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Review Article

Peptidomics as a robust tool for the characterization of food-derived bioactive peptides

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ABSTRACT- The proteome is intricately determined by the genome and is strongly influenced by a range of external factors, including tissue type, developmental stage, metabolic state, and various biological interactions. Proteomic analyses commonly employ techniques such as gel-based methods and shotgun proteomics. In recent years, the identification of bioactive peptides (BPs) has emerged as an important area of research in food science and technology. Food peptidomics, a specialized subfield of proteomics, focuses on the composition, interactions, and properties of peptides present in food matrices. This review highlights the growing importance of peptidomics as a powerful tool for discovering and monitoring BPs across a wide range of food products, including dairy, meat, fish and seafood, fruits and vegetables, cereals and legumes, and nuts. Advances in peptide identification, particularly through state-of-the-art mass spectrometry and high-resolution analytical techniques, have established peptidomics as a powerful approach for the efficient identification and characterization of BPs in diverse food matrices.

INTRODUCTION

The food industry is continuously striving to produce safe, nutritious, and high-quality products that satisfy the increasing consumer demand for functional foods (Hall et al., 2008; Ruan et al., 2026; Zhang et al., 2026a). In this context, both industry and academia face a significant challenge: devising strategies and products that not only support health maintenance but also reduce the risk of chronic diseases. Proteomics, as a high-throughput analytical platform, has emerged as a valuable tool to address these complex challenges (Gallardo et al., 2013). It offers considerable advantages in process optimization, safety assessment, quality control, and nutritional evaluation (Ijaz et al., 2026; Kussmann et al., 2006; Tissen et al., 2026).

Proteomics can be broadly defined as the large-scale examination of proteins within a biological system at a particular time. This includes the study of their modifications, interactions, cellular localization, abundance, and structural-functional relationships. In food science, proteomics facilitates the comprehensive identification and characterization of protein profiles in diverse food matrices (Amiri-Dashatan et al., 2018). Complementarily, peptidomics is a specialized subfield of proteomics that focuses on the composition, bioactivity, and dynamic behavior of peptides released from parent food proteins during processing, storage, and gastrointestinal digestion (Carrasco-Castilla et al., 2012; Le et al., 2026).

Given that proteins and peptides significantly affect the texture, yield, flavor, and nutritional value of food products, a thorough understanding of their structure and function is vital for optimizing food processing technologies. While previous reviews have addressed the general applications of proteomics in the food science, there has been less systematic attention devoted to the specific role of peptidomics in characterizing biologically active compounds, particularly bioactive peptides with health-promoting potential. Thus, the primary objective of this review is to provide a focused distinction that touches on current synthesis of recent advancements in both proteomics and peptidomics, emphasizing their integrated application for the identification and functional characterization of bioactive compounds across various food categories. To enhance orientation for the reader, this review is structured to first discuss analytical workflows, followed by a highlight of key findings in different food matrices, and finally to outline future perspectives and challenges in this rapidly evolving field.

PEPTIDOMICS AS A SUBSET OF PROTEOMICS

Peptidomics refers to the comprehensive analysis of endogenous peptides found in biological samples. It is

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recognized as a specific subdiscipline within proteomics. Proteomics encompasses the study of the proteome, which represents the complete set of proteins encoded by the genome. It is a term first introduced by Marc Wilkins and his team in the early 1990s, paralleling "genomics" and "genome." The proteome consists of all proteins present in a specific sample, such as tissue, organism, or cell culture, at any given moment, reflecting a dynamic profile that varies under different conditions, compared to the static nature of the genome (Kvasnička, 2003; Liebler, 2001).

As a relatively recent methodology, proteomics is extensively utilized in food science and technology to characterize proteins, analyze their conformations, interactions, nutritional value, biological roles, and functional properties, while also aiming to evaluate food quality (Carrasco-Castilla et al., 2012). Although peptidomics and proteomics are closely related, they differ significantly in their analytical methods. Traditional bottom-up proteomics typically involves enzymatic digestion (commonly with trypsin) and database-driven identification of multiple peptide fragments from each protein. In contrast, peptidomics employs a top-down approach that maintains the native structures of endogenous peptides and necessitates meticulous manual validation of tandem mass spectrometry (MS/MS) spectra for the identification of single peptides. However, this approach faces higher false-positive rates and increased analytical complexity (Schrader et al., 2014). The essential procedural steps for both methodologies are outlined in the following sections (Fig. 1).

Preparation steps

Adequate preparation is essential before evaluating the functional and biological properties of proteins. Protein separation methods generally rely on varying characteristics such as size, charge, and binding properties. Typically, tissue or microbial cells serve as sources for protein extraction. The purification process begins with cell lysis, resulting in the release of intracellular proteins into a solution termed the crude extract (Fig. 2). If necessary, additional techniques, such as differential centrifugation, may be employed to isolate specific organelles or prepare subcellular fractions. Subsequent methods for purifying protein molecules contained within extracts or organelles involve fractionating the extracts based on particular protein properties, such as size or charge. Initial fractionation commonly exploits differences in protein solubility. The solubility of proteins is often diminished at high ionic strengths, a phenomenon known as "salting out." In this context, salts can selectively precipitate some proteins while permitting others to remain soluble in the solution. Ammonium sulfate ($(NH_4)_2SO_4$) is frequently utilized in this process due to its high water solubility. It is important to note that certain treatments may need to be conducted on the solution containing the target protein prior to further purification steps. Dialysis, for instance, is typically employed to separate proteins from solvents, taking advantage of the larger size of the proteins (Nelson et al., 2008; O'Donnell et al., 2004). Proteomics, in general, integrates two key methods of separation and characterization, which will be discussed in subsequent sections.

Protein separation

The initial, crucial step in any proteomics study involves the separation of various proteins within a biological sample, a task that is both complex and challenging. The primary methods employed for the separation of complex protein mixtures include: (1) gel electrophoresis-based techniques and (2) liquid chromatography (LC).

