Institute of Food Science and Technology

APPLICATIONS OF ENDO - PEPTIDASES IN FOOD INDUSTRY

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INDEX

I. Definition

Protease, also known as **peptidases** or **proteinases**, are a unique class of enzymes that catalyze the hydrolysis of proteins by cleaving peptide bonds between amino acids. Peptidases are considered essential enzymes, constituting approximately 2.8% of the mammalian genome, with over 560 proteases identified among the 20,126 genes of humans (*Homo sapiens*).

Food-related proteases can originate from plants, animals, or microorganisms. While proteases are ubiquitous in nature, microbial-derived peptidases are the most accessible source. Microbial enzymes generally exhibit higher stability than those derived from plants and animals. Furthermore, plant-derived enzymes can be influenced by cultivation conditions and climate, while animal-derived proteases depend on livestock availability for slaughter. In contrast, microbial proteases are among the most widely used industrial enzymes globally due to the rapid cultivation and growth rates of microorganisms compared to plants and animals. Moreover, microbial proteases often have longer shelf lives and can be stored without significant loss of bioactivity.

*** Endopeptidases**:

Endopeptidases belong to the hydrolase class of enzymes and catalyze the hydrolysis of peptide bonds, thereby breaking down proteins. Endopeptidases are capable of proteolytic cleavage by cleaving peptide bonds at appropriate positions in the protein chain. These enzymes exhibit high selectivity in terms of chemical (chemo-), positional (regio-), and stereochemical (enantio-) specificity. They function under mild reaction conditions (pH 6–8), are easy to use, and typically do not require cofactors for activity.

Under certain conditions, endopeptidases can also facilitate peptide synthesis through a mechanism called reverse hydrolysis. In environments with low water concentrations (e.g., in organic solvents) or in the presence of water-absorbing agents, endopeptidases can reverse their hydrolytic function. Instead of breaking peptide bonds, these enzymes catalyze the condensation of two amino acids or an amino acid with a short peptide to form a new peptide bond.

This dual functionality makes endopeptidases highly versatile and valuable in both biochemical research and industrial applications.

II. Classification

Proteases are further distinguished based on their cleavage sites, including:

- **Exopeptidases**: Exopeptidases act by removing the terminal amino acid or dipeptide from the N-terminal or C-terminal of an oligopeptide or protein:
    - **Aminopeptidase**: These enzymes cleave amino acids from the N-terminus (Taylor, 1993).
    - **Carboxypeptidase**: These enzymes remove amino acids from the C-terminus (Skidgel and Erdos, 2006).
- **Endopeptidases**: Endopeptidases are capable of hydrolyzing peptide bonds within the interior of a protein or oligopeptide.

