

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY

Website: <http://journal.hibiscuspublisher.com/index.php/JEBAT/index>



Bioactive Peptides Derived from Waste Valorization: Sources, Processing Technologies, Bioactivities, Industrial Applications, and Future Perspectives

Mohd Yunus Shukor^{1*}

¹Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia.

*Corresponding author:
Mohd Yunus Shukor,
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang,
Malaysia.
Email: mohdyunus@upm.edu.my

History

Received: 10th Oct 2025
Received in revised form: 25th Nov 2025
Accepted: 30th Dec 2025

Keywords

Bioactive peptides
Waste valorization
Food by-products
Enzymatic hydrolysis
Subcritical water

SDG Keywords

SDG 3 Good Health and Well-Being
SDG 9 Industry, Innovation, and Infrastructure
SDG 12 Responsible Consumption and Production

Abstract

A large quantity of by-products rich in proteins that are often underutilized is often produced from agricultural production, aquaculture, slaughtering and industrial food processing. These byproducts can be used to generate biologically active peptide sequences. The recovery of these peptides is an important circular-bioeconomy strategy where these low-value residues can be transformed into high value functional ingredients with nutritional, technological or therapeutic potential. In this review the valorization of agricultural residues, aquaculture by-products, animal-processing waste and industrial food-processing streams as sources of bioactive peptides are discussed. The review covers pretreatment, protein extraction, enzymatic hydrolysis, subcritical-water processing, ultrasound- and microwave-assisted technologies, ultrafiltration, chromatographic purification, peptide identification, biological activities, industrial utilization and barriers to commercialization. To achieve controlled peptide liberation several methods are often used, of which the first is enzymatic hydrolysis, while subcritical-water technologies is the second most often used strategy that can provide an enzyme-free alternative with the potential for fast conversion of proteinaceous side streams. Membrane fractionation and chromatography are key in the enrichment and identification of active fractions, of which these fractions contained peptides and hydrolysates that have shown antioxidant, angiotensin-converting-enzyme inhibitory, dipeptidyl-peptidase-IV inhibitory, antidiabetic, antimicrobial, anti-inflammatory, metal-chelating, and techno-functional effects. Important feedstocks include rice bran, rapeseed meal, soybean meal, wheat germ, fish skins and frames, slaughterhouse blood, bovine plasma, porcine plasma, and brewer's spent grain. These bioactive peptides have applications in nutraceuticals, functional foods, pharmaceuticals, cosmeceuticals and natural food preservation systems. Challenges for scaling up requires better process yield, bitterness control, scalable purification, demonstrated gastrointestinal stability and bioavailability, rigorous safety assessment, regulatory approval and integrated techno-economic and life-cycle evaluation.

INTRODUCTION

The food system industries generates large amounts of biological residues in the course of crop production, aquatic-food processing, slaughtering, brewing, dairy manufacture and other food-industry operations with a lot of these residues contain substantial amounts of proteins that are traditionally diverted to animal feed, rendering, composting, fertilizer manufacture, anaerobic digestion, or disposal. Although these routes of utilization can be economically and environmentally useful they do not always capture the full biochemical value of protein-rich

side streams. Currently, the circular-bioeconomy approaches have increasingly focused on the fractionation and conversion of these materials into higher value ingredients, e.g. bioactive peptides [1-3], where these bioactive peptides are short sequences of amino acids that may exhibit physiological or technological activities after being liberated from the parent proteins. Bioactive peptides are released by endogenous proteolysis, by fermentation of food, gastrointestinal digestion, by chemical hydrolysis, by controlled protease treatment or by hydrothermal processing. The activity of bioactive peptides is dependent not only on amino-acid composition but also on

sequence, molecular mass, charge, hydrophobicity, terminal residues, conformation, solubility, digestive stability and accessibility to biological targets. Waste valorization becomes particularly attractive as the main agricultural or food commodity has already paid a large part of the upstream production cost. Hence, protein containing side streams such as rice bran, rapeseed meal, soybean meal, brewer's spent grain, fish frames, fish skins, blood, plasma and whey related materials can be relatively cheap substrates for peptide generation.

These low-value materials can be converted into functional food ingredients, nutraceuticals, natural antioxidants, antihypertensive peptide preparations, glycemic-modulating ingredients, or pharmaceutical leads via appropriately designed extraction, hydrolysis, fractionation, and purification processes. The concept is very much aligned with the circular-bioeconomy principles. Instead of considering wastes as terminal wastes, a cascading valorization system considers them as secondary raw materials, and the ideal technology is to first recover the high-value components, and then lower value parts in order. For example, a side stream of fish processing could offer oil, collagen, gelatin, protein isolates, bioactive peptides, minerals and finally residual biomass for use in feed, fertilizer or energy recovery. Similarly brewer's spent grain can provide dietary fiber, phenolic compounds, protein isolates, peptide-rich hydrolysates and fermentation substrates [4,5]. Nevertheless, the processes of peptide extraction may not be sustainably valorized since these processes need energy intensive drying, use of solvents, extensive chromatography, repeated centrifugation and low product recovery can negate environmental benefits that can be gained from the use of waste feedstocks. The route for a commercial success endeavor require simultaneous optimization of peptide activity, recovery efficiency, energy demand, water consumption, sensorial quality, regulatory acceptability and product value.

Major Waste Streams for Bioactive Peptides

Agricultural Residues and By-products

In agricultural processing, often large quantities of protein-rich side effluents are produced with examples include effluents from the processing of cereal bran, wheat germ, oilseed meals, soybean meal, rapeseed meal and protein residues after starch or oil recovery. Plant-based by-products are of particular interest as they are produced in high volumes and are often associated with relatively stable supply chains. Specific example include rice bran, a by-product of rice milling and contains proteins which have the potential to produce antioxidant and ACE-inhibitory peptides. Enzymatic hydrolysis can liberate antihypertensive and antioxidant peptide fractions [6,7,9,10]. Thai jasmine rice bran was also studied as a source of ACE-inhibitory peptides [8].

Another major protein-rich side product of oil extraction is rapeseed meal where it major uses is as animal feed. The controlled protein hydrolysis can lead to metal-chelating and antioxidant peptide fractions that inhibit lipid oxidation [11]. Moreover, in silico proteomic analysis shows that rapeseed proteins contain a large number of encrypted sequences with potential biological activity [12]. Soybean meal is another important by-product of plant-protein where ACE inhibitory and antioxidant peptide systems from soybean meal proteins have been reported [13,14]. Wheat germ is also a promising peptide feedstock where subcritical-water extraction followed by fractionation has yielded antioxidant peptide fractions able to interact with intestinal cell models [15]. In general, the by-products of plants are valuable due to their diversity such a peptide preparations from plant residues that can have biological

functions as well as techno-functional properties (e.g., solubility, emulsification, foaming and water binding) [2]. This combined attribute may increase their value in formulated foods.

Analysis of the VOSviewer Network on Valorization of Protein-Rich By-products into Bioactive Peptides

A structured bibliometric search was carried out in the Scopus database to further characterize the research landscape on the valorization of protein-rich waste and by-product streams to produce bioactive peptides. The search strategy was designed to capture four interrelated dimensions of the field: bioactive peptides and protein hydrolysates; protein-rich wastes, residues and by-products; peptide recovery and processing technologies; and biological or industrial applications. The aim of this approach was to cover studies on agricultural residues, aquaculture and seafood by-products, slaughterhouse and animal processing wastes and side streams from industrial food processing. A main Scopus search was performed in the fields title, abstract and keywords (TITLE-ABS-KEY) using the following search string:

Scopus search string:

TITLE-ABS-KEY (("bioactive peptide" OR "bioactive protein hydrolysate" OR "protein hydrolysate" OR "functional peptide" OR "food-derived peptide" OR "waste-derived peptide") AND ("food waste" OR "food by-product" OR "food processing by-product" OR "agro-industrial waste" OR "agricultural residue" OR "agricultural by-product" OR "aquaculture by-product" OR "fish processing waste" OR "fish by-product" OR "seafood by-product" OR "slaughterhouse waste" OR "animal by-product" OR "animal processing waste" OR "protein-rich waste" OR "proteinaceous waste" OR "industrial food waste") AND (valorization OR valorisation OR hydrolysis OR "enzymatic hydrolysis" OR "protein extraction" OR fractionation OR ultrafiltration OR purification OR "subcritical water" OR "ultrasound-assisted" OR "microwave-assisted"))).

This search string is a good compromise between specificity and coverage. The first group relates to the identification of target products, particularly bioactive peptides and protein hydrolysates. The second group identifies the major waste and by-product streams relevant to the review and the third group captures technologies associated with protein recovery, peptide liberation, fractionation and purification. Thus the search does not restrict the literature to one feedstock, biological activity or processing technology. The following complementary search formulation can be used to extend the retrieval and to reduce the risk of missing relevant publications that may use other terms to describe food-industry side streams:

TITLE-ABS-KEY (("bioactive peptide" OR "protein hydrolysate" OR "functional peptide" OR "bioactive hydrolysate") AND (waste OR by-product OR byproduct OR residue OR "side-stream" OR sidestream) AND (food OR agriculture OR aquaculture OR fish OR seafood OR slaughterhouse OR animal OR "food processing"))

Where greater emphasis is required on the biological functionality of waste-derived peptides, a supplementary Scopus search may be conducted using: TITLE-ABS-KEY (("bioactive peptide" OR "protein hydrolysate") AND (waste OR by-product OR residue OR "side-stream") AND (antioxidant OR antimicrobial OR "ACE inhibitory" OR "angiotensin-converting enzyme inhibitory" OR "DPP-IV inhibitory" OR "dipeptidyl peptidase IV inhibitory" OR antidiabetic OR "anti-inflammatory" OR "metal chelating" OR "techno-functional"))

This supplementary search captures major activities discussed in the review, particularly antioxidant, antihypertensive,

antidiabetic, antimicrobial, anti-inflammatory, metal-chelating, and techno-functional properties. A further search can specifically examine the transition of the field toward sustainability, circular bioeconomy, scale-up, and commercialization:

TITLE-ABS-KEY (("bioactive peptide" OR "protein hydrolysate") AND ("food waste" OR "food by-product" OR "agro-industrial waste" OR "aquaculture by-product" OR "animal by-product") AND (valorization OR valorisation OR "circular economy" OR "circular bioeconomy" OR scalability OR commercialization OR commercialisation OR "techno-economic analysis" OR "life cycle assessment" OR nutraceutical OR "functional food" OR pharmaceutical OR cosmeceutical))

The core dataset is proposed as the first search string of the main VOSviewer analysis. This is because including all biological activities, individual feedstocks, applications and commercialization terms at once might be a too restrictive query and exclude relevant fundamental papers. After retrieving the eligible Scopus records, the bibliographic information is then analyzed using VOSviewer. These include author keywords, index keywords, titles, abstracts, publication years, citation information and bibliographic data. Keyword co-occurrence mapping can then be used to identify the main thematic structures within the literature.