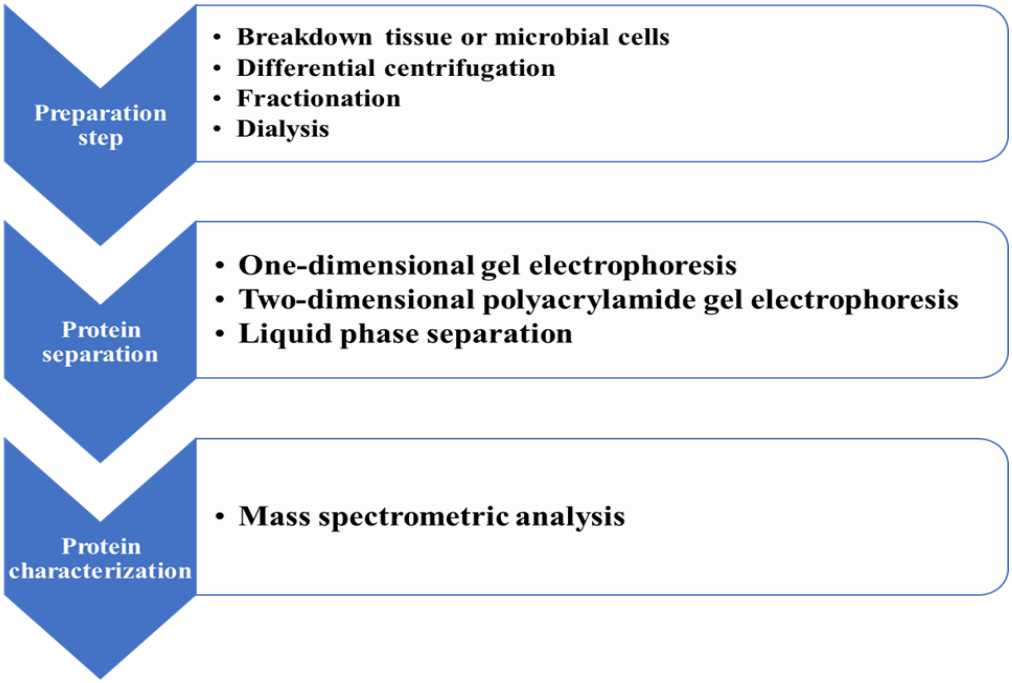

Fig. 1. Proteomics steps at a glance.

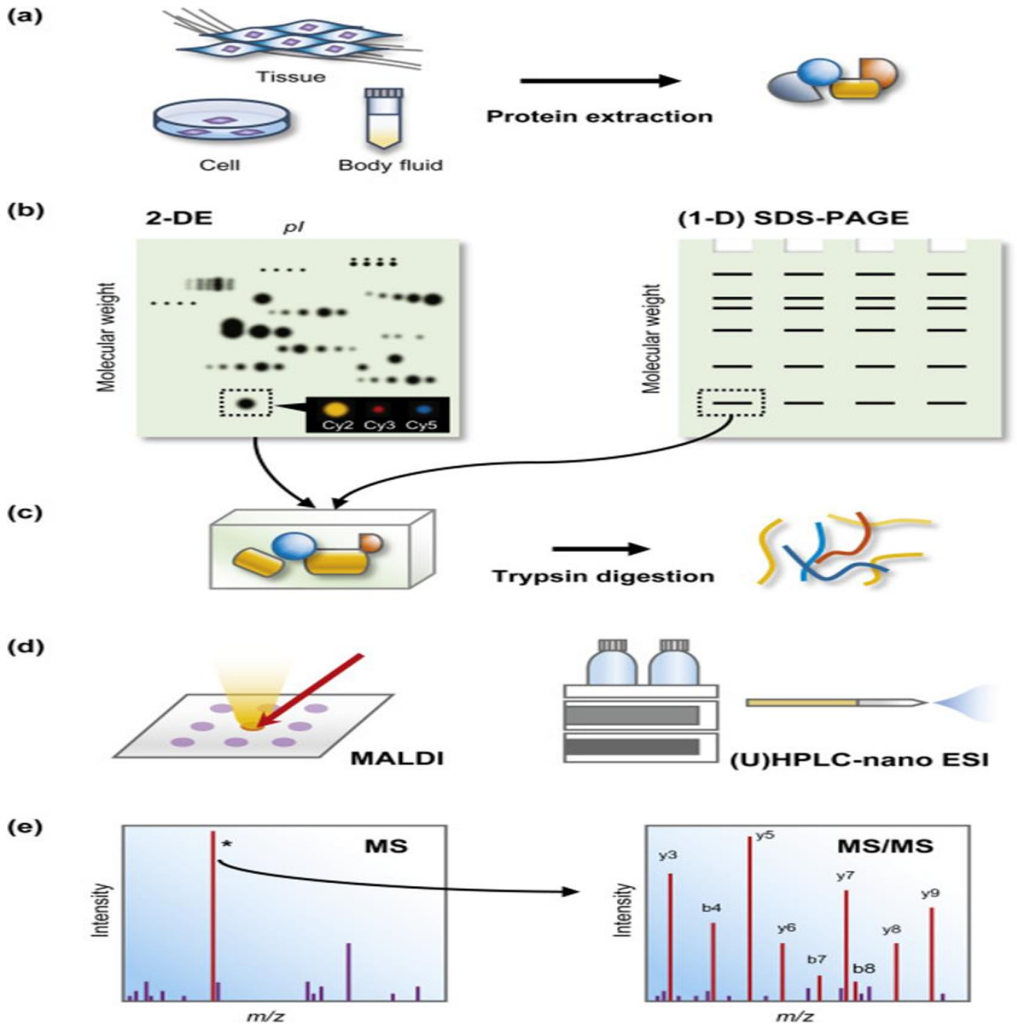

Fig. 2. Gel-based proteomics: (a) protein extraction, (b) protein separation by two-dimensional gel electrophoresis or sodium dodecyl sulfate polyacrylamide gel electrophoresis, (c) trypsin digestion and peptides collection, (d) peptide ionization *via* matrix-assisted laser desorption/ionization or nano electrospray ionization and induction to mass spectrometry, and (e) peptide measurement in mass spectrometry spectrum, followed by selection and isolation, subsequently fragmentation to get the sequence information from MS/MS spectrum. Reprinted with permission from Kim & Cho (2019).