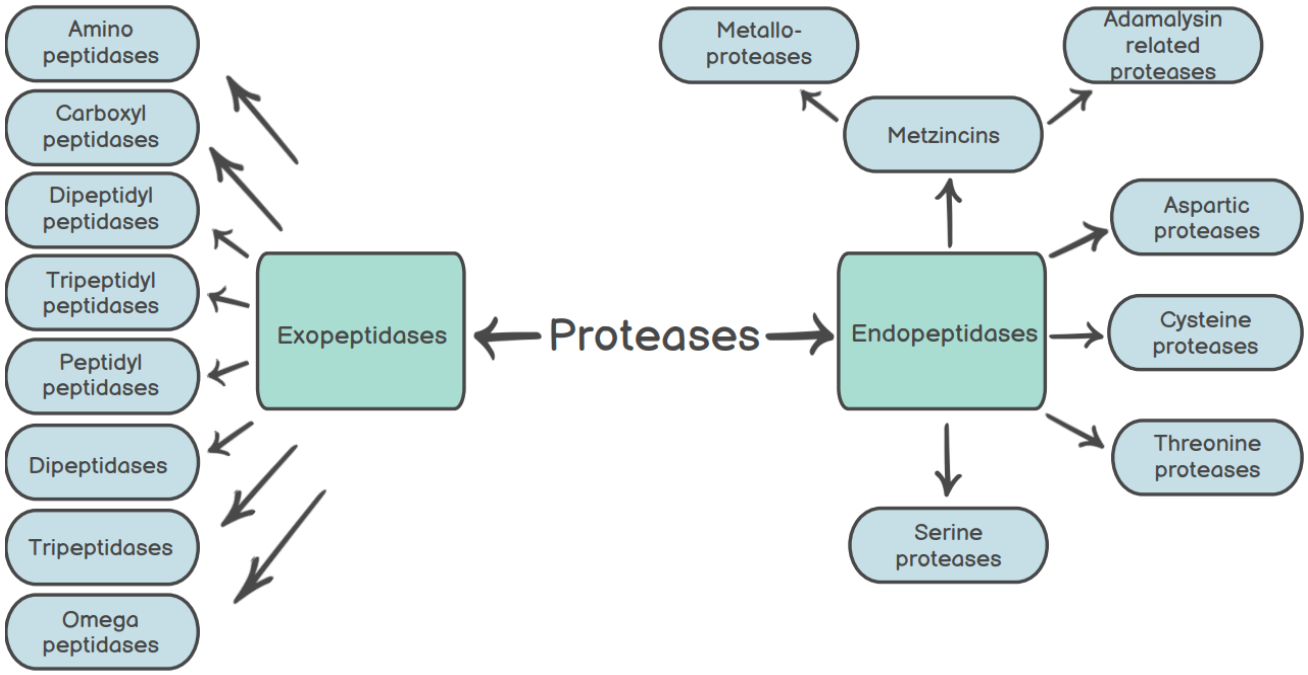


Figure 1. Classification of Protease Groups

Endopeptidases are classified based on their molecular mechanisms, which indirectly reflect structural similarities. In humans, there are five major groups:

- Aspartate proteases (a total of 21 types)
- Cysteine proteases (a total of 148 types)
- Metallo proteases (a total of 194 types)

- Serine proteases (a total of 175 types)
- Threonine proteases

	Based on their molecular mechanisms	Origin	Optimum pH	Molecular weight	Example
Endo-peptidases	Serine Proteases	• Microorganisms • Plants • Vertebrate animals	pH 9 to 13	18 to 35 kDa	Trypsin Chymotrypsin, subtilisin *Staphylococcus* V8 protease Papain Thermolysin Pepsin, etc.
	Cysteine Proteases	• Mammalian lysosomal cathepsins, the cytosolic calpains • Plants • Parasitic	Neutral pH (pH 7.0)	20 to 50 kDa	Papain, actinidin, bromelain Cathepsin B, etc.
	Aspartic Proteases		pH 3.4 to 5.5	30 to 45 kDa	Renin Penicillopepsin, Rhizopuspepsin, Endothiapepsin, etc.
	Metallo Proteases	Widely found in Bacteria, Fungi	pH 7.0 to 8.0	–	Thermolysin from *Bacillus thermoproteoliyticus,* etc.
	Threonine Proteases	Widely found in Bacteria and Eukaryotic cells	pH 6.5 to 8.5	19 to 700 kDa	HsIV protease, etc.

Table 1. Endo-peptidases classification

Additionally, peptidases can also be classified based on their optimal environmental conditions for catalytic activity, including acidophilic peptidases and alkaliphilic peptidases:

- **Alkaliphilic peptidases** (Serine peptidases, Metallo-peptidases, Cysteine peptidases), which remain stable at pH levels above 9.0, are extensively utilized in the food industry. These enzymes hydrolyze protein substrates such as casein, whey, soy, and meat, producing hydrolysates with high nutritional value.
- **Acidophilic peptidases** (Aspartic peptidases, Cysteine peptidases, Metallo-peptidases), by contrast, function optimally at moderately acidic pH levels ranging from 3.0 to 5.0. endopeptidases belonging to the Aspartic endopeptidase group are often associated with enzyme Pepsin. In the food industry, they are employed in cheese production, soy sauce fermentation, bread fermentation, and meat tenderization

III. Sources of Endo-peptidases enzyme

Plant species such as papaya and pineapple are abundant sources of endo-peptidases, which are essential enzymes in the food industry. Papain, chymopapain, glycyl endopeptidase, and Caricain are among the peptidases identified and described in papaya, primarily found in its latex. In pineapple, bromelain from the stem and fruit, as well as Comosain at lower levels, have been reported

- **Papain** is a protease enzyme derived from the fruit of *Carica papaya*, belonging to the cysteine protease group. It functions through the catalytic activity of cysteine at the enzyme's active site (AS).
- **Bromelain** is a natural protease enzyme, also classified as a cysteine protease, which works by catalyzing reactions involving cysteine at its active site (AS).