The network (**Fig. 1**) shows the three related research areas connecting protein-rich waste streams with peptide production, biological activity and value-added utilization. Among the 88 retained terms, the dominant node is Antioxidant Activity (132 occurrences; total link strength [TLS] 707), followed by Molecular Weight (75; TLS 432), Peptide Fractions (63; TLS 329) and Fish Protein Hydrolysates (60; TLS 296). These central nodes suggest that the field is mainly organized around the conversion of residual proteins into defined peptide fractions and the determination of their biological functionality.

Cluster 1: Peptide Production, Fractionation, and Bioactivity

The output showing red cluster forms the technological heart of peptide valorization. Antioxidant activity is highly related to peptide fractions, molecular weight, proteases, alcalase, fish protein hydrolysates and process optimization. These relationships indicate a close relationship between biological activity and controlled protein hydrolysis and subsequent separation on the basis of peptide properties. Enzymatic hydrolysis is of particular interest because the specificity of proteases and the hydrolysis conditions affect the peptide sequence, molecular size and functionality [4,16,19,43].

The importance of enrichment after hydrolysis is reflected in the high ranking of terms such as molecular weight, low-molecular-weight peptides, ultrafiltration and peptide fractionation, indicates. The low-molecular-weight fractions are often depicted with strong antioxidant or enzyme-inhibitory activities, but the activity also depends on the amino-acid sequence and physicochemical properties [7,33,36]. The close association of ACE Inhibitory Activity with peptide fractions, fish protein hydrolysates and proteases further reflects continued interest in antihypertensive peptides [8–10,22,24]. This cluster, in general, describes a production pathway from controlled hydrolysis to fractionation and molecular characterization to biological validation.

Cluster 2: Waste Valorization, Sustainability and Circular Bioeconomy

The green cluster focuses on animal by-products, sustainability, circular economy, agricultural by-products, agro-industrial waste, nutraceuticals, bioavailability and mechanisms, and the structure is indicative of a conceptual change from waste disposal to functional ingredient recovery. Animal-processing residues like blood and plasma are increasingly considered as peptide precursors instead of low-value wastes [1,3,25,28–31], and likewise, the presence of agricultural and agro-industrial residues along with the concepts of sustainability expands the feedstock base. Peptides derived from rice bran, rapeseed meal, soybean meal and brewer's spent grain have antioxidant, ACE-inhibitory, metal-chelating and antidiabetic activities [6,11–14,32]. The increasing connectivity with the terms "bioavailability" and "mechanisms" suggests moving beyond simple in-vitro screening to a more mechanistic understanding of the process of digestion, absorption and molecular mechanisms [15,37] where this work is critical for the conversion of promising hydrolysates into credible nutraceutical and functional-food ingredients.

Cluster 3: Marine By-products and Value-added Applications

The blue cluster is dominated by terms such as Bioactive Compounds, Skin By-products, Collagen, Fish Head By-products, Seafood By-products, Gelatin, and Food Products. It appears that there is a strong relationship among collagen, gelatin, seafood residues, and skin by-products. This strong relationship establish fish-processing waste as a major historical foundation of peptide valorization. Current works have shown that fish skin, frames, and collagen hydrolysates have yielded antioxidant and other bioactive peptides [16–19,22]. Connections with pharmaceutical, cosmeceutical, nutritional, and food applications demonstrate progression toward product development where this connection supports an integrated marine biorefinery concept in which proteins, collagen, gelatin, lipids, and other valuable fractions are recovered from the same processing streams [23,42].

Overlay Visualization Analysis: Research Trends and Emerging Directions

The temporal map shows a shift from established research on hydrolysis and marine proteins to sustainability and circular utilization (**Fig. 2**). Previous blue-purple themes include ACE Inhibitory Activity, Fish Protein Hydrolysates, Flavourzyme, Pepsin, and Fish Oil, highlighting the historical focus on enzymatic production and characterization of marine hydrolysates [16–19,22]. Intermediate green themes include Molecular Weight, Ultrafiltration, Antioxidant Activity and Process Optimization, with increased focus on control of hydrolysis and enrichment of active fractions [36,43]. The more recent yellow topics are Agri-food Waste, Sustainability, Circular Economy, Agricultural By-products, Mechanisms, Bioavailability and Circular Bioeconomy. These developments point to a change in research toward sustainable feedstocks, physiological relevance and translational utilization [2,11–14,47]. Subcritical-water hydrolysis as a green processing technology can further support the transition, combining protein extraction and hydrolysis, and reducing the reliance on conventional enzymatic processing [20,21,35,38,44,45,48].

Emerging Research Trends

An emerging trend is the growing sustainability and resource efficiency in circular valorization of agricultural and food wastes that involves peptide recovery [2,11,47], whereas bioavailability and mechanism-guided peptide discovery focus on gastrointestinal stability, absorption and molecular interactions instead of activity screening only [15,37]. Meanwhile, precision fractionation is focused on low-molecular-weight and sequence-defined peptides to reach higher potency and reproducibility [7,36,49] and green hydrolysis technologies like subcritical water present an emerging alternative for rapid protein conversion and integrated biorefinery processing [20,21,44,45,48].

Future Research Opportunities

Studies in the future should integrate controlled hydrolysis, membrane fractionation, mass spectrometry, sequence identification, gastrointestinal models, and mechanistic validation with particular opportunities exist in agricultural side streams and scalable green processing. Whether laboratory-scale peptide valorization can develop into commercially viable circular-bioeconomy processes depends on works on techno-economic, safety, regulatory, and bioavailability studies [37,47,49].

Wastes from aquaculture and seafood-processing

In the aquaculture and seafood industries, the processing of seafood generates large quantities of skins, bones, scales, heads, viscera, trimmings, frames and residual muscle tissue, and fish processing by-products are particularly rich in collagen, gelatin, myofibrillar proteins, connective tissue proteins and endogenous enzymes. Of the wastes generated, fish skin has been widely studied with a well-characterized antioxidant peptide was obtained from hoki-skin gelatin hydrolysate. This shows that collagen-rich residues can be utilized as valuable peptide substrates instead of low-grade wastes [17]. Hydrolysis of Alaskan pollock skin with enzymes also produced antioxidant hydrolysates [16]. Salmon collagen hydrolysates also reported to produce antioxidant and anti-freezing peptides [18].

Another example are tilapia heads where the enzymatic hydrolysis and chromatographic purification identified antioxidant peptides from frame proteins [19]. Fish frames are of particular interest because they contain residual muscle proteins and collagenous tissues and are produced in large quantities during fileting. Another potential source is hydrothermal processing, and subcritical-water extraction and hydrolysis of cod frames without conventional proteases yielded soluble protein and peptide fractions [21]. Subcritical water has also been used to convert fish meal into low-molecular-weight peptides and amino acids [20] despite the processing atmosphere can affect the distribution of the products. Recent reviews have indicated that fish processing residues represent valuable peptide resources for food, nutraceutical and functional applications, but also highlight limitations related to batch variability, oxidation, sensory quality, purification costs and regulatory characterization [22, 24].

Animal Processing Wastes

Slaughterhouse streams produce wastes such as blood, plasma, red blood cell fractions, connective tissue, skins, bones and other protein-rich materials. Of these, blood is particularly attractive because of the high concentrations of hemoglobin, albumin, globulins, fibrinogen and other proteins. However, it is also highly perishable and can impose a high biological oxygen demand if discharged untreated. Peptides with antioxidant, ACE-inhibitory, DPP-IV-inhibitory and multifunctional activities can be obtained by hydrolysis of animal by-products [1,3,25] with one study shows that ACE-inhibitory peptides were produced by

targeted proteolysis and activity-guided screening of bovine blood proteins [27]. Bovine plasma protein hydrolysates is also another source of ACE-inhibitory peptide [26]. Antioxidant peptide preparations can be obtained from chicken blood corpuscles [30]. Hydrolysates of porcine plasma have been shown to have antioxidant activity [29]. Recent work has suggested porcine blood as a peptide-rich functional ingredient for further food or nutraceutical development [28]. Porcine blood meal was also studied as a source of antioxidant peptides that can bind to hemin [31]. Goat blood is another emerging substrate with Laosam et al. [37] reported hydrolysates with peptides having dual ACE- and DPP-IV-inhibitory properties and investigated their behavior in simulated gastrointestinal digestion. These examples show how slaughterhouse proteins can be upgraded from low-value materials to sophisticated bioactive products. However, there are specific controls needed for food-grade traceability, hygienic collection, pathogen control, consumer acceptance and regulatory classification.

Industrial Food-Processing Wastes

Wastes from the industrial food processing include spent grain from brewing, residues from the dairy industry, fractions of whey, fermentation biomass, streams from starch processing, and other secondary materials generated at large-scale manufacturing facilities. Of these, brewer's spent grain is one of the most thoroughly studied. It is the main solid by-product of brewing, composed of residual proteins entrapped in a lignocellulosic matrix, and protein can be extracted by alkaline treatment followed by hydrolysis with proteolytic enzymes [4]. The protein hydrolysates have been demonstrated to inhibit ACE, α -glucosidase and DPP-IV activities [32].