Gel electrophoresis-based techniques

One-dimensional gel electrophoresis (1-DGE) is a straightforward and highly reproducible technique for separating complex protein mixtures based on their molecular weights, typically ranging from 7 to 300 kDa. It is particularly effective for specific applications such as immunoassays or for the separation of proteins following purification steps (Agrawal & Rakwal, 2006).

In 1975, O'Farrell introduced the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique, designed to separate cellular proteins under denaturing conditions. This methodology involves two distinct separation dimensions (Fig. 3). In the first dimension, isoelectric focusing (IEF) separates proteins according to their isoelectric points (*pI*). Subsequently, in the second dimension, proteins are separated by molecular weight in the presence of sodium dodecyl sulfate (SDS) (Issaq & Veenstra, 2008).

During IEF, a stable pH gradient is established, increasing from the anode to the cathode. In this environment, proteins possess a positive net charge when the pH is below their *pI*, resulting in their migration toward the cathode. The pH gradient facilitates the movement of proteins towards progressively higher pH values until they reach their *pI*, where their net charge becomes neutral, halting further migration (May et al., 2021).

Denaturing SDS-PAGE is performed by boiling proteins with excess SDS and a thiol reagent. This process masks the protein charge, forms anionic micelles, disrupts tertiary and secondary structures, and unfolds the protein molecules (Westermeier, 2014). An aliquot of the solubilized protein solution is loaded into wells prepared on top of the stacking gel. The stacking gel, characterized by low porosity and a lower buffer concentration and pH, concentrates the proteins into a narrow band before they migrate into the resolving gel, often referred to as the running or separating gel. Electrophoretic separation of individual proteins occurs in this resolving gel (Gallagher, 2006).

Despite their utility, 2D-PAGE-based methods present several technical limitations. These include low reproducibility, low sensitivity, and a limited dynamic range regarding the absolute quantity of proteins that can be loaded. Detecting highly acidic, alkaline, and hydrophobic proteins can be difficult (Shi et al., 2004).

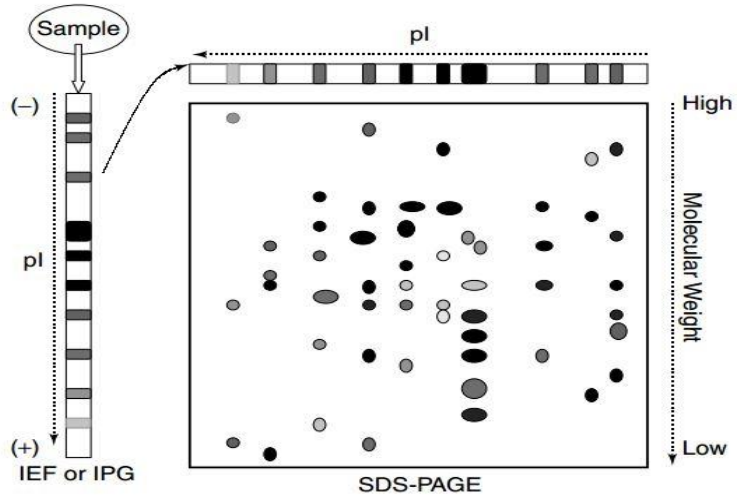

Fig. 3. Two-dimensional electrophoretic separation of proteins. Reprinted with permission from Zybailov & Washburn (2006).

Liquid phase separation

Liquid phase separation is increasingly utilized in proteomic studies for protein separation. This technique includes capillary electrophoresis (CE) and LC offering several advantages over 2D-PAGE, such as higher sensitivity, enhanced dynamic range, ease of automation, and reduced processing times (O'Donnell et al., 2004). CE primarily separates proteins based on the ratio of charge to size, enabling high efficiency and rapid separation (Sastre Toraño et al., 2019). However, the low concentration sensitivity of CE limits its ability to detect trace levels of analytes (Quirino et al., 2002).

Proteins can be purified through chromatographic methods based on their properties, including net charge, shape, size, binding capacity, and the stationary phase employed. Column chromatography is commonly used for this purpose. The sample is applied to the top of a column (stationary phase) and eluted using a washing buffer (mobile phase). The samples then accumulate at the bottom of the column, depending on the elution time and volume (Coskun, 2016). Individual proteins migrate through the column at varying rates, influenced by their distinct characteristics (Nelson et al., 2008).

For example, ion-exchange chromatography (IEC) separates proteins based on the electrostatic attraction between molecules of opposite charges. This technique employs an ion-exchanger matrix consisting of porous beads that provide a sufficient surface area for adsorption. A positively or negatively charged ligand is immobilized on this matrix, and a charged polymer can be grafted onto the matrix to enhance capacity (Jungbauer & Hahn, 2009).

Size exclusion chromatography (SEC) separates molecules based on size through differential pore permeation. Larger molecules elute from the column before smaller ones due to their shorter retention times, as they are unable to enter the smaller pores within the column (Kostanski et al., 2004). This technique may also be referred to as gel permeation chromatography (GPC) when using organic solvents as the mobile phase, or gel filtration chromatography (GFC) when using aqueous solutions (Batool & Menaa, 2020).

Affinity chromatography is another powerful method for protein separation, based on specific interactions between the target protein and an immobilized ligand (Lee & Lee, 2004). High Performance Liquid Chromatography (HPLC) represents an advanced form of column liquid chromatography. The HPLC system typically uses pumps to transport pressurized liquid solvents, including the sample mixture, into a column packed with solid adsorbent material. Each component of the sample interacts differently with the column, resulting in varied flow rates and subsequent separation (Goel & Sharma, 2019).