According to Rojas *et al.* (2018) protein hydrolysis enzyme extraction method (extraction solvent: using 0.1M phosphate buffer, pH 7, and a residual moisture content ratio of 20%), it has been confirmed that treatment through temperature does not affect the enzyme activity or the total protein content extracted. Additionally, green papaya peel and pineapple peel have been identified as the best residues for obtaining papain and bromelain.

Microbial proteases have become increasingly significant due to rising global demand, extending beyond the food industry to various industrial applications. While proteases from plant and

animal sources are abundant, microbial-derived enzymes now account for over 40% of global enzyme production, attributed to their high biodiversity and genetic modification flexibility. These advantages enhance their adaptability and efficiency in industrial processes. The following Table 2 presents a selection of endopeptidase enzymes

Food industry sector	Enzyme	Microorganisms
Baking	Endo-peptidases Exo-peptidases	*A. niger*
Dairy product	Serine-protease	*A. niger*
	Neutral proteases	*B. subtilis* *A. oryzae*
	Acid proteases	*Penicillium citrinum* *Rhizopus oryzae*
Meat product	Trypsin	*Bacillus subtilis* *Bacillus licheniformis* *Streptomyces griseus*
	Serine -protease	*Chryseobacterium sp*
	Acid protease Proteases	*Aspergillus oryzae* *B. licheniformis* *B. alcalophilus* *B. lentus* *Bacillus subtilis*

Table 2. Applications of Proteases in the Food Industry

A recent study conducted by Osorio et al. (2018) successfully expressed the endoprotease EP-HvB2, derived from barley, in wheat. This was further combined with the application of prolyl endopeptidase (PE) from either *Flavobacterium meningosepticum* or *Pyrococcus furiosus* to enhance gluten peptide degradation. The findings revealed a significant reduction in gluten content, ranging from 32% to 66%, demonstrating the potential of this approach in mitigating gluten-related disorders. Furthermore, the expression of glutenase in wheat grains markedly improved gluten digestibility, suggesting a promising strategy for individuals affected by Celiac Disease (CD). These results provide a foundation for genetic engineering-based interventions aimed at reducing gluten toxicity, thereby

offering a novel avenue for developing gluten-reduced wheat varieties suitable for gluten-sensitive populations

IV. Factors Affecting the Biological Activity

Peptidases catalyze the hydrolytic cleavage of peptide bonds in proteins. Some peptidases are involved in the digestion of intracellular and extracellular proteins, but most enzymes function in specialized biological processes such as zymogen activation, hormone release from precursors, protein cleavage, assembly, or receptor activation.

The bioactivity of peptidases can be influenced by factors such as temperature, pH, Enzyme concentration, and substrate concentration:

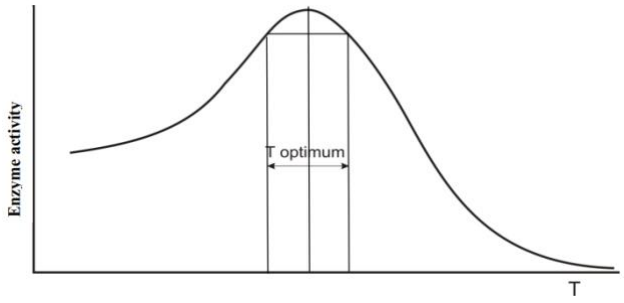
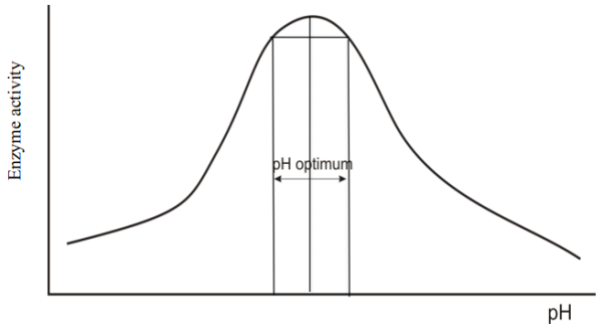
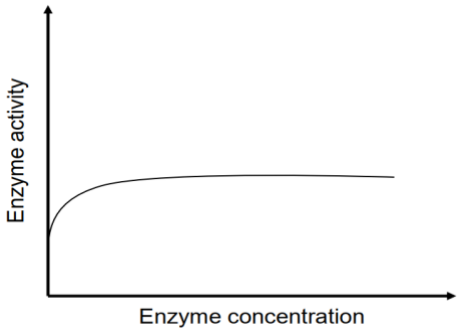
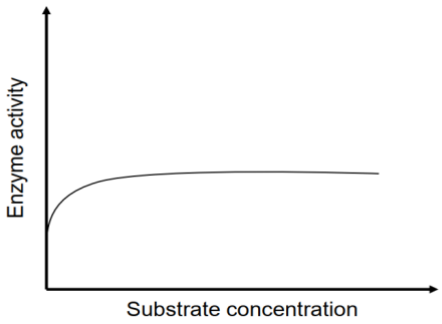
a. Temperature	
b. pH	
c. Enzyme concentration	
d. Substrate concentration	

