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REVIEW ARTICLE

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Bioactive peptide generation using flavourzyme: functional potentials and emerging applications

Geração de peptídeos bioativos usando flavourzyme: potenciais funcionais e aplicações emergentes

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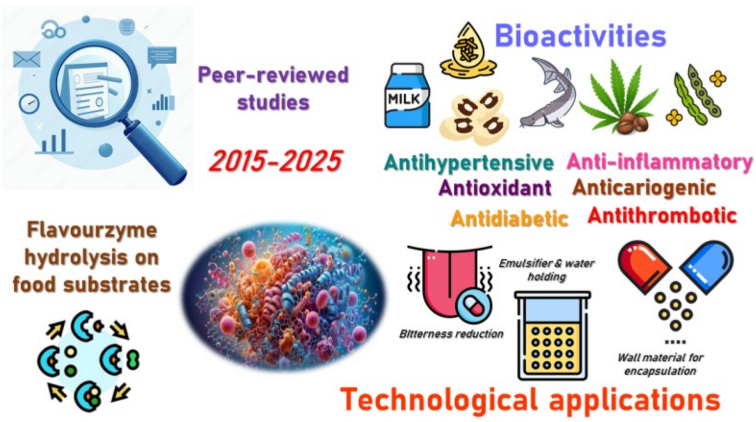
Abstract
Bioactive peptides derived from enzymatic hydrolysis have attracted considerable attention for their potential use in functional foods to prevent chronic diseases. This review aimed to analyze the functional potential of peptides generated by Flavourzyme-mediated hydrolysis, providing an overview of the scientific evidence on their enzymatic characteristics, substrate specificity, reported biological activities, and emerging applications in food systems. A narrative review approach was applied, focusing on peer-reviewed studies published between 2015 and 2025. Flavourzyme, a protease complex from *Aspergillus oryzae*, had endo- and exopeptidase activities that facilitate protein degradation and reduce peptide bitterness. The reported bioactivities of its hydrolysates include antioxidant, Angiotensin-Converting Enzyme (ACE) inhibition, dipeptidyl peptidase IV (DPP-IV) inhibition, and anti-inflammatory effects. These activities were observed across diverse protein sources, including dairy, legumes, cereals, and seafood by-products. The enzyme has also been used in sequential hydrolysis strategies with other proteases, improving bioactivity and sensory profiles. Additionally, Flavourzyme applications were identified in technological processes for texture improvement, emulsification, digestibility enhancement, and encapsulation. The results highlighted the enzyme's versatility and utility in designing functional ingredients with health-promoting effects. This work contributed to identifying the scope and relevance of Flavourzyme in advancing biotechnological strategies for functional food development.

Keywords: Hydrolysis. Proteases. Peptides. Bioactivity. Foodtech.

Resumo
Peptídeos bioativos derivados de hidrólise enzimática têm atraído considerável atenção por seu potencial de uso em alimentos funcionais para prevenir doenças crônicas. Esta revisão teve como objetivo analisar o potencial funcional de peptídeos gerados por hidrólise mediada por Flavourzyme, a fim de apresentar uma visão geral das evidências científicas sobre suas características enzimáticas, especificidade do substrato, atividades biológicas relatadas e aplicações emergentes em sistemas alimentares. Uma abordagem de revisão narrativa foi adotada, com foco em estudos revisados por pares publicados entre 2015 e 2025. Flavourzyme, um complexo de protease de *Aspergillus oryzae*, apresenta atividades de endo- e exopeptidase que facilitam a degradação de proteínas e reduzem o amargor dos peptídeos. As bioatividades relatadas de seus hidrolisados incluem efeitos antioxidantes, inibidores da enzima conversora de angiotensina (ECA), inibidores da dipeptidil peptidase IV (DPP-IV) e anti-inflamatórios. Essas atividades foram observadas em diversas fontes de proteína, como laticínios, leguminosas, cereais e subprodutos de frutos do mar. A enzima também tem sido usada em estratégias de hidrólise sequencial com outras proteases, o que tem melhorado a bioatividade e os perfis sensoriais. Além disso, foram identificadas aplicações da Flavourzyme em processos tecnológicos que envolvem melhoria de textura, emulsificação, aumento da digestibilidade e sistemas de encapsulamento. Os resultados destacaram a versatilidade e a utilidade da enzima no desenvolvimento de ingredientes funcionais com efeitos benéficos à saúde. Este trabalho contribuiu para a identificação do escopo e da relevância da Flavourzyme no avanço de estratégias biotecnológicas voltadas ao desenvolvimento de alimentos funcionais.