PROTEIN CHARACTERIZATION

Mass spectrometric analysis

Following the fractionation or separation process, individual proteins are typically identified and thoroughly characterized. Mass spectrometry (MS) is the predominant technique employed in this area of proteomic analysis (O'Donnell et al., 2004). MS measures the mass-to-charge ratio (m/z) of ions in the gas phase. A mass spectrometer consists of three main components: an ion source that transforms analytes into gas-phase ions, a mass analyzer that separates these ionized analytes based on their m/z ratio, and a detector that quantifies the number of ions at each m/z value (Han et al., 2008). The m/z measurements are conducted on molecules in the gas phase, though the heating or other processes required to vaporize a macromolecule often lead to rapid decomposition (Nelson et al., 2008).

To address the challenge of ion generation from large, nonvolatile analytes like proteins and peptides without significant fragmentation, two commonly used methods are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). These methods are classified as "soft" ionization techniques due to their minimal analyte fragmentation. Under certain conditions, even noncovalent interactions may be preserved during ionization (Aebersold & Mann, 2003).

MS can also be employed to sequence short polypeptide segments, facilitating the rapid identification of unknown proteins. This application is known as tandem MS or MS/MS. The MS/MS technique consists of two stages; initially, ions with a specific m/z are isolated from the other ions generated by the ion source. These isolated ions, referred to as parent or precursor ions, are then induced to undergo a chemical reaction, resulting in changes to either their mass (m) or charge (z). Typically, the internal energy of

the ions is elevated, leading to dissociation during this reaction. The resultant product ions are subsequently analyzed in the second stage of MS/MS (Glish & Vachet, 2003).

Additionally, ion trap (IT), time-of-flight (TOF), quadrupole (Q), and Fourier transform mass spectrometers (FTMS) are current analyzers utilized in proteomics research, each possessing unique designs and performance characteristics, along with distinct strengths and weaknesses. To leverage the advantages of each analyzer, these instruments can be employed independently or in combination (Fig. 4) (Aebersold & Mann, 2003).

IT analyzers facilitate the trapping and accumulation of ions over time within a physical device, offering MS/MS capabilities characterized by exceptional sensitivity and rapid data acquisition. When integrated with data-dependent acquisition, IT technology supports high-throughput analysis. However, limitations such as restricted resolution, low ion-trapping capacity, and space-charging effects result in inaccuracies in mass measurements (Domon & Aebersold, 2006).

TOF analyzers determine the mass-to-charge (m/z) ratio of analyte ions by measuring the time required for ions to traverse a specific-length tube under vacuum conditions. TOF mass analyzers serve as a fundamental basis for analytical platforms utilizing both ESI and MALDI (Domon & Aebersold, 2006).

Q mass analyzers are frequently employed in conjunction with ESI sources for LC-coupled applications. A Q analyzer comprises four parallel rods with a hyperbolic cross-section, where diagonally opposite rods are linked to radio frequency and direct-current voltage sources, creating a quadrupole field. Ions generated at the ion source are electrostatically accelerated into this quadrupole field, and mass selection is achieved by adjustably varying the radio frequency and direct current amplitudes, allowing only ions of a particular m/z to reach the detector for any specified pair of these amplitudes (Zybailov & Washburn, 2006).

FTMS, or ion-cyclotron resonance mass spectrometry (ICR-MS), utilizes a cubic cell placed within a strong magnetic field. This cell comprises three sets of plates: one for trapping, one for transmission, and one for reception. A continuous beam of ions is generated externally, directed into the trap, and pulsed into the cell for mass analysis. Inside the cell, ions are confined by the magnetic field, resulting in cyclotron motion. This motion is periodic and defined by the cyclotron frequency, which corresponds to the frequency at which an ion completes its orbit (Lane, 2005). Despite numerous attempts to advance FTMS through radio frequency ion traps, electrostatic traps, and multiple reflection systems, various technical challenges have hindered their adoption in mainstream mass spectrometry. These circumstances facilitated the development and rise of a new variant within the FTMS family: the Orbitrap analyzer (Zubarev & Makarov, 2013).

Mass spectrometry-based proteomics plays a crucial role in identifying and elucidating physiological processes, providing a powerful technological framework for the study of proteins within diverse biological contexts. This platform has broadened research opportunities by applying techniques such as "top-down" proteomics, which analyzes proteins in their intact form, and "bottom-up" proteomics, which investigates proteins using peptides produced during proteolysis (Klont et al., 2018).

A significant challenge in top-down proteomics is the scarcity of high-resolution separation techniques for complex protein samples. The most commonly employed method for analyzing intricate intact proteins is reversed-phase liquid chromatography (RPLC) coupled online with mass spectrometry. Efforts to enhance the separation capabilities of RPLC have led to the development of various methods aimed at improving the efficiency of one-dimensional (1D) RPLC separation, such as utilizing ultra-high-pressure liquid chromatography (UPLC) systems with longer columns or smaller particle sizes. However, 1D RPLC alone may not sufficiently cover the proteome in top-down proteomics due to the extreme complexity, limited sample loading capacities, and extensive dynamic ranges of intact protein samples. Furthermore, CE has been applied as a complementary separation method to RPLC in proteomics studies, assisting in the separation of peptides and proteins (Wang et al., 2018).