Table 3. Factors influencing the biological activity of enzymes

In addition, peptidases are influenced by inhibitors. Some inhibitors perform functions beyond merely blocking the activity of peptidases, such as acting as growth factors, receptor clearance signals, or participating in cancer cell development.

Fundamentally, inhibitors employ various mechanisms to prevent substrates (S) from binding to the active site (AS) of peptidases. Enzyme-substrate reaction inhibitors are classified into three main groups: Competitive, Non-competitive, and Uncompetitive inhibitors

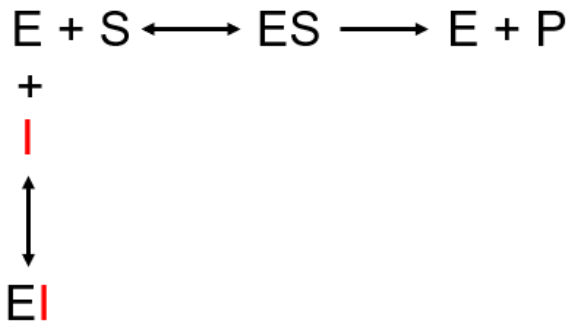
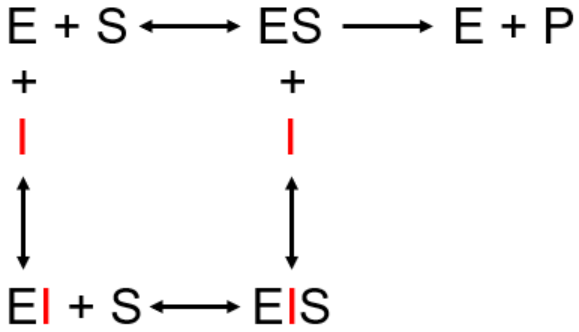
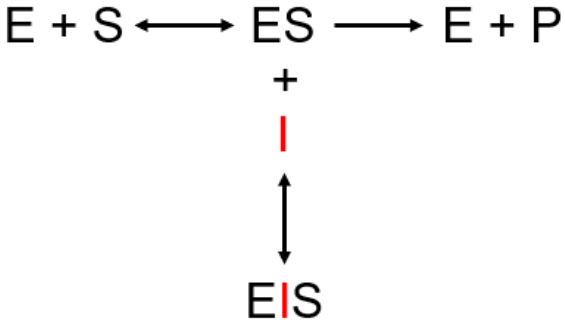
Enzyme inhibitors	Description
Competitive	
Non-competitive	
Uncompetitive	

Table 4. Enzyme inhibitors

*** Inhibitory Effects of Food Preservatives on Endopeptidases**

The alteration in the activity of pancreatic enzymes, specifically chymotrypsin and trypsin, under the influence of food preservatives such as sodium benzoate (E211), potassium sorbate (E202), and sorbic acid (E200), has been well-documented.

Elena N. Esimbekova *et al.* (2017) reported that food preservatives significantly inhibit trypsin and chymotrypsin activity, even at concentrations well below permissible limits in food products. Sodium benzoate and sorbic acid were found to inhibit chymotrypsin at concentrations 14 and 70 times lower than their acceptable daily intake (ADI), respectively. Additionally, prolonged exposure further reduces chymotrypsin activity in the pancreas. These findings suggest that consuming food preservatives may negatively impact protein digestion, particularly in individuals with a history of pancreatitis.