Palavras-chave: Hidrólise. Proteases. Peptídeos. Bioatividade. Tecnologia de alimentos.

Graphical Abstract



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ROYALDATASET

1. Introduction

Bioactive peptides derived from food proteins have attracted increasing attention due to their physiological effects and potential to prevent chronic diseases. These short sequences of amino acids exhibit a range of biological activities, including antioxidant, antihypertensive, antidiabetic, and anti-inflammatory effects, positioning them as valuable components in the development of functional foods and nutraceuticals (Ghulam et al., 2025; Intiquilla et al., 2019; Peighambardoust et al., 2021).

Advancements in protein hydrolysis technologies, particularly enzymatic processes that offer efficient, reproducible, and mild conditions compatible with food-grade applications, support the growing interest in these peptides (Helal et al., 2023; Mazorra-Manzano et al., 2018). Proteolytic enzymes are central to the production of bioactive peptides from diverse food matrices. Alcalase has been extensively studied for its broad substrate specificity and high hydrolytic efficiency, making it a preferred enzyme in research and industry (Ahmadifard et al., 2016; Rocha et al., 2015). Its application often yields hydrolysates with enhanced antioxidant and antihypertensive activities. However, the use of Alcalase is frequently associated with the generation of bitter peptides due to the release of hydrophobic amino acid residues (Hernandez & de Mejia, 2019; Mao et al., 2023), which may limit its utility in formulations where sensory properties are critical.

Flavourzyme, a protease preparation from *A. oryzae*, presents a distinct enzymatic profile, combining endo- and exopeptidase activities. This dual action facilitates the production of peptides with reduced bitterness by exopeptidase-mediated removal of terminal residues, thereby improving the sensory quality of protein hydrolysates (Saetang et al., 2025; Tang et al., 2018). Despite its promising characteristics, Flavourzyme remains underrepresented in the literature compared to Alcalase, possibly due to the facility and speed of Alcalase to produce low molecular peptides with a higher probability of exhibiting positive biological effects (Oliveira et al., 2024). The above highlights a significant gap in strategies that would enable greater application of Flavourzyme to obtain bioactive peptides. Available studies suggest that Flavourzyme can effectively hydrolyze a range of protein substrates and generate peptides with antioxidant and antihypertensive activities (F. B. Ahtesh et al., 2021; Alahmad et al., 2023), though it often yields lower degrees of hydrolysis and bioactivity than Alcalase under similar conditions (Jogi et al., 2024; Karimi et al., 2020).

The relative paucity of reviews explicitly focused on Flavourzyme underscores the need for a more detailed examination of its properties, applications, and potential in peptide-based food design (Alahmad et al., 2023; Bjørlie et al., 2024; Jogi et al., 2024). Addressing this gap is timely, given the increasing demand for functional food ingredients that offer health benefits and meet sensory expectations (Peighambardoust et al., 2021). A better understanding of Flavourzyme's enzymatic behavior, substrate interactions, and the bioactivities of resulting peptides may contribute to more refined strategies in food biotechnology (Rezvankhah et al., 2021a; Saetang et al., 2025).