Shotgun proteomics or bottom-up protein analysis

Proteins exhibit a wide range of physicochemical properties, making the chromatographic separation of intact proteins challenging. As a result, separating complex mixtures using a single method is often ineffective. Additionally, the high molecular weight of proteins and their incomplete elution from chromatographic columns present significant obstacles, hindering comprehensive identification through MS. To address these issues, enzymatic reactions, such as trypsin digestion, are employed to partially hydrolyze protein mixtures for chromatography-based proteome analysis. However, as the complexity of the protein mixture increases, the number of resultant peptides also rises. For example, the digestion of a single protein by trypsin typically produces around 25 peptides; thus, a sample containing 10,000 proteins could yield approximately 250,000 peptides. In contrast, techniques for the chromatographic separation and mass spectrometric identification of peptides are well-established and straightforward. Nonetheless, similar to electrophoretic separations, highly complex peptide mixtures cannot be adequately resolved using the peak capacity of one-dimensional chromatography alone. Therefore, multidimensional LC coupled with mass spectrometry has become a standard approach in proteome analysis, commonly referred to as shotgun proteomics (Pischetsrieder & Baeuerlein, 2009). This shotgun proteomics strategy, which involves digesting proteins into peptides and sequencing them with MS/MS, has gained significant acceptance in the scientific community.

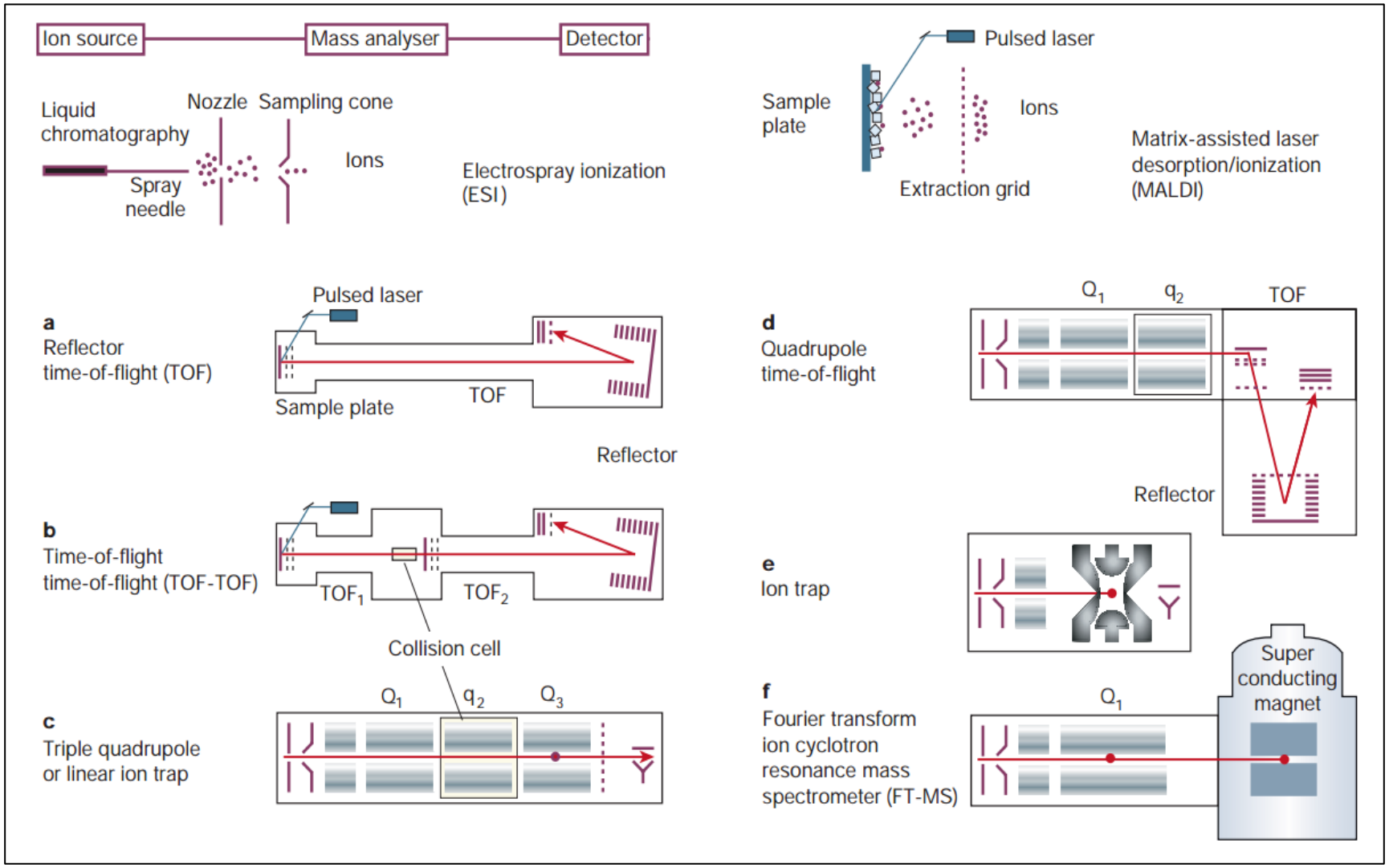

Fig. 4. Mass spectrometers used in proteome research. Reprinted with permission from Aebersold & Mann (2003).

The database search method is predominantly utilized for identifying peptides based on acquired MS/MS spectra. Furthermore, the shotgun proteomics approach facilitates higher data throughput and enhanced protein detection sensitivity compared to traditional methods that require extensive protein separation prior to MS identification, such as 2D gels. Additionally, shotgun proteomics offers advantages over analytical techniques that rely on MS/MS sequencing of intact proteins. The sequencing of such large proteins is inherently challenging, as it necessitates costly MS instrumentation, which can limit the technique's widespread adoption in standard academic laboratories (Matthiesen, 2007).

FOOD BIOACTIVE PEPTIDES

Currently, the presence of protein hydrolysates and bioactive peptides (BPs) in various foods is not considered in the evaluation of dietary protein quality. Bioactive proteins and peptides are associated with several advantages, and their effects can be influenced by multiple factors. Although some dietary proteins exert effects in their natural form, the peptides generated during digestion, hydrolysis, or fermentation often garner the most interest (Babazadeh et al., 2022). The concentration of BPs produced must be sufficient to elicit a desired response while remaining under levels that could pose safety concerns or adverse effects. Moreover, certain peptides exert systemic effects and require adequate absorption to manifest these benefits. While numerous in vitro studies have been conducted, it is imperative that further in vivo investigations assess the safety, dose-response relationships, and efficacy of these peptides. Therefore, standardized methods for measuring health benefits and BP content should be developed (Rutherfurd-Markwick, 2012).