V. Application

Endopeptidases have been widely employed in traditional food industries, including processes such as cheese production, bread quality improvement, and protein preparation (Rao *et al.*, 1998). Due to their high enantioselectivity, these enzymes, similar to lipases, also find applications in the chemical industry, where they act as catalysts in various reactions involving non-natural substrates. In addition, in the food sector, peptides and amino acids are more reactive than whole proteins and can create specific flavors when interacting with other components in food, such as sugars and fats. The flavor of food can depend on the content of free amino acids and peptides. For example, vegetable hydrolysates derived from soy and wheat proteins are commonly used as flavor enhancers in soups and seasonings.

A. Cheese and dairy products

The development of cheese flavor and texture is largely controlled by complex biochemical reactions, wherein proteins, carbohydrates (particularly lactose), and lipids are broken down by the activity of bacteria and their endogenous enzymes, combined with the addition of exogenous enzymes. These processes are significantly influenced by environmental conditions within the cheese, such as moisture content, pH, and salt concentration.

Cheese flavor is primarily generated through the proteolysis and lipolysis processes, along with the formation of other compounds, including hydrocarbons, alcohols, aldehydes, ketones, esters, free fatty acids, and nitrogen- and sulfur-containing compounds. Strains of *Lactococcus lactis* and other lactic acid bacteria (LAB) are commonly employed as starter cultures in the production of aged cheeses, ensuring consistent acidification and flavor characteristics.

Traditional cheese production primarily relies on the use of rennin (chymosin) enzymes derived from the stomach of dairy cows. However, due to the lengthy maturation process, microbial Aspartic peptidases from filamentous fungi such as *Aspergillus oryzae*, *Penicillium oxalicum*, *Mucor bacilliformis*, *Mucor circinelloides*, *Mucor miehei*, *Mucor pusillus*, *Endothia parasitica*, *Rhizomucor pusillus*, and *Rhizopus oligosporus* have been introduced as effective substitutes.

Recently, the identification and manipulation of bacterial genes associated with protein degradation and sulfur metabolism have highlighted their correlation with flavor and aroma development in cheese. Casein proteolysis represents the principal and most complex pathway occurring during cheese ripening. This multi-step process is mediated by indigenous milk peptidases, rennin (chymosin), and the proteolytic enzymes of LAB. Recent studies have established that LAB contain at least 16 essential peptidases, which are pivotal for casein degradation and flavor formation. These peptidases are categorized into endopeptidases and exopeptidases based on their cleavage sites within the peptide substrate.

The four primary casein types in bovine milk include α_{s1}-, α_{s2}-, β-, and κ-casein. Rennin initiates milk coagulation by cleaving κ-casein. In the initial stage of maturation, α_{s1}-casein is degraded by rennin, and the extent of this breakdown directly influences the textural properties of the cheese. In some cases, peptidases derived from casein hydrolysis can preventing the development of bitterness in the final cheese product.

The proteolytic activity of LAB is not only essential in cheese production but also plays a crucial role in the manufacturing of other dairy-based products such as yogurt, kefir, and various fermented milk products. *Lactobacillus* species are widely employed as starter cultures due to their complex proteolytic systems, which enable them to break down casein into small peptides and free amino acids. Furthermore, some special peptidases (aminopeptidases) play a significant role in acidification and flavor development by removing amino acids from the amino terminus of various

peptides. This enzymatic activity ensures efficient protein hydrolysis and significantly improves the overall sensory attributes of dairy products.

B. The Meat Industry

Meat tenderness is one of the most critical attributes for assessing meat quality, influencing chewability and overall palatability. Tenderness results from the degradation of key structural proteins and the fragmentation of muscle fibers. Several enzymatic reactions occur post-mortem in meat muscles, primarily involving calpains and cathepsins, which hydrolyze myofibrillar proteins. Certain proteolytic enzymes derived from bacteria are utilized to cleave muscle proteins, including both myofibrillar and connective tissue proteins, thereby enhancing the tenderness, flavor, and overall palatability of meat products. A study conducted by Mageswari *et al.* (2016) demonstrated that a protease enzyme derived from a novel *Chryseobacterium* sp. increased the relative fragmentation ratio of myofibrils in meat by up to 221% compared to control samples. Based on differences in relative fragmentation, a cold-active serine protease from this novel *Chryseobacterium* sp. has shown significant potential for improving meat tenderness. Additionally, Arihara (2006) provided evidence that proteases from *Aspergillus oryzae* and bacterial sources such as *Bacillus licheniformis, B. alcalophilus, B. lentus,* and *B. subtilis* contribute to increased meat tenderness and enhanced flavor.