In line with the above, this study aimed to analyze the functional potential of bioactive peptides generated through enzymatic hydrolysis with Flavourzyme by synthesizing current scientific evidence on their biochemical characteristics, substrate interactions, reported biological activities, and technological applications in food systems. The goal was to identify their role in developing functional foods with health-promoting properties.

2. Methodology

2.1. Search Strategy

A narrative synthesis was developed to collate evidence on Flavourzyme (protease from *A. oryzae*) in food-protein hydrolysis, associated bioactivities (antioxidant, ACE-inhibitory, DPP-IV, anti-inflammatory), and techno-sensory aspects (e.g., debittering, sequential hydrolysis) within a 2015–2025 window aligned with the manuscript's scope. Searches covered Web of Science Core Collection, Scopus, and PubMed/MEDLINE, combining controlled and free-text terms for the enzyme and outcomes (*e.g.*, "Flavourzyme", "*A. oryzae* protease", "bioactive peptide(s)", "antioxidant", "ACE inhibitory", "DPP-IV", "sequential hydrolysis", "bitterness/debittering").

2.2. Eligibility criteria

Eligible records were peer-reviewed articles (original research and reviews) published 2015–2025, in English, that used food-relevant matrices (*e.g.*, dairy, legumes, cereals, seafood by-products). They explicitly applied Flavourzyme alone or in sequence with other proteases, while reporting biochemical, bioactive, sensory, or techno-functional outcomes. Items excluded were conference abstracts, theses, patents, non-peer-reviewed sources, non-food applications, and reports lacking minimum methodological detail on hydrolysis conditions or outcome measurement.

2.3. Selection and appraisal

Records were screened by title/abstract and then full text against the stated criteria. Given the narrative design, no formal risk-of-bias instrument was applied; appraisal emphasized adequate reporting of enzyme identity and conditions, standardized *in vitro* assays (*e.g.*, DPPH/ABTS, ACE, DPP-IV), stated controls/replication, and consistency of findings across multiple sources before inclusion in the synthesis.

2.4. Data extraction and synthesis

From each included study, the following were charted: substrate/source, enzymatic strategy (Flavourzyme alone and sequential), key hydrolysis conditions when reported, and primary outcomes, enzyme features, and debittering; bioactivities (antioxidant, ACE-I, DPP-IV, anti-inflammatory); and emerging/technological applications. The synthesis maintains the 2015–2025 focus on supporting like-for-like comparisons across matrices and enzymatic strategies.

3. Flavourzyme: Origin, optimal conditions, and functional benefits

Flavourzyme is a proteolytic enzyme complex obtained from *A. oryzae*, composed of both endopeptidases and exopeptidases. This dual enzymatic nature facilitates cleavage of internal peptide bonds and terminal amino acids, thereby enabling efficient protein hydrolysis and a diverse peptide profile. Its enzymatic activity is optimized at approximately 50 °C and within a pH range of 5.5 to 7.0, conditions commonly encountered in food processing systems (Alahmad et al., 2023; D. Wang et al., 2018; Xia et al., 2023; H. Zhang et al., 2022). Deviations from this range, particularly in temperature, lead to rapid loss of activity due to protein denaturation (Rezvankhah et al., 2023).

The enzymatic versatility of *A. oryzae* has enabled widespread use of Flavourzyme in industrial hydrolysis processes to improve nutritional and sensory attributes (Kim et al., 2024; Merz et al., 2015). One of the most studied advantages of Flavourzyme is its ability to reduce bitterness in protein hydrolysates. This property is linked to its exopeptidase activity, which removes hydrophobic amino acid residues typically responsible for off-flavors. In contrast to Alcalase, which predominantly generates low-molecular-weight peptides with bitter profiles, Flavourzyme promotes the formation of less bitter, higher molecular weight fractions (Foong et al., 2015; Karami et al., 2023; Rezvankhah et al., 2021a). Sequential hydrolysis using Alcalase followed by Flavourzyme has demonstrated effective debittering outcomes, improving palatability while preserving functional properties (Matos et al., 2025; O'Sullivan et al., 2017). This approach has proven effective in protein systems derived from lentils, maize, and seafood waste (Chiang et al., 2019; Suaste et al., 2023).