These hydrolysates can be relevant to antihypertensive and glycemic-management applications with one specific ACE inhibitory peptides have been isolated from BSG protein hydrolysates [33]. Subsequent work showed broader bioactivity in alkaline and enzyme extracted preparations [34]. Food products have also had peptide hydrolysates of BSG added to them with Bazsefidpar et al. [39] proved that the use of BSG protein hydrolysate as a functional ingredient in muffins allows the evaluation of biological activity together with sensory quality. This difference is important because commercial success will ultimately be driven by performance in real food matrices, not just biochemical assays. Another industrial substrate of interest is made up by protein streams related to whey, in which hydrolysis of whey protein by subcritical water can be used to generate smaller peptides components without conventional enzymatic treatment [35].

Upstream Processing and Pretreatment

Stabilization

A major challenge to fresh biological residues is they are prone to rapid deterioration due to the presence of endogenous enzymes, microbial growth, lipid oxidation and high moisture content. Rapid stabilization of fish residues, blood, wet brewer's spent grain and dairy streams is therefore necessary with refrigeration and freezing are common approaches but energy intensive. Spray drying and freeze drying increase the cost of processing but drying can improve the storage stability. Wet processing at the production site may be more efficient than drying and subsequent rehydration, where possible. Defatting may be necessary for lipid-rich materials. Oxidized lipids can produce off-flavors and reactive carbonyl compounds that react with proteins and peptides. For the marine side streams stabilization against oxidation is of particular importance.

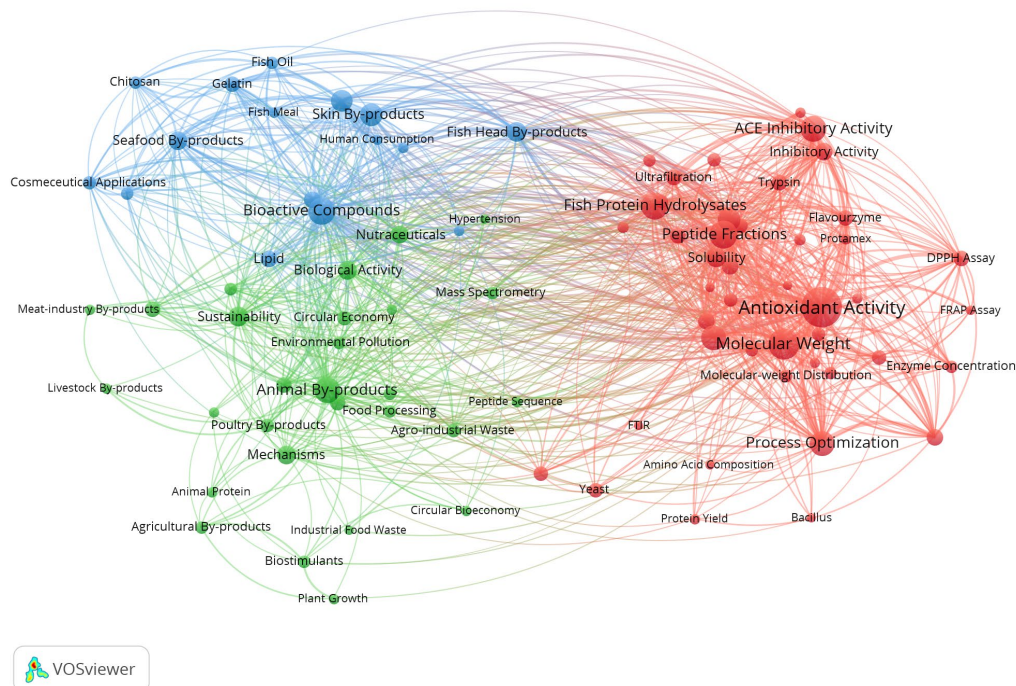


Fig. 1. Network visualization of protein-rich by-product valorization into bioactive peptides where the node size represents keyword occurrence, while connecting lines indicate the strength of keyword relationships. Cluster colours distinguish peptide production and bioactivity, sustainable waste valorization, and marine-derived product development, demonstrating strong integration between processing technologies, biological functions, feedstocks, and applications.

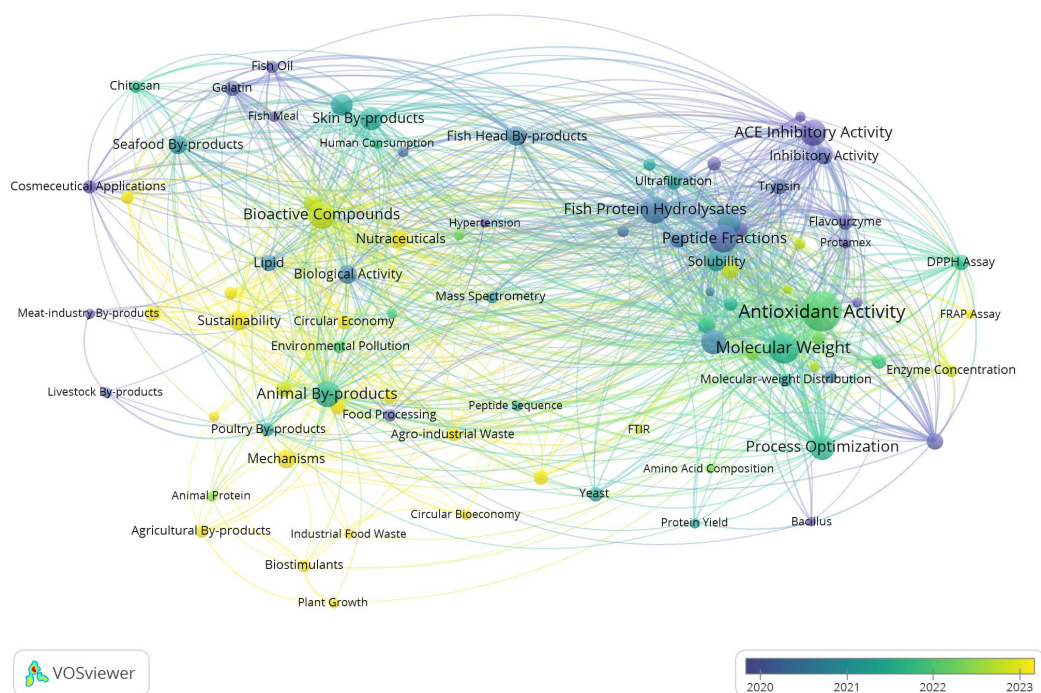


Fig. 2. Overlay visualization illustrates the temporal progression of bioactive-peptide valorization research. Blue-purple nodes represent earlier emphasis on enzymatic hydrolysis and marine protein sources, green nodes indicate subsequent molecular characterization and process optimization, while yellow nodes highlight emerging sustainability, circular-economy, agricultural-waste, bioavailability, and mechanistic themes driving future research and translation.

Treatment Chemical

Chemical treatment by alkaline extraction is a common method for plant by-products, which include brewer's spent grain and oilseed meals. Increasing pH improves protein solubility by altering the charge of the protein and disrupting intermolecular interactions, and proteins can be extracted and then isolated by isoelectric precipitation. Celus et al. [4] demonstrated the enzymatic hydrolysis of BSG proteins after protein recovery, and the subsequent fractionation work indicated that the processing history has a strong influence on the characteristics of the hydrolysate [36]. A big issue with alkaline treatment is it can damage chemically the peptides. pH and temperature that are too high may favor racemization, deamidation, lysinoalanine formation or destruction of sensitive amino acids. Hence mild conditions are preferable. Acid treatment can be used to adjust pH, demineralize or process collagen, but prolonged contact can change proteins and require additional neutralization.

Physical Pretreatment

In order to enhance the accessibility of enzymes, the particle size can be reduced, or the protein structure can be altered using methods such as grinding, homogenization, milling, high pressure processing, ultrasound, extrusion and microwave treatment. The physical reduction in size is especially important for plant residues because proteins can be physically entrapped within fiber-rich structures. BSG disruption of the lignocellulosic matrix enhances protein recovery while ultrasound can cause cavitation, microstreaming and localized shear which improve mass transfer. On the other hand, microwave treatment can cause rapid volumetric heating and protein unfolding. Such technologies could improve the hydrolysis efficiency, but comparisons with conventional thermal controls are required to distinguish true process-intensification effects from simple temperature effects.

Extraction and Hydrolysis Method

Hydrolysis by Enzymes

Enzymatic hydrolysis is the most common method for food-grade bioactive peptide production. A major enzyme used are proteases that work under relatively mild conditions and allow partial control of cleavage specificity. Commonly used enzymes are alcalase, trypsin, pepsin, papain, bromelain, Flavourzyme, Protamex, Neutrase, chymotrypsin and a number of microbial protease preparations. The hydrolysis efficiency is affected by enzyme specificity, enzyme to substrate ratio, substrate concentration, pH, temperature, reaction time, mixing conditions, pretreatment history and targeted degree of hydrolysis [10].

Degree of hydrolysis can be expressed as:

$$DH (\%) = (h / h_{tot}) \times 100$$

where h represents the number of hydrolyzed peptide bonds and h_{tot} is the total number of peptide bonds available in the substrate.

A major issue is higher degree of hydrolysis may not necessarily correspond to greater bioactivity. Progressive proteolysis may initially release active peptides but subsequently cleave them into fragments with lower activity. Thus, optimization should target biological function rather than simply maximal peptide-bond cleavage. Examples of successful enzymatic hydrolysis include antioxidant peptides from tilapia frames [19], antioxidant preparations from Alaska pollock skin [16], ACE-inhibitory peptides from rice bran [10], antioxidant soybean-meal peptides [14], and multifunctional peptides derived from animal blood proteins [27, 37].

Hydrolysis in subcritical water

Subcritical water forms when liquid water is maintained at a temperature above ambient pressure but below the critical temperature for vaporization, and under these conditions its ionic product and its dielectric constant are changed drastically. Subcritical water can act as both the reaction medium and the hydrolytic agent. Subcritical-water hydrolysis is an emerging method for obtaining bioactive peptides according to Rivas-Vela et al. [44], and Álvarez-Viñas et al. [45] pointed out its potential for incorporation into agro-food-waste biorefineries. A major advantages of this approach are reduced reliance on commercial enzymes, and a shorter processing time. In addition it is suitable for wet feedstocks, scarce or no use of organic solvents, concurrent protein extraction and hydrolysis and potential for continuous operation.

