mechanical strength and low heat stability.¹⁴

➤ *Protein-Based Materials*

Protein-based substances that are edible, biodegradable, and useful for producing films include gelatin, whey protein, soy protein isolate, and zein. Nevertheless, they have disadvantages such as the possibility of enzyme interactions and temperature sensitivity.¹⁴

➤ *Lipid-Based and Nano-emulsions*

Lipid-Based and Nano-emulsions, including Liposomes and Solid lipid nanoparticles, offer excellent barrier systems, but their high cost and instability during cooking are drawbacks.¹⁵

➤ *Novel Biopolymers for Meat Processing*

Although they lack validation, emerging bio-based carriers such as edible plant-derived gums, resistant starches, and nanocellulose show promise for usage in meat systems.¹⁶

VI. MECHANISMS OF CONTROLLED RELEASE FOR ENCAPSULATED ENZYMES IN MEAT

Moisture diffusion during margination, muscle contraction and ionic changes, low-temperature cook-in (sous-vide) conditions, mechanical tumbling/injection pressure, pH or salt gradients, and other factors are commonly known to cause release.^{17,18}

VII. BENEFITS OF ENCAPSULATION

This affords several potential benefits which include Protection of the structural integrity of enzymes against environmental stress like oxidation, pH, salt, and ionic strength,¹⁹ Protease activity can be released in a controlled,

delayed, or sustained manner to lower the risk of over-tenderization,²⁰ Improved shelf life, storage stability, and possibly less enzyme autolysis before use,²¹ Control of enzyme activity in meat tissue both spatially and temporally.²²

VIII. IMMOBILIZATION

Attaching enzymes to solid or semi-solid substrates, such as gels, membranes, beads, and polymer matrices, is known as immobilization. This technique promotes stability throughout processing, minimizes enzyme loss, and allows for reuse.²³

Immobilization offers several advantages which include, important characteristics include localized proteolytic activity, increased resistance to denaturation, possibility for reuse in industrial systems, and better thermal stability.¹²

IX. NEW DEVELOPMENTS: ENZYME ENCAPSULATION FOR FOOD USES

A study used a spray-drying technique with cross-linked chitosan to successfully microencapsulate a food-grade protease complex (Flavourzyme®). Under ideal circumstances, the method produced high encapsulation efficiency ($\approx 78.6\%$) and activity yield ($\approx 88\%$), indicating the possibility of producing stable protease-loaded microcapsules on a wide scale for use in food applications.²⁴

Encapsulation techniques of fixed formalin and paraffin,²⁵ microcapsule biosensors,²⁶ penicillinase immobilised phase change microcapsules,²⁷ height difference nanoindentation,²⁸ and self-assembly technology,²⁹ are a few of the promising encapsulation and immobilization technologies that have been investigated recently.

Furthermore, encapsulation, immobilization, and stabilization have recently been identified as strategic approaches to address problems associated with free enzyme tenderization in more comprehensive assessments of enzyme usage in meat processing.

X. THE POTENTIAL REVOLUTION IN MEAT TENDERIZATION THROUGH ENCAPSULATION/IMMOBILIZATION

Technologies for encapsulation or immobilization could, if effectively implemented:

- Deliver proteases slowly and in a controlled manner to avoid over-tenderization and maintain preferred meat texture.^{30,31,32,33}
- Enhance the stability of enzymes throughout handling, distribution, and storage, particularly important for developing nations with weak cold chains.^{34,35,36,37}

- Improve uniformity throughout a cut by enabling consistent enzyme distribution and penetration into deeper muscle layers, particularly when combined with marinating, injecting, or tumbling.^{38,39}
- Utilise reusable immobilized systems that are appropriate for industrial-scale operations to cut down on enzyme waste and processing expenses.^{40,41,42,43}
- Improve control over the kinetics of proteolysis to enable consistent processing procedures for various meat kinds.^{44,45}
- Modulate release profiles to potentially reduce sensory adverse effects (bitterness, off flavours). For instance, rather than an aggressive breakdown that releases bitter peptides all at once, slow release may result in mild, progressive proteolysis.^{46,47,48,49}
- Encapsulation/immobilization is therefore a viable paradigm shift, particularly for producers looking for scalability, cost effectiveness, and consistent quality.^{50,51}

XI. CONCLUSION

By offering regulated and effective enzyme delivery systems, encapsulation and immobilization technologies have the potential to completely transform meat tenderization. Though there are still a lot of unanswered questions about enzyme kinetics, release inside meat, impacts on meat quality and sensory qualities, variance between meat kinds, and regulatory approval, however recent developments, such as the microencapsulation of proteases through spray-drying, offer promise. To validate these technologies for industrial usage, cooperative research involving food engineering, materials science, meat biochemistry, and sensory science is required.

RECOMMENDATIONS

In order to fully utilize encapsulated or immobilized enzymes in meat tenderization, future studies should focus on:

- Creating and testing edible, salt-and heat-stable encapsulating materials (such as biopolymer gels and polysaccharide-protein composites) that are appropriate for processing and cooking meat.
- Comparing free, encapsulated, and immobilized enzymes in systematic trials on a variety of meat types (beef, goat, poultry), cuts (lean, collagen-rich), and processing techniques (marination, injection, tumbling). Texture (shear force), water-holding capacity, microstructure, cooking loss, and sensory evaluation should also be determined.
- Creating and verifying computational and mathematical models of the diffusion and release kinetics of enzymes inside muscle tissue that take temperature, mechanical

forces, water distribution, fibre orientation, and connective tissue barriers into account.

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