The peptide profiles generated by Flavourzyme exhibit a broad range of molecular weights, often skewed toward higher-mass peptides in the 1-10 kDa range, associated with favorable bioactivities such as ACE inhibition and antioxidative potential (F. B. Ahtesh et al., 2021; Naji & Çam, 2025; Shi et al., 2017). These hydrolysates also show increased levels of free amino acids, reaching up to 84.2% in some substrates, contributing to improved solubility and nutritional value (O'Sullivan et al., 2017; Waglay & Karboune, 2016). Optimization of hydrolysis conditions can modulate peptide size distribution, enhancing functional properties while reducing undesirable sensory effects (Chiang et al., 2019; Yao et al., 2021).

Bioactive properties reported for Flavourzyme-derived peptides include antioxidant activity and ACE-inhibitory effects, both of which are relevant for mitigating oxidative stress and regulating blood pressure (F. B. Ahtesh et al., 2021; Sukkhown et al., 2021; Tejasari et al., 2021). The enzymatic mechanism favors the production of medium- to large-sized peptides while removing terminal bitter residues. It supports its application in functional food formulations designed for health promotion and improved organoleptic quality.

Given these multifaceted advantages, several studies have explored Flavourzyme's enzymatic parameters and functional contributions across various food matrices (F. B. Ahtesh et al., 2021; Chiang et al., 2019; Foong et al., 2015; Karami et al., 2023; Matos et al., 2025; O'Sullivan et al., 2017; Tejasari et al., 2021). **Fig. 1** presents a consolidated overview of its origin, optimal operational conditions, and key functional roles, offering a practical reference for its application in food hydrolysis processes (Alahmad et al., 2023; Merz et al., 2015; Rezvankhah et al., 2023; D. Wang et al., 2018; Xia et al., 2023; Yao et al., 2021; Y. Zhang et al., 2021).

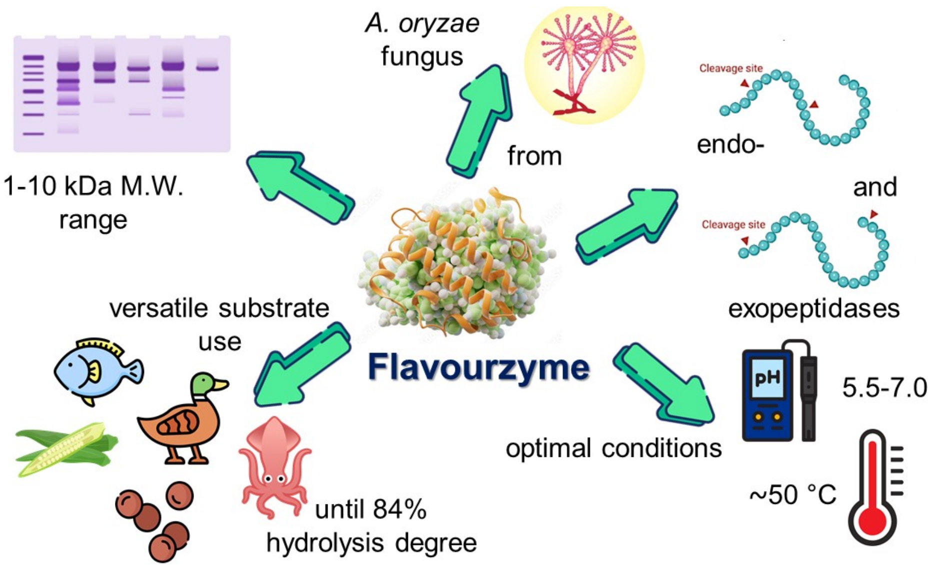

Fig. 1 Overview of Flavourzyme origin, functions, and working parameters.