Subcritical water has been successfully used to process cod frames for the production of protein-rich extracts [21] and Barea et al. [20] have demonstrated the production of small peptides and amino acids from fish meal. Subcritical-water extraction was also used for the processing of wheat-germ protein to obtain antioxidant peptide fractions, which were tested with Caco-2 intestinal cell models [15]. Also, whey protein and pea protein have been studied under hydrothermal conditions [35, 38]. An important drawbacks of subcritical water are it hydrolysis is nonselective, and a possible destruction of amino acids at high processing severity, production of Maillard reactions, a need for high-pressure equipment requirements and possible generation of undesirable thermal products.

Ultrasound and microwave assisted processing

Another technology is the use of ultrasound and microwave technologies, which are generally considered as tools for process intensification rather than as standalone peptide production approaches. Ultrasound may enhance solvent penetration, tissue disruption and enzyme access through the process of cavitation that can expose buried regions of the protein and improve mass transfer. Microwave treatment causes rapid heating and may result in unfolding of protein structures and therefore increased susceptibility to proteases. But over-heating can denature enzymes or cause unwanted reactions. Industrial adoption requires considerations of energy efficiency, reactor geometry, penetration depth, acoustic-field uniformity, maintenance, and throughput.

Isolation and Purification

Ultrafiltration

A key technology for the industrial fractionation of peptide hydrolysates is ultrafiltration where complex hydrolysates can be separated into broad fractions according to size using membranes with molecular weight cut offs of 10, 5, 3 or 1 kDa. The target outputs are lower molecular weight fractions that tend to have a higher biological activity, as smaller peptides may have better binding ability to the enzyme targets and also better absorption ability in the gastrointestinal tract. However, peptide activity is not only defined by molecular size, but also by amino-acid sequence, charge, hydrophobicity and structural conformation. The main advantages of membrane processing are continuous operation, moderate processing temperatures, relatively low solvent consumption, scalability, compatibility with diafiltration and the possibility to retain and recycle enzymes. The main disadvantages of ultrafiltration include membrane fouling, concentration polarization, non-ideal molecular weight selectivity, peptide adsorption, cleaning requirements and potential loss of valuable sequences.

Gel Filtration Chromatography

Gel filtration or size-exclusion chromatography is sometimes called gel permeation chromatography. Gel filtration separates peptides according to their hydrodynamic volume. It is useful for activity-guided fractionation, it is often used after the ultrafiltration step as a polishing step. Its disadvantages are low throughput as sample processing volume is often less than 5% of the column volume, sample dilution, relatively limited resolution and high solvent consumption in industrial scale.

Reverse-Phase HPLC

Reverse-phase HPLC is one of the most important chromatographic techniques for the separation of individual bioactive peptide. The main factor affecting separation is the peptide hydrophobicity. RP-HPLC has been employed for purification of peptides from fish gelatin, rice bran, BSG and other protein hydrolysates. Fractions obtained after chromatographic separation can be tested for biological activity and analyzed by mass spectrometry. However, a main drawback is the total preparative RP-HPLC purification for production of nutraceutical or functional-foods can be economically unrealistic. Therefore, commercial products are more likely to be standardized peptide-rich fractions rather than purified sequences.

Major Biological Activities

Antioxidant activity

Food spoilage and in many biological disorders are caused by oxidative stress. Peptide antioxidants may act by donating hydrogen, electron transfer, radical scavenging, chelation of transition metals, inhibition of lipid peroxidation, or modulation of cellular antioxidant pathways. The amino acids histidine, tyrosine, tryptophan, cysteine, methionine, phenylalanine, leucine and proline in bioactive peptides can be involved in the antioxidant behavior but this activity is dependent upon on the position in the sequence. Mendis et al. [17] showed antioxidant activity for a peptide purified from hoki-skin gelatin hydrolysate. Antioxidant peptides were isolated from proteins of tilapia-frame by Fan et al. [19]. Antioxidant and anti-freezing peptides from salmon collagen hydrolysate were also reported by Wu et al. [18].

Plant sources are too important. Beaubier et al. [11] used rapeseed meal protein hydrolysates to identify peptides with metal chelating properties that could restrict lipid oxidation. Peptides from rice-bran and soybean-meal were also reported to possess antioxidant properties [6,14]. An important interpretation of antioxidant activity of bioactive peptides needs caution because assays such as DPPH, ABTS, FRAP and ORAC measure different reaction mechanisms. Hence, any claims for physiological antioxidant benefit can only be substantiated after cell and animal studies are completed.

ACE Inhibition and Antihypertensive Effects

Blood-pressure regulation is controlled by angiotensin-converting enzyme (ACE) through the catalysis of angiotensin II synthesis and degradation of bradykinin. Therefore, ACE-inhibitory bioactive peptides have attracted great attention as antihypertensive ingredients derived from food. In this connection rice-bran proteins have been well studied where Wang et al. [9], Suwannapan et al. [8], Zhang et al. [10] and Scarabattoli et al. [7] reported ACE-inhibitory peptide preparations from rice-bran protein.

Another major source is BSG, of which Connolly et al. [32] demonstrated ACE inhibitory activity in BSG hydrolysates and ACE-inhibitory peptides were subsequently isolated from a BSG protein isolate by Connolly et al. [33]. ACE-inhibitory sequences may be released from animal blood proteins through the hydrolysis of bovine plasma protein [26,27].

Antidiabetic Activity

Glycemic regulation can be affected by food-derived peptides inhibiting DPP-IV, α -glucosidase or α -amylase. Inhibition of DPP-IV is of particular interest because this enzyme degrades incretin hormones that are involved in insulin secretion. The DPP-IV and α -glucosidase inhibitory activity of BSG protein hydrolysates as well as the ACE inhibition was demonstrated by Connolly et al. [32]. Follow-up work on peptides derived from BSG also showed potential modulation of glycemic response [40]. Similarly, antidiabetic relevant activity was shown with BSG hydrolysates incorporated in muffins [39]. Hydrolysates of goat blood have been recently reported to contain peptides with dual ACE- and DPP-IV-inhibitory activity [37], suggesting possible cardiometabolic applications.

Antimicrobial Effect

Certain food-derived peptides exhibit interaction with microbial cell membranes or intracellular targets leading to cellular growth inhibition. Antimicrobial peptides are typically positively charged and amphiphilic, with hydrophobic residues that promote binding to negatively charged microbial surfaces. Antimicrobial activity is often reported in peptide literature but its translation into foods is not straightforward. This is because salts, fats, proteins, pH and food microstructure can greatly reduce activity relative to laboratory buffers. The most promising and sustainable sources of antimicrobial and multifunctional peptides are fish-processing by-products [23, 42].

Activity of Anti-inflammatory

Anti-inflammatory effects of peptide hydrolysates may be exerted their through modulation of oxidative stress, NF- κ B signaling, production of cytokines and related molecular mechanisms. The evidence is still not as extensive as for antioxidant or ACE inhibitory activity and many reports are based on cell based models. However, as a multifunctional substance, the antioxidant and anti-inflammatory effects of peptide hydrolysates may be overlapped. As pointed out by the reviews of animal and plant by-products [1-3], the anti-inflammatory activity is an emerging field in the future research of peptide valorization.

Industrial Applications

Nutraceuticals

One of the most accessible routes of commercialization is the use of nutraceuticals, since peptide-rich hydrolysates can be prepared as powders, capsules, sachets, beverages or medical-nutrition products. ACE-inhibitory rice-bran, BSG and blood-derived preparations are of particular interest for cardiovascular health applications. Antioxidant hydrolysates of fish skin, fish frames, soybean meal, rapeseed meal and animal plasma could be developed as functional ingredients related to oxidative stress. Robust chemical standardization is required for commercialization. Important parameters are molecular-weight distribution of the peptides, degree of hydrolysis, free amino-acid content, marker peptides and residual parent proteins, sodium content, moisture content, microbial specifications and biological potency.

Functional Food

Food applications provide a pragmatic approach to incorporate waste-derived peptides into daily diets. Brewer's spent grain (BSG) protein hydrolysate was tested as a functional ingredient in muffins, showing that peptide bioactivity can be incorporated in a conventional food matrix [39]. Other possible delivery forms include bakery products, sports drinks, protein drinks, soups, snack bars, dairy and dairy alternative products, nutritional supplements, fermented foods, and specialized clinical foods. Sensory compatibility matters. Strong bitterness or fishy, metallic or oxidized notes may limit inclusion levels.

Medicinal Products

In principle, purified bioactive peptides can be pharmaceutical leads if they exhibit sufficiently potent and selective biological activity. ACE- or DPP-IV-inhibitory activity sequences are obvious candidates, but pharmaceutical development requires evidence far beyond that required for food ingredients. Studies required include pharmacokinetics, toxicology, dose response relationships, mechanism of action, reproducibility of manufacturing, stability, clinical efficacy and regulatory approval. Even if eventual pharmaceutical manufacture depends on synthetic peptide production rather than continued extraction from variable waste materials, waste-derived discovery can identify useful sequences.

Cosmeceuticals

Marine collagen and gelatin hydrolysates are particularly interesting for cosmeceuticals because they can provide antioxidant, moisturizing, film-forming, anti-aging or matrix support functions. Fish skin and collagen derived peptides from hoki, salmon, seabass and related species are attractive nonmammalian sources [17, 18, 41]. However, the extrapolation of in vitro antioxidant assays to the topical efficacy should not be made. Evaluation of skin penetration, formulation stability, irritation potential and activity in appropriate dermal models is needed.

Challenges and Limitations

Yield Optimization

Maximizing protein recovery does not necessarily maximize peptide bioactivity. Aggressive extraction conditions may improve yield but chemically alter proteins. Similarly, prolonged hydrolysis may generate more soluble nitrogen while destroying key active sequences.