Identifying all peptides in hydrolyzed food proteins poses a challenge due to the complexity of these mixtures, which typically contain hundreds of peptides, each varying in chain length and abundance (Agyei et al., 2018). Bioactivity is initially detected in crude protein hydrolysates, which are subsequently fractionated by peptide size, often through ultrafiltration. Following purification by methods such as RP-HPLC or GPC, the hydrolysate demonstrating the highest biological activity is further separated into its constituent peptides. Individual peptide segments are then identified using a combination of protein sequencing and mass spectrometry. To validate biological activity, assays are subsequently performed with synthetic versions of the peptides (Ryan et al., 2011).

PEPTIDOMICS IN FOOD SCIENCE: FROM BIOACTIVE PEPTIDES TO FUNCTIONAL HEALTH APPLICATIONS

Interest in BPs has significantly increased over the past decade. BPs are protein fragments ranging from 2 to 20 amino acids in length, resulting in considerable heterogeneity. Their potential as nutraceuticals that enhance health and nutrition is gaining attention, especially in relation to diseases and disorders lacking effective treatment strategies. In this context, naturally occurring BPs found in various foods may serve as adjuvants in disease management (Piovesana et al., 2015).

The approach adopted in this analysis of dietary protein-derived BPs is fundamentally empirical. However, advancements in proteomics and peptidomics hold promise for researchers to integrate novel and established methods, thereby enhancing BP production from proteins. Indeed, several studies have successfully incorporated various proteomics and peptidomics techniques within the traditional framework of BP research (Carrasco-Castilla et al., 2012).

Peptidomics of milk and dairy products

Bovine milk, with a protein concentration of 32 g/L, constitutes approximately 85% of global protein consumption and is a crucial protein source for humans. The primary protein fractions in milk include α_s-casein, β-casein, κ-casein, α-lactalbumin, and β-lactoglobulin. These proteins not only serve as a vital source of bioactive components but also significantly influence essential processing characteristics such as solubility, thermal stability, water binding, renneting, and foam formation due to their nutritional value. Consequently, milk proteins are considered valuable ingredients for the development of functional foods aimed at enhancing human health. Nevertheless, further investigation is necessary to fully evaluate the therapeutic potential of milk-derived bioactive factors, particularly since much of the current evidence regarding the bioactive effects of food proteins and peptides is derived from in vitro studies (Punia et al., 2020; Vargas-Bello-Pérez et al., 2019). This underscores the need for robust analytical methods to isolate and characterize bioactive peptides within complex dairy matrices to better understand their beneficial properties

In addressing this gap, multiple studies have effectively utilized advanced peptidomics and proteomics techniques to identify bioactive peptides in milk (Table 1). For instance, Zanutto-Elgui et al. (2019) fractionated and identified antioxidant and antimicrobial peptides from bovine and goat milk that had been hydrolyzed by *Aspergillus oryzae* and *Aspergillus flavipes* through solid-state fermentation, using 2D-PAGE and ESI-MS/MS. Similarly, Tonolo et al. (2020) identified several antioxidant peptides from fermented milk proteins *via* LC-MS/MS, demonstrating that these peptides exhibited antioxidant capacity both in vitro and in cellular models (Caco-2 cells). Additionally, the potential of peptidomics for identifying antihypertensive peptides has been highlighted. In a study by Amorim et al. (2019), MALDI-TOF/MS was utilized to analyze protein extracts from non-fermented bovine milk and kefir beverages, and Shotgun proteomics was applied to sequence tryptic digestion products. The peptides from fermented kefir milk were primarily derived from serum albumin (50%), caseins (17%), β-lactoglobulin (13%), lactotransferrin (10%), and α-lactalbumin (5%), with 35 peptides exhibiting potential antihypertensive activity identified. Another significant study by Santini et al. (2020) characterized kefir milk using 2D-PAGE and LC-MS/MS, identifying milk-derived polypeptides from κ-, α_{s1}-, and α_{s2}-caseins, as well as from glycosylation-dependent cell adhesion molecules, such as lactophorin, in commercial organic kefir milk (Fig. 5).

The use of protein hydrolysates as natural antioxidants also presents a promising strategy for producing antioxidant-enriched foods (Nooshkam & Madadlou, 2016a, 2016b). A recent study successfully characterized a hydrolysate derived from milk protein concentrate through proteomics methodologies. SDS-PAGE and high-efficiency GFC revealed a molecular weight of less than 3 kDa for the hydrolysate, identifying 25 peptides with known biological activity through EASY-nLC-Orbitrap MS/MS and bioinformatics. The hydrolysate demonstrated significant antioxidant activity, as evidenced by tests measuring hydroxyl-radical scavenging activity, reducing power, and both ABTS and DPPH assays (Cui et al., 2022). The effect of processing conditions on peptide profiles has been examined. Pisanu et al. (2015) assessed the impact of milk pasteurization on the peptide profiles of cheeses made from raw versus pasteurized ovine milks. Characterization of peptidic extracts via LC-MS/MS and bioinformatics analysis led to the identification of 187 peptides (originating from α_{S1}-casein, β-casein, and α_{S2}-casein). Notably, the biological activity of 37 identified peptides was predicted, revealing that cheese produced from raw milk contained a significantly higher abundance of peptides with immunomodulating and ACE-inhibitory properties, thereby highlighting the effect of processing on bioactive potential.