4. Bioactive peptides generated by Flavourzyme: Sources, activities, and applications

The hydrolysis of food proteins using Flavourzyme has generated peptides with diverse biological activities. Its dual endo- and exopeptidase action enables the cleavage of both internal and terminal peptide bonds, facilitating the release of multifunctional peptides from various protein substrates, including dairy, legumes, cereals, fish, and meat. Whey and skim milk proteins have been effectively hydrolyzed with Flavourzyme, yielding peptides with ACE inhibitory activity and high degrees of hydrolysis (F. Ahtesh et al., 2016).

In plant sources, cowpea protein hydrolysates reached hydrolysis degrees close to 59% (Gerónimo-Alonso et al., 2025), while flaxseed protein hydrolysates exceeded 70% when treated with Flavourzyme (Karamać et al., 2016). Rice bran, mung bean, and adzuki bean proteins have also been explored, producing peptide fractions with DPP-IV inhibitory potential and antioxidant capacity (Karami et al., 2023; Uraipong & Zhao, 2016).

Flavourzyme-derived peptides have been evaluated for several bioactivities. Antioxidant effects, assessed through assays such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), have been observed in hydrolysates from sturgeon skin, duck meat, squid by-products, and corn-soy blends (Gui et al., 2022; Mirzaee et al., 2024; Sukkhown et al., 2021; L.-S. Wang et al., 2015). These peptides typically have low molecular weights (<1 kDa), which favors higher reactivity with free radicals (Hwang et al., 2016).

The use of Flavourzyme in combination with other proteases, particularly Alcalase, has enhanced the antioxidant activity of hydrolysates by generating a broader range of peptides (Hunsakul et al., 2022; Rezvankhah et al., 2021a). Regarding antihypertensive potential, peptides obtained from green lentil and dairy proteins using Flavourzyme alone or sequentially with Alcalase demonstrated ACE-inhibitory activity, with lower IC_{50} values than single-enzyme treatments (Rezvankhah et al., 2021a). Hydrophobic residues, particularly at the C-terminus, have been linked to decreased potency (Kasiwut et al., 2019; Zheng et al., 2020). Also, fermentation combined with Flavourzyme treatment accelerated peptide release and improved the functional profile (F. B. Ahtesh et al., 2016).

Peptides with DPP-IV inhibitory properties, which modulate glucose metabolism and delay incretin degradation, have also been generated using Flavourzyme. Studies using chickpea, rice, and soy proteins revealed that these hydrolysates enhanced Glucagon-like peptide-1 (GLP-1) secretion and resisted enzymatic degradation *in vitro* and *in vivo*, indicating their relevance in type 2 diabetes management (Ishikawa et al., 2015; Lammi et al., 2018; Mora-Melgem et al., 2023). Molecular docking analyses further supported the binding affinity of these peptides to DPP-IV active sites (M. Zhang et al., 2022).

Anti-inflammatory effects have been reported in hydrolysates from hempseed, chicken feather meal, and lentils. These peptides modulated the production of inflammatory mediators, including nitric oxide (NO), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and IL-10, in macrophage or monocyte models (Cheng et al., 2023; Cruz-Chamorro et al., 2022; Inkanuwat et al., 2019). The specific sequences responsible for these effects were often released more efficiently under sequential hydrolysis strategies.

Sequential hydrolysis combining Flavourzyme with enzymes such as Alcalase or Trypsin has emerged as a strategy to enhance both peptide yield and bioactivity. In lentil, oat, and pea proteins, this approach increased the degree of hydrolysis and produced peptides with improved ACE inhibition, antioxidant potential, and sensory properties (Asen & Aluko, 2022; Ji et al., 2024; Rezvankhah et al., 2021a). These combinations complement the substrate specificity of each enzyme and improve the techno-functional properties of the resulting hydrolysates.