Optimization should therefore integrate multiple objectives:

Optimal Process = f(yield, bioactivity, sensory quality, cost, purity, sustainability)

Response-surface methodology, factorial design, proteomics, kinetic modeling, and computational peptide prediction can improve process optimization.

Bitterness and Flavor

Bitter taste is one of the most common sensory defects of protein hydrolysates. Proteolysis exposes hydrophobic residues which may interact with bitter-taste receptors. Fish hydrolysates may also develop marine or oxidized flavors. Metallic or heme-related notes may be present in blood-derived products.

Several complementary strategies can improve the bitterness and sensory acceptability of protein hydrolysates. Limited release of hydrophobic peptide sequences is achieved by controlled proteolysis and removal of terminal amino acids involved in bitter taste by exopeptidases. Membrane fractionation may be used to separate the low molecular weight bitter peptides from the more desirable fractions. Selective adsorption may be used to remove the hydrophobic peptides with activated carbon, resins or other suitable materials. Encapsulation, which involves the physical separation of bitter compounds from taste receptors, and the protection of sensitive peptides during processing or digestion, is another useful approach. Flavor-masking agents may further improve product palatability, e.g., sweeteners, acids, salts, and compatible natural flavors.

Fermentation can alter the peptide profiles through microbial proteolysis and activity of aminopeptidase, thus decreasing bitterness and possibly generating additional bioactive compounds. Finally, the addition of hydrolysates in highly flavored foods such as cocoa beverages, savory soups, fermented products or spiced formulations can mask undesirable sensory notes. The best approach should maintain bioactivity and nutritional value and consumer acceptance. One of the big challenges is that often the hydrophobic peptides are responsible for the desired ACE-inhibitory or antioxidant activity. Thus debittering can unintentionally remove functional components too.

Scalability in Industry

Many peptide studies involve laboratory scale procedures including freeze drying, multiple centrifugations, multiple chromatography steps, dialysis and analytical HPLC. Commercial production should be based on scalable and resource-efficient operations that preserve peptide activity while reducing the costs of manufacturing. Continuous reactors can offer stable operating conditions, consistent product quality and higher throughput than batch systems. Tangential-flow membrane systems are especially suitable for the concentration of hydrolysates, separation of peptide fractions and reduction of membrane fouling. By providing adequate mixing, high-solids hydrolysis can increase reactor productivity and decrease the amount of water that must be removed downstream.

Spray drying offers a rapid, economical method of converting liquid hydrolysates into stable powders for storage, shipping and formulation. Enzyme recycling and membrane enzyme reactors can reduce protease consumption by retaining the enzymes within the processing system but allowing smaller peptides to pass through the membrane. At larger scale, a simplified chromatographic polishing step is generally more practical than extensive purification of individual peptides. Finally, processing wet residues directly at their source can reduce spoilage, transportation costs, energy-consuming drying and deterioration of proteins before hydrolysis. Subcritical-water technologies may offer opportunities for continuous processing, although pressure-rated equipment adds to capital cost [44, 45].

Table 1. Representative waste-derived peptide preparations, recovery strategies, bioactivities, and applications.

Waste Source	Specific Waste Matrix	Isolated Peptide / Fraction	Extraction / Isolation Method	Key Biological Activity / Characteristic	Target Application / Use	Ref
Agricultural	Rapeseed meal	Chelating peptide fraction	Enzymatic hydrolysis and peptide fractionation	Metal chelation; inhibition of lipid oxidation	Natural antioxidant	[11]
Agricultural	Rapeseed meal	Low-MW antioxidant peptide pool	Hydrolysis and chromatographic separation	Antioxidant activity	Functional food ingredient	[11]
Agricultural	Rapeseed meal	Predicted bioactive peptide set	In silico proteomic profiling and simulated proteolysis	Predicted multifunctional activity	Peptide discovery platform	[12]
Agricultural	Plant-origin by-products	Low-MW peptide hydrolysates	Protein extraction, enzymatic hydrolysis, ultrafiltration	Antioxidant/functional activity	Functional foods	[2]
Agricultural	Rice bran	ACE-inhibitory peptide fraction	Enzymatic hydrolysis and chromatographic purification	ACE inhibition	Antihypertensive nutraceutical	[9]
Agricultural	Rice bran	Antioxidant peptide fraction	Enzymatic hydrolysis	Radical-scavenging activity	Natural antioxidant	[9]
Agricultural	Rice bran	ACE-inhibitory peptide fraction I	Hydrolysis, fractionation and peptide identification	ACE inhibition	Cardiovascular functional food	[10]
Agricultural	Rice bran	ACE-inhibitory peptide fraction II	Hydrolysis, fractionation and peptide identification	ACE inhibition	Nutraceutical	[10]
Agricultural	Rice bran	Low-MW ACE-inhibitory hydrolysate	Enzymatic hydrolysis and molecular-weight fractionation	ACE inhibition	Antihypertensive ingredient	[7]
Agricultural	Rice bran	Low-MW antioxidant hydrolysate	Enzymatic hydrolysis and fractionation	Antioxidant activity	Functional food	[7]
Agricultural	Rice bran	Antioxidant peptide hydrolysate	Bacillus licheniformis protease treatment	Radical scavenging	Nutraceutical	[6]
Agricultural	Rice bran	α -Chymotrypsin peptide hydrolysate	α -Chymotrypsin hydrolysis	Antioxidant activity	Food antioxidant	[6]
Agricultural	Jasmine rice bran	ACE-inhibitory peptide fraction	Enzymatic hydrolysis and peptide purification	ACE inhibition	Functional food	[8]
Agricultural	Soybean meal	ACE-inhibitory peptide fraction	Hydrolysis, screening and activity validation	ACE inhibition	Antihypertensive nutraceutical	[13]
Agricultural	Soybean meal	Antioxidant peptide fraction	Enzymatic hydrolysis and peptide screening	Cellular antioxidant activity	Cytoprotective functional ingredient	[14]
Aquaculture	Fish meal	Small-peptide fraction	Subcritical-water hydrolysis	Generation of low-MW peptides	Nutraceutical ingredient	[20]
Aquaculture	Tilapia frame	Antioxidant peptide fraction I	Enzymatic hydrolysis and chromatography	Radical scavenging	Functional food	[19]
Aquaculture	Tilapia frame	Antioxidant peptide fraction II	Enzymatic hydrolysis and chromatography	Antioxidant activity	Nutraceutical	[19]
Aquaculture	Alaska pollack skin	Antioxidant hydrolysate	Protease hydrolysis	Radical-scavenging activity	Natural antioxidant	[16]
Aquaculture	Cod frames	Bioactive protein/peptide extract	Subcritical-water extraction and hydrolysis	Low-MW peptide generation	Fish biorefinery ingredient	[21]
Aquaculture	Hoki fish skin gelatin	Purified antioxidant peptide	Enzymatic hydrolysis and chromatography	Antioxidant; lipid-peroxidation inhibition	Nutraceutical/cosmeceutical	[17]
Aquaculture	Seabass skin gelatin	Antioxidant peptide fraction I	Enzymatic hydrolysis, gel filtration and RP-HPLC	Radical scavenging	Functional food	[41]
Aquaculture	Seabass skin gelatin	Antioxidant peptide fraction II	Enzymatic hydrolysis and peptide purification	Antioxidant activity	Nutraceutical	[41]
Aquaculture	Seabass skin gelatin	Antioxidant peptide fraction III	Enzymatic hydrolysis and LC-based identification	Antioxidant activity	Cosmeceutical ingredient	[41]
Aquaculture	Salmon collagen	Antioxidant peptide fraction	Bacterial extracellular protease hydrolysis	Radical scavenging	Food antioxidant	[18]
Aquaculture	Salmon collagen	Anti-freezing peptide fraction	Protease hydrolysis and peptide isolation	Cryoprotective property	Frozen-food applications	[18]
Aquaculture	Fish skin hydrolysates	Bioactive low-MW peptide fractions	Enzymatic hydrolysis and purification	Multifunctional biological activity	Nutraceutical	[22]
Aquaculture	Fish-processing residues	Antioxidant peptide-rich fraction	Enzymatic hydrolysis and membrane separation	Antioxidant activity	Functional foods	[23]
Aquaculture	Fish-processing residues	Functional peptide hydrolysate	Protein recovery and enzymatic hydrolysis	Bioactive and techno-functional properties	Food applications	[42]
Aquaculture	Threadfin bream frame	Enzyme-generated peptide hydrolysate	Controlled enzymatic hydrolysis	Antioxidant/functional activity	Food and nutraceutical use	[43]
Animal	Bovine blood	ACE-inhibitory peptide fraction	Proteolysis prediction, synthesis and activity testing	ACE inhibition	Antihypertensive lead	[27]
Animal	Bovine plasma	ACE-inhibitory peptide	Enzymatic hydrolysis and purification	ACE inhibition	Cardiovascular nutraceutical	[26]
Animal	Chicken blood corpuscles	Antioxidative peptide	Enzymatic hydrolysis and peptide isolation	Cellular antioxidant activity	Functional food	[30]

Table 1. Representative waste-derived peptide preparations, recovery strategies, bioactivities, and applications-Continue.