The breakdown of proteins into peptides and amino acids also contributes to the distinctive flavors of meat, enhancing its palatability. In addition to improving meat quality, proteolytic can extend meat shelf life by delaying lipid oxidation and inhibiting microbial growth due to the lower pH and the production of antimicrobial compounds.

Fermented meat products often have high sodium content. Given the increasing consumer demand for healthier food options, it is crucial to reformulate these products to reduce sodium levels and develop innovative, functional meat products that align with health-conscious trends.

C. The Baking Industry

In addition, protein degradation plays a significant role in the production of baked goods such as bread, cakes, crackers, and waffles. Wheat flour contains gluten, a protein fraction responsible for the unique characteristics of bakery doughs. The use of endo-peptidases can improve the rheological properties of dough and reduce mixing time due to their ability to cleave internal peptide bonds within gluten proteins. (Ter *et al*., 2023)

Lactic Acid bacteria (LAB) and their proteolytic enzymes modify the gluten network structure, improving the dough's ability to rise and resulting in bread with greater loaf volume achieved in a shorter fermentation time. Furthermore, proteolytic fermentation enhances the nutritional quality of whole-wheat baked products by slowing starch digestibility, thereby promoting a lower glycemic response. It also increases protein digestibility, regulates the concentration and bioavailability of bioactive compounds, and improves the bioavailability of essential minerals (Ter *et al*., 2023)

Moreover, peptidases derived from Lactic Acid bacteria (LAB) can degrade gluten proteins into smaller peptides with reduced immunogenic activity. This process offers an alternative for producing gluten-free baked goods suitable for individuals with gluten intolerance or celiac disease (CD). The breakdown of gluten also increases the concentration of free amino acids, enhancing product flavor and influencing the rheological quality of sourdough. (Ter *et al*., 2023)

Wheat flour is a crucial component in baking, directly influencing the freshness and quality of bread. Rao *et al.* (1998) demonstrated that the use of Endo- and Exo-proteinases from *Aspergillus oryzae* reduces mixing time and increases loaf volume. Additionally, bacterial proteases can enhance dough strength, further improving bread quality.

D. The Brewing Industry

In the brewing industry, lactic acid bacteria (LAB) are employed to regulate microbial populations, acidify wort, and produce traditional sour beers such as Lambic, Gueuze, Berliner Weisse, Flemish red ale, and kettle-soured beers. The proteolytic activity of LAB contributes to breaking down proteins in beer, thereby reducing haze formation - a major quality issue that can shorten shelf life and negatively impact flavor. While LAB is advantageous for sour beer production, it can spoil non-sour beers. Therefore, maintaining proper fermentation conditions is crucial to ensuring the quality of both sour and non-sour beers by preventing contamination with spoilage organisms

Another significant application of endo-peptidases in the brewing industry is the production of gluten-free beer for individuals with celiac disease (CD). CD is an autoimmune disorder triggered by the ingestion of alcohol-soluble gluten fractions-gliadins, hordeins, secalins, and avenins-found in wheat, barley, rye, and oats. A gluten-free diet is currently the only treatment for CD, which includes avoiding traditional beers. Although gluten-free beers are available in many countries, their quality

often falls short compared to conventional beers, and the range of products remains limited due to the market.

Research has demonstrated that brewing processes can help reduce gluten content in beer. The use of processing aids, typically added to stabilize beer by removing haze-active proteins, also helps lower gluten levels. This is because haze formation in beer is associated with proline-rich barley hordeins. Lopez and Edens (2005) demonstrated that the addition of Prolyl Endopeptidase (PE) from *Aspergillus niger*, an enzyme that degrades proline-rich gluten fractions, during wort production can facilitate gluten reduction in beers brewed from traditional raw materials. Furthermore, data indicate that under optimal conditions (50°C and pH 3.0–6.0), even a small amount of Prolyl Endopeptidase can physically stabilize beer without compromising foam stability.