Flavourzyme enables the targeted generation of peptides with diverse health-promoting functions across various protein sources. Its use offers promising applications in developing functional foods to manage hypertension, oxidative stress, and metabolic disorders. The variety of bioactive peptides produced through Flavourzyme hydrolysis depends on several factors, including the type of protein substrate, the hydrolysis conditions, and whether the enzyme is applied alone or in combination with others.

Table 1 summarizes studies published between 2015 and 2025, outlining the protein sources, intended bioactivities, enzymatic strategies employed, and principal references. This overview highlights the versatility and potential of Flavourzyme in advancing functional food innovations.

Table 1 Functional activities of bioactive peptides generated by Flavourzyme from different protein sources.

Enzymatic strategy	Protein source	Bioactive activities	References
Flavourzyme alone	Duck meat	Antioxidant (DPPH, metal chelating)	L.-S. Wang et al. (2015)
Flavourzyme alone	Rice bran	ACE-inhibitory, DPP-IV inhibition	Uraipong & Zhao (2016)
Flavourzyme alone	Chicken feather meal	Anti-inflammatory (NO inhibition)	Inkanuwat et al. (2019)
Flavourzyme alone	Sturgeon skin	Antioxidant	Gui et al. (2022)
Flavourzyme alone	Mung bean, adzuki bean	DPP-IV inhibitory	Karami et al. (2023)
Flavourzyme alone	Chickpea	DPP-IV inhibition, glucose modulation	Mora-Melgem et al. (2023)
Flavourzyme alone	Corn and soy blend	Antioxidant	Mirzaee et al. (2024)
Flavourzyme alone	Cowpea	Antithrombotic, anticariogenic	Gerónimo-Alonso et al. (2025)
Flavourzyme alone	Casein from cow and water buffalo	ACE and α-amylase inhibition	Naji & Çam (2025)
Flavourzyme alone or with fermentation	Whey and skim milk	ACE-inhibitory, antioxidant	F. Ahtesh et al. (2016); Eberhardt et al. (2021)
Flavourzyme + Alcalase	Oat globulin	ACE-inhibitory	Zheng et al. (2020)
Flavourzyme + Alcalase	Lentils	ACE-inhibitory, antioxidant, anti-inflammatory	Rezvankhah et al. (2021b)
Flavourzyme + Alcalase	Pea protein	Antioxidant, sensory improvement	Asen & Aluko (2022)
Flavourzyme + Alcalase	Hempseed	Anti-inflammatory (cytokine modulation)	Cruz-Chamorro et al. (2022)
Flavourzyme + Alcalase	Rapeseed	Antidiabetic (T2DM model)	Ji et al. (2024)

DPPH (2,2-Diphenyl-1-picrylhydrazyl); ACE (Angiotensin-Converting Enzyme); DPP-IV (Dipeptidyl Peptidase IV); NO (Nitric Oxide); T2DM (Type 2 Diabetes mellitus)

5. Emerging technological applications of Flavourzyme in functional foods

The integration of Flavourzyme into functional food development extends beyond its proteolytic efficiency, its bitterness-reducing properties, and even the allergenicity of food proteins. Recent approaches have explored its role in enhancing the biofunctionality and processing performance of food products by modulating solubility, emulsification, and foaming capacities through controlled hydrolysis. These techno-functional improvements are relevant to the design of novel textures and delivery systems for dairy analogs, plant-based beverages, and protein-enriched formulations.

Flavourzyme has generated hydrolysates with enhanced emulsifying and water-holding capacities in plant protein applications, which are critical for meat analogs and dairy substitutes. For example, hydrolysates from lentils and peas treated with Flavourzyme displayed improved oil absorption and dispersibility, facilitating incorporation into low-fat products and protein drinks (Asen & Aluko, 2022; Rezvankhah et al., 2021b). Its combination with Alcalase in sequential treatments further enhances these properties by modifying peptide size and charge distribution.