Animal	Porcine plasma	Antioxidant hydrolysate	Enzymatic hydrolysis	Radical-scavenging activity	Natural antioxidant	[29]
Animal	Porcine blood	Peptide-rich functional hydrolysate	Sequential enzymatic hydrolysis	High peptide content	Food fortification	[28]
Animal	Porcine blood meal	Hemin-interacting antioxidant peptide fraction	Hydrolysis, chromatography, spectroscopy and docking	Antioxidant/hemin interaction	Nutraceutical	[31]
Animal	Porcine blood meal	Low-MW antioxidant peptide fraction	Enzymatic hydrolysis and purification	Antioxidant activity	Functional ingredient	[31]
Animal	Goat blood	Dual ACE/DPP-IV inhibitory hydrolysate	Enzymatic hydrolysis, fractionation and GI digestion	ACE and DPP-IV inhibition	Cardiometabolic nutraceutical	[37]
Animal	Meat by-products	Bioactive peptide hydrolysates	Proteolytic hydrolysis	Antioxidant/ACE inhibitory	Functional foods	[25]
Animal	Meat-processing by-products	Multifunctional peptide fractions	Protein recovery and hydrolysis	Antioxidant and metabolic bioactivities	Nutraceuticals	[3]
Industrial food processing	Brewer's spent grain	Protein hydrolysate	Protein extraction and enzymatic hydrolysis	Technofunctional properties	Functional food ingredient	[4]
Industrial food processing	Brewer's spent grain	Fractionated peptide hydrolysate	Hydrolysis followed by fractionation	Improved peptide functionality	Food formulation	[36]
Industrial food processing	Brewer's spent grain	ACE-inhibitory hydrolysate	Enzymatic hydrolysis	ACE inhibition	Antihypertensive food	[32]
Industrial food processing	Brewer's spent grain	DPP-IV inhibitory hydrolysate	Enzymatic hydrolysis	DPP-IV inhibition	Antidiabetic functional food	[32]
Industrial food processing	Brewer's spent grain	α -Glucosidase inhibitory hydrolysate	Enzymatic hydrolysis	α -Glucosidase inhibition	Glycemic-management food	[32]
Industrial food processing	Brewer's spent grain	ACE-inhibitory peptide fraction	Protein isolation, hydrolysis and peptide purification	ACE inhibition	Nutraceutical	[33]
Industrial food processing	Brewer's spent grain	Bioactive alkaline-extracted hydrolysate	Alkaline extraction and enzyme treatment	Multifunctional bioactivity	Functional food	[34]
Industrial food processing	Brewer's spent grain	Glycemic-modulating peptide fraction	Protein isolation and peptide purification	Potential modulation of glycemic response	Antidiabetic food	[40]
Industrial food processing	Brewer's spent grain	Functional muffin peptide ingredient	Enzymatic hydrolysis and bakery incorporation	Antioxidant/antidiabetic activity	Functional bakery product	[39]
Industrial food processing	Whey protein stream	Low-MW subcritical-water hydrolysate	Subcritical-water hydrolysis	Protein conversion to smaller peptides	Functional protein ingredient	[35]

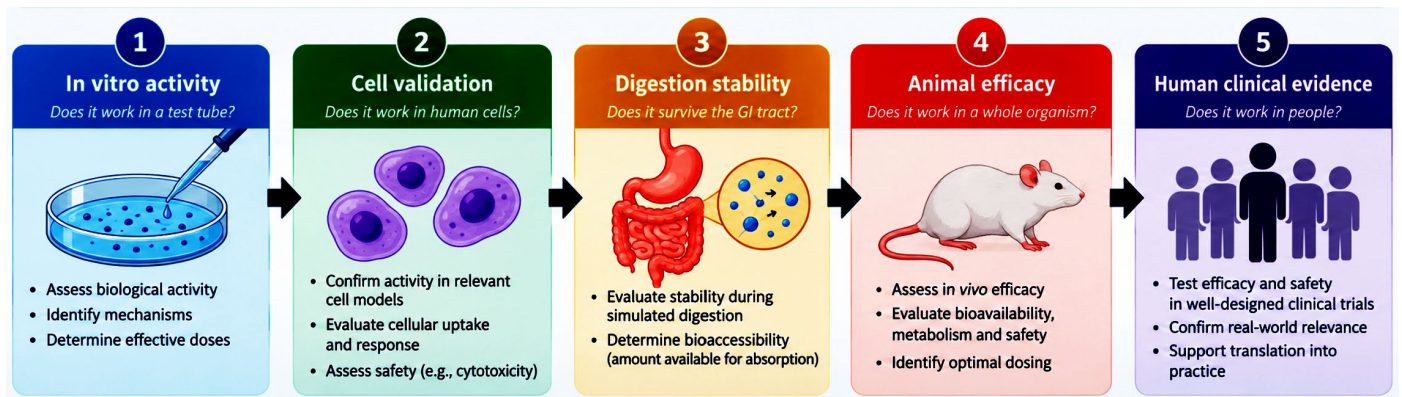


Fig. 3. A 5-step horizontal flow chart showing steps of testing: In vitro activity, Cell validation, Digestion stability, Animal efficacy, and Human clinical evidence, prompt. *ChatGPT*, GPT-4o version, OpenAI, 15 May 2026, chatgpt.com.

Stability in the gastrointestinal tract and bioavailability

Peptides intended for oral administration must be resistant or positively modified during gastrointestinal digestion. Biological availability is influenced by gastric pepsin, pancreatic enzymes, intestinal brush-border peptidases, epithelial transport, hepatic metabolism, and renal clearance. The wheat-germ study of Zhang et al. [15] is significant in that peptide fractions were tested for intestinal transport in Caco-2 cells. Laosam et al. [37] also studied the peptides of goat blood after gastrointestinal digestion. Future work should use an integrated translational framework to establish whether the peptide activity observed *in vitro* is preserved under physiological conditions. Standardized simulated-digestion protocols are required to evaluate peptide stability and transformation during gastrointestinal digestion. Then LC-MS-based sequence tracking should reveal peptides that are intact or produce active metabolites. Models of epithelial transport may help to clarify intestinal permeability and mechanisms of transport, and animal pharmacokinetic studies should be used to assess absorption, distribution, metabolism and elimination. Systemic bioavailability can be directly demonstrated by measuring concentrations of peptides and metabolites in plasma. Ultimately, well-designed, controlled human intervention studies are needed to confirm efficacy, effective dosage, safety, and clinically significant health outcomes.

Safety

Protein-rich waste streams may contain biological, chemical and processing-related hazards that must be controlled during valorization. Microbial contamination can occur in wet substances such as blood, fish residues and dairy by-products if they are poorly handled, slow to stabilize or stored improperly. Other possible risks include heavy metals, residues of veterinary drugs, allergens, mycotoxins, products of lipid oxidation and biogenic amines. Processing contaminants may also be generated by thermal or chemical treatments or residual extraction chemicals may be present in the final product. Safety assessment should include the sourcing of raw materials, hygienic collection, storage conditions, screening for contaminants, extraction solvents, the severity of processing and the specifications of the final product.

Essential elements are validated decontamination procedures, traceability systems, good manufacturing practices, and routine microbiological and chemical analyzes. Special attention should be paid to labeling of allergens and to the possibility that hydrolysis may reduce, retain or expose immunoreactive protein sequences. Such controls are necessary to ensure that peptide ingredients used in foods, nutraceuticals, cosmetics or pharmaceuticals comply with applicable safety and regulatory requirements. Requirements for allergen labeling may also apply to ingredients derived from animals, dairy, soy, cereal and marine. Safety must be evaluated at both the raw material and finished product stages.

Challenges of Regulation

Regulatory requirements vary by intended use and jurisdiction. For example, a peptide hydrolysate marketed as a general food ingredient needs to be supported by different evidence than a peptide hydrolysate marketed with an antihypertensive or antidiabetic health claim. A purified peptide as a therapeutic drug would have to have much more extensive clinical and manufacturing data.

Meat and animal by-products are particularly hard to regulate because food-grade traceability must be maintained from collection to processing. Limitations in the valorization of animal-derived peptide ingredients include regulatory and safety barriers [47].

Future prospects

In the future it is anticipated that the progress in the valorization of waste derived peptides will be built on the integration of protein recovery, controlled hydrolysis, peptide identification and product validation. This is all within an integrated biorefinery framework. The choice of enzyme and the degree of hydrolysis should be optimized for each substrate as these variables influence peptide sequence, bioactivity, solubility and sensory performance [3,43]. Emerging technologies like low-solvent technologies, which include subcritical-water processing can reduce the consumption of chemicals and allow simultaneous extraction and hydrolysis of proteins [20,44,45]. Advances in peptidomics, *in silico* screening methods via molecular docking and structure-activity modeling is anticipated to accelerate discovery pipelines. These advances would also guide the synthesis of peptides with specified physiological targets [9,12,27]. Current assay limitations include the identification by chemical or enzyme-inhibition assays requires confirmation by simulated digestion, intestinal-transport models, animal studies, and well-designed human trials [15,24,37]. Future research should also consider scale-up, batch variability, membrane fractionation, bitterness, allergenicity, microbial safety and regulatory acceptance [36,39,47]. In the final stage, techno-economic analysis and life-cycle assessment calculations are needed to demonstrate if these processes provide environmental and economic benefits over conventional methods.

Bioinformatics and proteomics

Before the experimental hydrolysis, proteomic databases can identify the parent proteins and predict the encrypted peptide sequences. The value of *in silico* methods for rapeseed meal is presented in Garbacz et al. [12]. Bioinformatics can rank enzyme combinations likely to release ACE inhibitory, antioxidant, DPP-IV inhibitory or antimicrobial sequences.

Exact Hydrolysis

Instead of maximizing the extent of hydrolysis, future processing strategies should be aimed at controlled peptide-bond cleavage to release target sequences and avoid their degradation. Complementary enzyme specificities can be utilized in sequential protease systems to improve the selective production of bioactive peptides. Immobilized enzymes can lead to better operational stability, simplified separation and repeated use and enzyme-recycling systems can reduce protease consumption and production costs. Membrane reactors combine hydrolysis and product separation, enabling the removal of smaller peptides from the reaction zone before their further degradation. Fermentation offers an alternative biological pathway in which the microbial proteases and peptidases generate characteristic peptide patterns. Physical pretreatments, either alone or in combination (ultrasound, high-pressure processing, microwave treatment), and enzymatic hydrolysis can improve substrate accessibility and reduce processing time. Spectroscopic, chromatographic or mass spectrometric techniques for real-time peptide monitoring could additionally aid process control, e.g. by identifying the optimal endpoint and ensuring constant peptide composition, activity and product quality. Such systems may improve reproducibility and reduce enzyme consumption.

Artificial Intelligence and Molecular Modeling

Molecular modeling and artificial intelligence are becoming increasingly useful in accelerating the discovery of bioactive peptides from protein-rich waste streams. Molecular docking can predict peptide binding orientations and the interaction energies and important amino acid contacts with targets such as angiotensin-converting enzyme (ACE), dipeptidyl peptidase-IV (DPP-IV), antioxidant associated proteins and cellular receptors. Subsequently, machine-learning models may be used to further analyze large peptide libraries, identify sequence–activity relationships and prioritize candidates on the basis of predicted potency, stability, toxicity and bioavailability.