Peptidomics of meat and meat products

The meat sector is a keystone element of the larger food and beverage industry, influencing both consumer behavior and economic policy. In addition to providing essential nutrients, meat serves as a substantial source of biologically active substances, particularly tissue-specific proteins and peptides. The meat proteome is recognized as a reservoir of BPs that exhibit diverse biological activities, i.e., antioxidant, antihypertensive, immunomodulating, opioid, mineral-binding, prebiotic, antimicrobial, and cholesterol-lowering effects. These bioactivities may be present naturally in raw tissues or generated through technological processing, enzymatic hydrolysis, or fermentation. Recent advancements in analytical techniques, particularly proteomics, have established that the proteome and peptidome of tissues contain not only constitutive structural and functional proteins but also unique molecules that contribute to maintaining normal physiological homeostasis (Chernukha et al., 2018).

Emerging evidence indicates that bioactive peptides can be released naturally during the post-mortem aging of fresh meat. Liu et al. (2017) investigated the endogenous generation of BPs in duck meat over a seven-day aging period, establishing that small peptides (< 5 kDa) were progressively liberated due to the muscle protein degradation. Notably, peptides that formed after three days of aging showed the highest antioxidant activity. Through SEC fractionation and nano LC-MS/MS sequencing, the most potent fraction was enriched with hydrophobic amino acids, likely responsible for the observed bioactivity. Supporting these findings, Fu et al. (2017) reported similar results in beef, specifically identifying multifunctional BPs released during post-mortem aging using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). This indicates that the phenomenon is not species-specific but characteristic of muscle tissue maturation.

Extending the timescale from days to months, peptidomics approaches have been employed to identify naturally generated peptides during the long-term processing of meat products. Mora et al. (2019) utilized tandem MS to characterize peptides resulting from the hydrolysis of myosin heavy chain protein during a nine-month dry-cured ham processing period. The size, sequence, and known bioactivities of several identified peptides highlighted the potential of the myosin heavy chain as a significant precursor of BPs in cured meats.

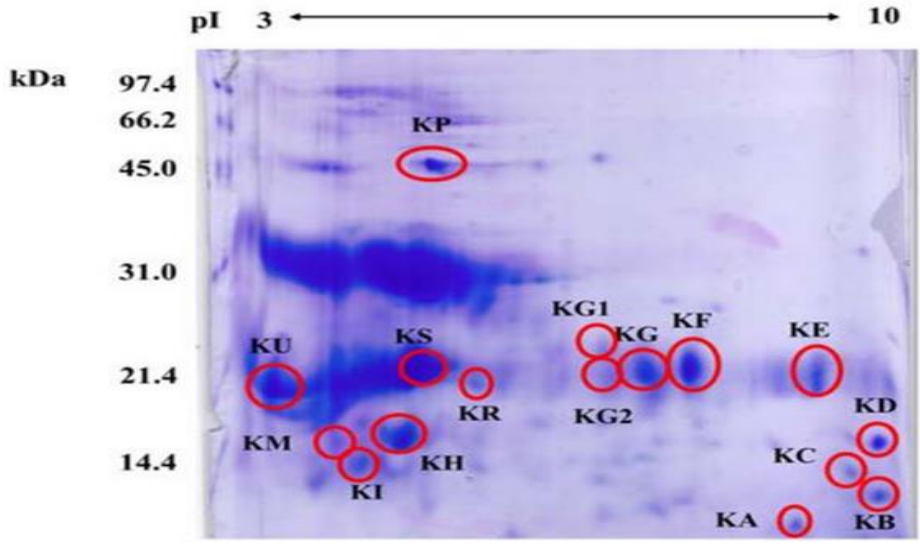

Fig. 5. Two-dimensional electrophoresis of the kefir milk proteins. Reprinted with permission. The spots KA, KB, and KC were identified as κ-casein derived polypeptides, which contain a YIPIQYVLSR sequence that corresponds to the casoxin C, an opioid antagonist. The KH, KI, and KU spots are α_{s1}-casein originated fragments containing FFVAPFPEVFGK sequence, a well-known Angiotensin-converting-enzyme inhibitory peptide. The KM spot is a peptide derived from the α_{s2}-casein, whereas the KE, KF, KG, KG1, and KG2 spots assign to the glycosylation-dependent cell adhesion molecule, i.e., lactophorin, which is highly expressed during lactation by the mammary epithelial cells (Santini et al., 2020).

Table 1. Summary of proteomics and peptidomics analysis studies in dairy products

Purpose	Method	Bioactivity	Reference
Peptidomic profiling analysis of endogenous peptides in buffalo milk during lactation stages	▪ LC-MS/MS	▪ Anti-inflammatory properties	(Zhang et al., 2026b)
Goat milk protein hydrolysates	▪ LC-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity	(Zhang et al., 2025a)
Bioactivity characterization of donkey milk	▪ Nano HPLC-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity	(Piovesana et al., 2015)
Identification of potentially bioactive peptides in goat milk kefir	▪ LC-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity ▪ Antibacterial activity ▪ Antithrombotic activity ▪ Dipeptidyl peptidase IV (DPP-IV) inhibitor	(Izquierdo-González et al., 2019)
Peptide profile of yogurt	▪ MALDI-TOF-MS	▪ ACE-inhibitory	(Giacometti Cavalheiro et al., 2020)
Peptide profile of Cheddar cheese	▪ SDS-PAGE ▪ LC/MS	▪ ACE-inhibitory	(Hao et al., 2021)
Characterization of whey protein concentrate hydrolysate	▪ SDS-PAGE ▪ Size exclusion-HPLC ▪ HPLC-MS/MS ▪ MALDI-TOF MS	▪ Antimicrobial activity ▪ Antidiabetic activity ▪ Antithrombic activity ▪ Hypotensive activity ▪ Opioid activity	(Hinnenkamp & Ismail, 2021)
Characterization of functional peptides in four traditional cheeses	▪ LC-MS/MS	▪ Antibacterial activity ▪ ACE-inhibitory	(Robinson et al., 2021)
Separation and characterization of ACE-inhibitory peptides from fermented goat milk	▪ SDS-PAGE ▪ SEC ▪ LC-MS/MS	▪ ACE-inhibitory	(Aslam et al., 2019)
Protein and peptide profiles of regional 'Coalho' cheeses	▪ SDS-PAGE ▪ MALDI-TOF/MS	▪ Mineral-binding ability ▪ Antimicrobial activity ▪ Antihypertensive activity ▪ Antioxidant activity	(Silva et al., 2016)
Separation and bioactivity analysis of peptides from Camembert-type cheese affected by milk heat treatment and incorporating adjunct culture	▪ MALDI-TOF/MS	▪ ACE-inhibitory ▪ Antimicrobial activity ▪ Immunomodulator ▪ Opioid	(Galli et al., 2019)
Fractionation and identification of peptides with antimicrobial activity from the Canastra minas cheese	▪ RPLC ▪ MALDI-TOF/TOF/MS	▪ Antimicrobial activity	(Fialho et al., 2018)
Fractionation and sequence determination of antibacterial peptides from different Italian cheese varieties	▪ RP-FPLC ▪ HPLC ▪ ESI-IT/MS	▪ Antibacterial activity	(Rizzello et al., 2005)