Ghionno *et al*. (2016) reported that adding PE during fermentation effectively reduces gluten content without negatively affecting beer quality or sensory attributes. The combination of enzyme addition and optimizing brewing steps (such as vigorous wort boiling, lower temperatures, extended fermentation time, and filtration) further enhances gluten reduction. Importantly, PE can be incorporated into conventional brewing processes without requiring additional steps or significant costs, as it is commonly used as a stabilizer in breweries.

In addition, haze formation in beer is also a big challenge for researchers. During the brewing and winemaking processes, proteins and polyphenols are extracted from plant tissues. Depending on their concentration and nature, these compounds can interact to form haze. In beer, proteins are the primary contributors to haze, whereas in wine, polyphenols play a more significant role.

Protein-polyphenol aggregates in bottled beer create chill haze, with proline content in proteins being a key factor in haze formation. One approach to mitigate haze is the use of acidic proteolytic enzymes like papain. Although papain is cost-effective and reduces haze, its proteolytic activity can negatively impact beer foam stability. Furthermore, peptide bonds involving proline residues are difficult to cleave completely. Papain is inefficient at hydrolyzing proline-rich proteins, limiting its effectiveness in haze reduction.

Recent studies suggest that proline oligo-peptidase, a Serine peptidase, is a promising alternative for addressing these challenges. This enzyme can effectively reduce haze by targeting proline-rich peptide bonds, overcoming the limitations of conventional proteases like papain

The use of Proline endo-peptidases from *A. niger* at low dosages can effectively stabilize beer to the same extent as conventional Polyvinylpolypyrrolidone (PVPP) treatments. However, this enzymatic method results in a final product with higher polyphenol content and greater antioxidant capacity compared to PVPP-treated beer. Notably, the enzyme's impact on beer foam stability is minimal, making it a superior alternative to traditional stabilization methods.

E. Beverages and Fruit-juice Industry

Fruit-based beverages are complex colloidal systems that contain both suspended solids, such as proteins and pectic compounds, and soluble solids, such as sugars, organic acids, phenolic compounds, and vitamins. The combined use of pectinase (which helps degrade pectin) and protease enzymes has shown a synergistic effect, resulting in reduced turbidity and decreased potential for haze formation in fruit juice. A study by Landbo *et al*. (2006) demonstrated that the combination of Enzeco protease and gallic acid yielded remarkable results, as phenolic compounds exhibited significant potential in reducing oxidative reactions in the human body. However, the combination of pectinase and Enzeco protease had an unexpected negative effect by increasing turbidity in berry juices.

F. Fermentation in Food Using Proteases

Food fermentation using carbohydrate as raw material can be categorized into three primary types: lactic acid fermentation, alcoholic fermentation, and acetic acid fermentation. Lactic acid fermentation involves three metabolic pathways: glycolysis, proteolysis, and lipolysis. In the glycolysis stage, carbohydrates are broken down into pyruvate. During lipolysis, triacylglycerols are converted into free fatty acids and glycerol. In proteolytic fermentation, proteolytic microorganisms, primarily lactic acid bacteria (LAB), are employed to hydrolyze large protein molecules into smaller peptides or free amino acids. This enzymatic breakdown improves protein digestibility and enhances the bioactivity of the resulting products

During LAB metabolism, various organic acids are produced through proteolytic fermentation, including lactic acid, acetic acid, formic acid, succinic acid, citric acid, pyroglutamic acid, azelaic acid, caproic acid, linoleic acid, and lipoteichoic acid. These organic acids enhance flavor, preserve nutritional value, improve food stability, and offer several health benefits. Certain organic acids, such as propionic acid (E280) and benzoic acid (E210), function as preservatives, preventing food spoilage and extending shelf life. Citric acid, malic acid, fumaric acid, and tartaric acid can serve as acidulants

to regulate or maintain food pH levels. Additionally, ascorbic acid is a well-known antioxidant that stabilizes food by delaying oxidative deterioration of fats, oils, and other food components

Furthermore, organic acids produced during proteolytic fermentation have demonstrated anti-inflammatory, antimicrobial, immunomodulatory, anti-obesity, and growth-promoting properties. These bioactivities underscore the potential of LAB-derived organic acids for functional food applications

During proteolysis, certain LAB strains synthesize essential vitamins such as B2 (riboflavin), B6 (pyridoxine), B9 (folate), and K. This natural vitamin production offers a safe, cost-effective alternative to synthetic vitamins, whose use remains controversial. This process enhances the bioactive potential of foods and expands the application of LAB in nutritional fortification.