Recent works have been focused on computational screening and molecular-interaction analyzes for the search of potentially active peptides from soybean meal and animal-processing by-products [13,31]. The integration of these approaches with peptidomics and proteomic databases allows to minimize considerably the time and cost of conventional screening [9,12,27]. However, computational results should be considered hypotheses rather than proof of biological activity. The selected sequences must then be synthesized and tested using biochemical assays, digestion stability studies, cell models and, when appropriate, animal or human studies to verify their efficacy, selectivity and safety [15, 24, 37].

Integrated Green Extraction and Biorefinery

Subcritical water, ultrasound, membrane processing and other green technologies can reduce the dependence on the conventional solvents and improve the process integration. Cascading biorefineries, where several valuable fractions are recovered in sequence, will most likely produce the greatest sustainability gain. Fish residues can be used to obtain oil, proteins, peptides, collagen, gelatin and minerals. BSG could provide fibers, proteins, peptide hydrolysates, phenolics and fermentation substrates. Life-cycle assessment and techno-economic analysis should thus be standard components of peptide valorization research.

Clinical Validation

The current limitations of existing studies are the heavy reliance on *in vitro* assays that cannot fully mimic gastrointestinal digestion, intestinal absorption, peptide metabolism or interactions in living tissues. For example, the strong antioxidant, ACE-inhibitory or DPP-IV inhibitory activity under laboratory conditions may not necessarily translate into clinically meaningful effects. Future studies should apply a stepwise validation strategy with standardized simulated digestion procedures, intestinal transport studies and cell studies before animal and controlled human trials are performed [15,24,37].

Attentions to the following issues need to be addressed such as peptide stability, absorption, circulating metabolites, effective dose, pharmacokinetics and possible interactions with foods or medications. Clinical studies should be randomized, placebo-controlled, with well-defined participant populations and validated biomarkers, and of sufficient power, and the safety assessment should also cover allergenicity, immunogenicity, toxicity and contaminants from the waste-derived raw materials [23,47]. Establishing reproducible efficacy and safety in humans will be critical to substantiating health claim. In addition, establishing appropriate dosage recommendations and supporting regulatory approval of peptide-based functional foods, nutraceuticals or therapeutic products are also important.

Future studies should progress through a translational hierarchy (Fig. 3) where the validation pipeline consists of a sequential process starting with (1) *in vitro* activity assessment to ascertain fundamental biological mechanisms and efficient doses in test tube tests, (2) cell validation to evaluate uptake, response, and cytotoxicity in human cell models, and (3) digestion stability testing to quantify bioaccessibility throughout simulated gastrointestinal transit. Further validation continues to (4) animal efficacy studies to measure *in vivo* bioavailability, metabolism, safety, and optimal dosing in whole-organism models, leading to (5) human clinical evidence through rigorous trials to determine real-world efficacy, safety, and translational applicability to clinical practice.

CONCLUSION

Agricultural, aquaculture, animal-processing and industrial food-processing wastes are a large reservoir of protein-rich functional peptide precursors. Potential biological value peptide rich hydrolysates can be produced from rice bran, soybean meal, rapeseed meal, wheat germ, fish skins, frames, collagen, slaughterhouse blood, plasma, brewer's spent grain, and whey-related streams. Enzymatic hydrolysis is still the main production technology because it allows food compatible processing and limited control of the peptide release. One promising alternative is subcritical-water hydrolysis, which can simultaneously hydrolyze and extract proteins. Active components can be enriched and characterized by membrane fractionation, gel filtration, RP-HPLC and mass spectrometry. The most evidenced activities are antioxidant, ACE-inhibitory, DPP-IV-inhibitory, glycemic-modulating, metal-chelating, antimicrobial and anti-inflammatory effects. These functions point toward prospective applications in nutraceuticals, functional foods, natural preservatives, pharmaceuticals and cosmeceuticals. However, successful commercialization requires a transition from laboratory research aimed at discovery to integrated process development. The main priorities are to improve active-peptide yield, reduce bitterness, enhance process scalability, prove gastrointestinal stability and bioavailability, confirm safety, meet regulatory requirements and prove environmental and economic superiority over competing waste-management pathways. Bioactive peptides from waste are more than a niche category of functional ingredients. When incorporated into biorefineries and supported by extensive analytical and clinical validation, they can be a viable approach to link food waste reduction with high-value ingredient production and circular bioeconomy development.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests or personal relationships.

ETHICS APPROVAL

Not applicable

ACKNOWLEDGEMENT

This research is self-funded.

DATA AVAILABILITY STATEMENT

Not applicable

AI USAGE DECLARATION

The author used generative AI technologies during the preparation of this work, solely for language editing, image construction, polishing and refinement. All of the authors reviewed and edited the content. The authors take full responsibility for the final content of the publication.

REFERENCES

- Bechaux J, Gatellier P, Le Page JF, Drillet Y, Sante-Lhoutellier V. A comprehensive review of bioactive peptides obtained from animal byproducts and their applications. *Food Funct.* 2019;10(10):6244-6266. <https://doi.org/10.1039/C9FO01546A>
- Görgüç A, Gençdağ E, Yılmaz FM. Bioactive peptides derived from plant origin by-products: Biological activities and technofunctional utilizations in food developments – A review. *Food Research International.* 2020;136:109504. <https://doi.org/10.1016/j.foodres.2020.109504>
- López-Pedrouso M, Zaky AA, Lorenzo JM, Camiña M, Franco D. A review on bioactive peptides derived from meat and by-products: Extraction methods, biological activities, applications and limitations. *Meat Science.* 2023;204:109278. <https://doi.org/10.1016/j.meatsci.2023.109278>
- Celus I, Brijs K, Delcour JA. Enzymatic Hydrolysis of Brewers' Spent Grain Proteins and Technofunctional Properties of the Resulting Hydrolysates. *Journal of Agricultural and Food Chemistry.* 2007;55(21):8703-8710. <https://doi.org/10.1021/jf071793c>
- Wen C, Zhang J, Duan Y, Zhang H, Ma H. A Mini-Review on Brewer's Spent Grain Protein: Isolation, Physicochemical Properties, Application of Protein, and Functional Properties of Hydrolysates. *Journal of Food Science.* 2019;84(12):3330-3340. <https://doi.org/10.1111/1750-3841.14906>
- Noptana R, McClements DJ, McLandsborough LA, Onsaard W, Onsaard E. Rice bran-derived peptides with antioxidant activity: effects of enzymatic hydrolysis using *Bacillus licheniformis* and α -chymotrypsin. *Foods.* 2026;15(3):516. <https://doi.org/10.3390/foods15030516>
- Scarabattoli L, Fanzaga M, Aiello G, Boschin G, d'Adduzio L, Morelli CF, et al. ACE-inhibitory activity and antioxidant properties of a low-MW rice bran protein hydrolysate. *LWT.* 2025;217:117381. <https://doi.org/10.1016/j.lwt.2025.117381>
- Suwannapan O, Wachirattanapongmetee K, Thawornchinsombut S, Katekaew S. Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from Thai jasmine rice bran protein hydrolysates. *International Journal of Food Science & Technology.* 2020;55(6):2441-2450. <https://doi.org/10.1111/ijfs.14495>
- Wang X, Chen H, Fu X, Li S, Wei J. A novel antioxidant and ACE inhibitory peptide from rice bran protein: biochemical characterization and molecular docking study. *LWT - Food Science and Technology.* 2017;75:93-99. <https://doi.org/10.1016/j.lwt.2016.08.047>
- Zhang L, Miao J, Guo J, Liu J, Xia Z, Chen B, et al. Two Novel Angiotensin I-Converting Enzyme (ACE) Inhibitory Peptides from Rice (*Oryza sativa* L.) Bran Protein. *Journal of Agricultural and Food Chemistry.* 2023;71(9):4153-4162. <https://doi.org/10.1021/acs.jafc.2c07270>
- Beaubier S, Durand E, Lenclume C, Fine F, Aymes A, Framboisier X, et al. Chelating peptides from rapeseed meal protein hydrolysates: identification and evaluation of their capacity to inhibit lipid oxidation. *Food Chem.* 2023;422:136187. <https://doi.org/10.1016/j.foodchem.2023.136187>
- Garbacz K, Wawrzykowski J, Czelej M, Waśko A. In Silico Proteomic Profiling and Bioactive Peptide Potential of Rapeseed Meal. *Foods.* 2025;14(14):2451. <https://doi.org/10.3390/foods14142451>
- Jiang M, Liu Y, Xue Y, Zhang M, Liu J, Wu L, et al. Angiotensin-Converting Enzyme Inhibitory Peptides from the Soybean Meal: Screening, Interaction, and Their Protective Effects on Ang II-Induced EA.Hy926 Injury. *Journal of Agricultural and Food Chemistry.* 2026;74(12):10207-10219. <https://doi.org/10.1021/acs.jafc.5c13574>
- Yu Y, Ma S, Han Y, Zhang S, Yang M, Du Z, et al. A novel antioxidant peptide from soybean meal alleviates H₂O₂-induced oxidative damage via the Keap1-Nrf2-HO-1 pathway. *Food Research International.* 2025;206:116084. <https://doi.org/10.1016/j.foodres.2025.116084>
- Zhang J, Wen C, Li C, Duan Y, Zhang H, Ma H. Antioxidant Peptide Fractions Isolated from Wheat Germ Protein with Subcritical Water Extraction and Its Transport Across Caco-2 Cells. *Journal of Food Science.* 2019;84(8):2139-2146. <https://doi.org/10.1111/1750-3841.14720>
- Jia J, Zhou Y, Lu J, Chen A, Li Y, Zheng G. Enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. *Journal of the Science of Food and Agriculture.* 2010;90(4):635-640. <https://doi.org/10.1002/jsfa.3861>
- Mendis E, Rajapakse N, Kim SK. Antioxidant Properties of a Radical-Scavenging Peptide Purified from Enzymatically Prepared Fish Skin Gelatin Hydrolysate. *Journal of Agricultural and Food Chemistry.* 2005;53(3):581-587. <https://doi.org/10.1021/jf048877v>
- Wu R, Wu C, Liu D, Yang X, Huang J, Zhang J, et al. Antioxidant and anti-freezing peptides from salmon collagen hydrolysate prepared by bacterial extracellular protease. *Food Chem.* 2018;248:346-352. <https://doi.org/10.1016/j.foodchem.2017.12.035>
- Fan J, He J, Zhuang Y, Sun L. Purification and Identification of Antioxidant Peptides from Enzymatic Hydrolysates of Tilapia (*Oreochromis niloticus*) Frame Protein. *Molecules.* 2012;17(11):12836-12850. <https://doi.org/10.3390/molecules171112836>
- Barea P, Melgosa R, Illera AE, Alonso-Riaño P, Díaz de Cerio E, Benito-Román O, et al. Production of small peptides and low molecular weight amino acids by subcritical water from fish meal: effect of pressurization agent. *Food Chem.* 2023;418:135925. <https://doi.org/10.1016/j.foodchem.2023.135925>
- Melgosa R, Marques M, Paiva A, Bernardo A, Fernández N, Sá-Nogueira I, et al. Subcritical Water Extraction and Hydrolysis of Cod (*Gadus morhua*) Frames to Produce Bioactive Protein Extracts. *Foods.* 2021;10(6):1222. <https://doi.org/10.3390/foods10061222>
- Abuine R, Rathnayake AU, Byun HG. Biological activity of peptides purified from fish skin hydrolysates. *Fisheries and Aquatic Sciences.* 2019;22(1):10. <https://doi.org/10.1186/s41240-019-0125-4>
- Ghalamara S, Brazinha C, Silva S, Pintado M. Valorization of Fish Processing by-Products: Biological and Functional Properties of Bioactive Peptides. *Current Food Science and Technology Reports.* 2024;2(4):393-409. <https://doi.org/10.1007/s43555-024-00045-5>
- Zhang X, et al. Review of antioxidant and angiotensin-converting-enzyme inhibitory peptides derived from fish: preparation, structure-relationship, bioavailability and ameliorating strategies. *Food Biosci.* 2024;62:104955. <https://doi.org/10.1016/j.fbio.2024.104955>
- Mora L, Reig M, Toldrá F. Bioactive peptides generated from meat industry by-products. *Food Research International.* 2014;65:344-349. <https://doi.org/10.1016/j.foodres.2014.09.014>
- Lee SH, Song KB. Isolation of an Angiotensin Converting Enzyme Inhibitory Peptide from Irradiated Bovine Blood Plasma Protein Hydrolysates. *Journal of Food Science.* 2003;68(8):2469-2472. <https://doi.org/10.1111/j.1365-2621.2003.tb07047.x>
- Zhang T, Nie S, Liu B, Yu Y, Zhang Y, Liu J. Activity Prediction and Molecular Mechanism of Bovine Blood Derived Angiotensin I-Converting Enzyme Inhibitory Peptides. *PLOS ONE.* 2015;10(3):e0119598. <https://doi.org/10.1371/journal.pone.0119598>
- Moreno-Mariscal C, Moroni F, Pérez-Sánchez J, Mora L, Toldrá F. Enzymatic Hydrolysis of Porcine Blood as a Strategy to Obtain a Peptide-Rich Functional Ingredient. *International Journal of Molecular Sciences.* 2025;26(20):9863. <https://doi.org/10.3390/ijms26209863>
- Xu X, Cao R. Antioxidant activity of hydrolysates derived from porcine plasma. *J Sci Food Agric.* 2009;89:1897-1903. <https://doi.org/10.1002/jsfa.3670>
- Zheng Z, Si D, Ahmad B, Li Z, Zhang R. A novel antioxidative peptide derived from chicken blood corpuscle hydrolysate. *Food Research International.* 2018;106:410-419. <https://doi.org/10.1016/j.foodres.2017.12.078>