In dairy, Flavourzyme-modified casein and whey proteins have been incorporated into yogurt and cheese formulations to improve digestibility and texture, and to reduce the antigenicity of β-lactoglobulin, a common allergen in the dairy industry (Sun et al., 2025). These applications align with the growing demand for hypoallergenic and easily digestible products for sensitive populations, such as infants and older adults. Furthermore, hydrolysis with Flavourzyme has been associated with lower antigenicity in some food proteins, offering potential for reduced allergenicity (Tejasari et al., 2021).

Microencapsulation systems also benefit from Flavourzyme-derived hydrolysates, which can act as wall materials or emulsifying agents in spray-dried or freeze-dried powders. Such applications enable the stabilization of bioactive compounds and probiotics in functional ingredients. Additionally, antioxidant peptides enhance stability in lipid-rich matrices, potentially extending shelf life in formulated products (Mirzaee et al., 2024).

Flavourzyme's versatility continues to be explored in novel fermentation and co-processing systems, where its integration into biotechnological platforms may yield multifunctional peptides with synergistic effects. Indeed, current research is focused on supporting this enzyme on inorganic materials to enable its reuse and improved applicability in scale-up systems, as well as on incorporating *in silico* tools, such as virtual screening, to save time during *in vitro* and *in vivo* bioactivity determination (Jiang et al., 2025; Zhao et al., 2025).

This opens pathways for developing tailored hydrolysates targeting specific health benefits, including glycemic regulation, muscle health, or cognitive support. These technological advances position Flavourzyme as a multifunctional tool in formulating next-generation functional foods, offering opportunities to enhance nutritional and structural aspects through protein engineering.

6. Concluding remarks and perspectives

This review synthesized current evidence on the use of Flavourzyme to generate bioactive peptides, focusing on its enzymatic characteristics, substrate versatility, and functional outcomes. Flavourzyme, derived from *A. oryzae*, combines endo- and exopeptidase activities, enabling effective protein hydrolysis and bitterness reduction. These enzymatic properties have been associated with the production of peptides with antioxidant, antihypertensive, DPP-IV-inhibitory, and anti-inflammatory activities. The reviewed literature reported applications across various protein sources, including dairy, legumes, cereals, and marine organisms, demonstrating Flavourzyme's adaptability and effectiveness under varied hydrolysis conditions.

Flavourzyme has been studied independently and in sequential hydrolysis strategies alongside other proteases, such as Alcalase or Trypsin, with evidence suggesting improved hydrolysate functionality in terms of peptide size distribution, solubility, and sensory properties. Beyond bioactivity, techno-functional attributes such as emulsifying capacity, protein digestibility, and reduced allergenicity were also enhanced, supporting its integration into functional food formulations. Additionally, advances in encapsulation and i*n vitro* digestion models indicated that peptides produced with Flavourzyme can remain stable and bioaccessible under gastrointestinal conditions.

Future research may benefit from refining enzymatic protocols through kinetic modeling, exploring underutilized protein substrates, and investigating synergistic effects in multi-enzyme systems. There is also a growing need to bridge *in vitro* findings with *in vivo* and clinical data to validate health-related claims and establish translational relevance. As interest in sustainable, health-oriented food design grows, Flavourzyme remains a promising tool for developing functional ingredients aligned with evolving nutritional demands and biotechnological innovations.

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Authors' Contributions

G.M.R.-S. and E.P.-E.: Conceptualization; J.G.P.-F. and Y.N.A.-V.: Data curation; E.C.-L. and R.P.-S.: Formal analysis; L.G.G.-O. and Y.N.A.-V.: Investigation; J.G.P.-F. and L.G.-C.: Methodology; J.G.P.-F.: Software; E.P.-E. and G.M.R.-S.: Supervision; L.G.G.-O. and R.P.-S.: Validation; E.P.-E. and E.C.-L.: Visualization; J.G.P.-F. and Y.N.A.-V.: Writing - original draft; E.P.-E. and L.G.-C.: Writing – review & editing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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