Exopolysaccharides (EPS) produced by LAB and fungi during proteolysis consist primarily of carbohydrates, proteins, DNA, and phospholipids. LAB strains produce a variety of exopolysaccharides, including dextran, inulin, and alternan. In the food industry, exopolysaccharides are used as additives, particularly in fermented dairy products such as yogurt and cheese, due to their thickening properties and ability to enhance texture.

Proteolytic fermentation by LAB also influences foaming properties, such as foam formation and stability-critical functional attributes of proteins. The food industry focuses on these foaming characteristics because they significantly affect mouthfeel and sensory properties. Common foods relying on protein foaming include beer, bread, and cakes. The foaming ability of proteins is influenced by various physicochemical properties, such as surface tension, hydrophobicity, electrostatic interactions, and molecular weight.

Incorporating proteolytic LAB into milk fermentation reduces pH, facilitating milk coagulation and improving gel structure, thus enhancing the production of diverse fermented dairy products. The protein coagulation process in milk is complex and irreversible like yoghurts, …

Proteolytic fermented foods exhibit promising bioactivities, including antioxidative, antihypertensive, antihyperglycemic, anti-inflammatory, antimicrobial, and anticancer properties. These functional foods offer natural alternatives to synthetic chemicals and pharmaceuticals.

Chen *et al*. (2023) demonstrated that exogenous proteases significantly improve protein utilization and fermentation quality in soy sauce production. Endogenous proteases secreted by *Aspergillus oryzae* play a crucial role in nitrogen utilization. Additionally, the supplementation of natural protease (pH = 7) during the "moromi" stage has been shown to accelerate protein degradation by enhancing endogenous enzyme activity and increasing peptide release. Exogenous protease also enhances flavor expression and fermentation quality by increasing Maillard peptides and the lactic-to-acetic acid ratio.

Hagen *et al.* (1996) compared the effects of proteinase enzymes produced by *Lactobacillus paracasei* and *Bacillus licheniformis* on dry sausage ripening. The results indicated that *L. paracasei*-derived proteinase increased starter bacteria levels, D-lactic acid concentration, and pH reduction rate, leading to significant changes in chromatographic profiles. Additionally, flavor, color, hardness, and other sensory attributes developed 14 days earlier than in control samples without enzyme supplementation.

Overall, proteolytic fermentation is a powerful tool for enhancing sensory perception in the food industry, providing desirable flavors, textures, and colors while mitigating sensory defects without the need for artificial additives

VI. The novelty in research on endopeptidases

A recent study by Claudia et al. (2018) employed advanced biotechnological tools and techniques, specifically the biolistic (gene gun) method, to insert genes encoding the endopeptidase enzymes EP-HvB2 and PE-FmPep into wheat genotypes. This genetic modification enabled the wheat plants to autonomously degrade gluten proteins—the primary trigger of Celiac disease (CD). CD is an autoimmune disorder induced by gluten, and currently, the only known treatment is adherence to a strict gluten-free diet. The findings of the study demonstrated that certain transgenic wheat lines exhibited up to a 72% reduction in immunogenic gluten proteins, as well as a 67% decrease in indigestible peptides. Notably, the introduced enzymes retained their activity under simulated gastrointestinal conditions and even after the baking process. This approach offers a promising strategy for managing Celiac disease through the consumption of regular wheat-based products, eliminating the need for complete gluten exclusion or reliance on costly dietary supplements.

VII. The Global Protease Enzyme Market

According to a Global Market Insights report, food enzymes (including proteases, lipases, carbohydrase, polymerases and nucleases, phytases, and catalases) were valued at USD 2 billion in 2016, and they are expected to reach USD 3.6 billion by 2024, recording a compound annual growth rate (CAGR) of 7.6% from 2016 to 2024. In the food industry, proteases are primarily used in the meat industry (30% for enhancing product quality and 30% for meat tenderizing). For enzymes in dairy production, rennet derived from microorganisms and animals accounts for 60%. Proteases represent 60% of the global industrial enzyme market, with microbial proteases accounting for 40% of this amount, representing a two-thirds share of commercial protease production in the total global market. The global demand for proteases is evenly divided between microbial and plant sources, with prices ranging from USD 10/kg to USD 30/kg for microbial protease and from USD 10/kg to USD 15/kg for plant-based protease.

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