31. Jia R, Du J, Cao J, Wang J, Zhang Y, Teng W, et al. Antioxidant peptides from porcine blood meal: Mechanisms of interaction with hemin via spectroscopy and molecular docking. *Food Chemistry*. 2026;517:149475. <https://doi.org/10.1016/j.foodchem.2026.149475>
32. Connolly A, Piggott CO, FitzGerald RJ. In vitro α -glucosidase, angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory properties of brewers' spent grain protein hydrolysates. *Food Research International*. 2014;56:100-107. <https://doi.org/10.1016/j.foodres.2013.12.021>
33. Connolly A, O'Keeffe MB, Piggott CO, Nongonierma AB, FitzGerald RJ. Generation and identification of angiotensin converting enzyme (ACE) inhibitory peptides from a brewers' spent grain protein isolate. *Food Chemistry*. 2015;176:64-71. <https://doi.org/10.1016/j.foodchem.2014.12.027>
34. Connolly A, Cermeño M, Crowley D, O'Callaghan Y, O'Brien NM, FitzGerald RJ. Characterisation of the in vitro bioactive properties of alkaline and enzyme extracted brewers' spent grain protein hydrolysates. *Food Res Int*. 2019;121:524-532. <https://doi.org/10.1016/j.foodres.2018.12.008>
35. Espinoza AD, Morawicki RO, Hager T. Hydrolysis of whey protein isolate using subcritical water. *J Food Sci*. 2012;77(1):C20-C26. <https://doi.org/10.1111/j.1750-3841.2011.02462.x>
36. Celus I, Brijs K, Delcour JA. Fractionation and Characterization of Brewers' Spent Grain Protein Hydrolysates. *Journal of Agricultural and Food Chemistry*. 2009;57(12):5563-5570. <https://doi.org/10.1021/jf900626j>
37. Laosam P, Yue Y, Luasiri P, Suwanangul S, Pongsamai N, Chokchaichamnankit D, et al. Valorization of Goat Blood: Hydrolysate Production, Identification, Stability, and Bioavailability upon Gastrointestinal Digestion of Peptides with Dual ACE and DPP-IV Inhibitory Properties. *Foods*. 2026;15(10):1783. <https://doi.org/10.3390/foods15101783>
38. Vo H, Saldaña MDA. Hydrolysis of pea protein concentrate in subcritical water media with addition of citrus pectin and citric acid. *The Journal of Supercritical Fluids*. 2023;195:105866. <https://doi.org/10.1016/j.supflu.2023.105866>
39. Bazsefidpar N, Ghandehari Yazdi AP, Karimi A, Yahyavi M, Amini M, Ahmadi Gavligi H, et al. Brewers spent grain protein hydrolysate as a functional ingredient for muffins: antioxidant, antidiabetic, and sensory evaluation. *Food Chem*. 2024;435:137565. <https://doi.org/10.1016/j.foodchem.2023.137565>
40. Connolly A, Piggott CO, FitzGerald RJ. Isolation of peptides from a novel brewers spent grain protein isolate with potential to modulate glycaemic response. *Int J Food Sci Technol*. 2017;52:146-153. <https://doi.org/10.1111/ijfs.13260>
41. Sae-Leaw T, Karnjanapratum S, O'Callaghan YC, et al. Purification and identification of antioxidant peptides from gelatin hydrolysate of seabass skin. *J Food Biochem*. 2017;41:e12350. <https://doi.org/10.1111/jfbc.12350>
42. Ghalamara S, Brazinha C, Silva S, Pintado M. Exploring Fish Processing By-Products as an Alternative Source of Bioactive Peptides: A Review on Extraction and Food Applications. *Current Food Science and Technology Reports*. 2024;2(4):377-391. <https://doi.org/10.1007/s43555-024-00041-9>
43. Behere GS, Surasani VKR, Shamasundar BA, Rathod NB, Pandey PK. Influence of enzyme source and degree of hydrolysis on bioactivity and functional properties of protein hydrolysates from threadfin bream (*Nemipterus japonicus*) frame waste for food and nutraceutical applications. *Frontiers in Marine Science*. 2026;13:1776400. <https://doi.org/10.3389/fmars.2026.1776400>
44. Rivas-Vela CI, Amaya-Llano SL, Castaño-Tostado E, Castillo-Herrera GA. Protein hydrolysis by subcritical water: a new perspective on obtaining bioactive peptides. *Molecules*. 2021;26(21):6655. <https://doi.org/10.3390/molecules26216655>
45. Álvarez-Viñas M, Rodríguez-Seoane P, Flórez-Fernández N, Torres MD, Díaz-Reinoso B, Moure A, et al. Subcritical Water for the Extraction and Hydrolysis of Protein and Other Fractions in Biorefineries from Agro-food Wastes and Algae: a Review. *Food and Bioprocess Technology*. 2021;14(3):373-387. <https://doi.org/10.1007/s11947-020-02536-4>
46. Connolly A, et al. Identification of bioactive peptides from brewers' spent grain and contribution of Leu/Ile to bioactive potency. *J Funct Foods*. 2019;60:103455. <https://doi.org/10.1016/j.jff.2019.103455>
47. Gagaoua M, Das AK, Fu Y, Dib AL, Nanda PK. Meat by-products as a source of bioactive peptides and functional ingredients: Regulatory and safety barriers to valorization. *Current Opinion in Green and Sustainable Chemistry*. 2024;47:100910. <https://doi.org/10.1016/j.cogsc.2024.100910>
48. Lee JW, Lee S, Hong GP. Subcritical water hydrolysis for food applications: Temperature-dependent conversion and bioactivities of meat proteins. *npj Science of Food*. 2026;10(1):114. <https://doi.org/10.1038/s41538-026-00770-2>
49. Paksin P, Sringarm K, Tangjaidee P, Klangpetch W, Rachtanapun P, Onsaard E, et al. Selective production of novel ACE-inhibitory peptides from brewer's spent grain via two-stage enzymatic hydrolysis and integrated fractionation. *Future Foods*. 2026;13:100877. <https://doi.org/10.1016/j.fufo.2025.100877>