LC-MS/MS: Liquid chromatography tandem mass spectrometry; ACE: Angiotensin-converting-enzyme; MALDI-TOF-MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; RPLC: Reversed-phase liquid chromatography; RP-FPLC: Reversed-phase fast protein liquid chromatography; ESI-IT/MS: electrospray ionization-ion trap mass spectrometry.

Furthermore, extrinsic factors such as rearing systems and carcass composition have been examined for their effects on peptide profiles and functionality. Kęska et al. (2020) compared organic and conventional pork, as well as various carcass components (shoulder, loin, and ham), in terms of the meat proteome and the antioxidant capacity of its peptides. LC-MS/MS analysis revealed that myofibrillar proteins were the primary source of the identified peptides. While the overall proteome remained largely unchanged by meat origin or carcass portion, the peptides' abilities to chelate iron ions and exert reducing activity were significantly influenced by these factors. Specifically, pork ham from conventional farming systems demonstrated the most potent antioxidant effects, suggesting that production choices may have important implications for both human health and the oxidative stability of meat products, particularly concerning lipid oxidation.

While previous studies focused mainly on antioxidant properties, additional biological activities have also been explored. Takeda et al. (2020) assessed the ACE-inhibitory activities of pepsin-digested meats from venison, wild boar, beef, and pork. SDS-PAGE (Fig. 6) and SEC profiles indicated distinct molecular weight distributions among the digests, with digested venison exhibiting the highest ACE-inhibitory efficacy, correlating with its higher anserine content and lower carnosine content compared to the other samples. Further fractionation and LC-MS/MS analysis identified five ACE-inhibitory peptides from venison, with the sequence isoleucine-lysine-glutamic acid-valine-threonine-glutamic acid-arginine (IKEVTER) demonstrating the most potent activity.

In addition to in vitro digestion studies, research has also focused on characterizing BPs directly extracted from finished meat products. Escudero et al. (2013) purified

water-soluble peptides from Spanish dry-cured ham using SEC and RP-HPLC, followed by sequence identification *via* nano LC-MS/MS. This resulted in the identification of 73 peptide sequences, with molecular masses ranging from 374 to 1610 Da and chain lengths of 5–14 amino acid residues. Several synthesized peptides, particularly those with low molecular weight and favorable physicochemical properties, were effective ACE inhibitors in both in vitro and in vivo assays. Collectively, these findings indicate that Spanish dry-cured ham can be considered as a natural source of bioactive peptides with beneficial health implications, reinforcing the notion that meat and meat products possess significant functional potential beyond traditional nutritional value.

Peptidomics of fish and seafoods

Fish and their derivatives are important nutritional resources for humans due to their beneficial fatty acid composition, high-quality proteins, and substantial concentrations of minerals and vitamins (Carrera et al., 2013; J. Han et al., 2025; Yin et al., 2026). Recently, there has been an increased emphasis on seafood products, underscoring the necessity to ensure their safety, authenticity, traceability, and health benefits (Abel et al., 2022; Piñeiro et al., 2003; Rybicka et al., 2022). Fish hydrolysates have garnered significant attention for their abundant nutritional value and versatility. BPs derived from fish proteins found in skin, muscle, and waste materials exhibit various properties, including antioxidant, antimicrobial, antihypertensive, anti-obesity, and cryo-protective effects (Yi et al., 2018). The utilization of proteomics and in silico techniques can facilitate the rapid identification of proteins and peptides in fish and seafood products/by-products, as well as predict their potential bioactivities (Table 2).

Panjaitan et al. (2018) conducted an analysis of BPs derived from Giant Grouper (*Epinephelus lanceolatus*) roe proteins using SDS-PAGE and nLC-ESI-MS/MS techniques. The BIOPEP-UWM database suggested that the peptides exhibited significant bioactivities, primarily showing inhibitory effects on dipeptidyl peptidase-IV (DPP-IV) and ACE. Pepsin was identified as the most effective enzyme for the production of BPs, theoretically yielding 47 ACE-inhibitory peptides and 82 DPP-IV-inhibitory peptides. Consequently, Giant Grouper roe has the potential to serve as a raw material for the development of health-promoting products.

Furthermore, the characterization of the jumbo squid (*Dosidicus gigas*) skin proteome through shotgun LC-MS/MS led to the identification of 1,004 unique peptides belonging to 219 distinct proteins. Bioinformatics analyses indicated that the proteome of jumbo squid skin may provide novel BPs with antitumor, antimicrobial, and antihypertensive properties (Carrera et al., 2020).

In a separate study, LC-MS Orbitrap was utilized to investigate BPs (< 10 kDa) in the skin and residual materials of Atlantic herring (*Clupea harengus* L.). A total of 66 peptides were identified, with approximately 50% of these matches associated with motifs that may influence cardiovascular activity. Additionally, the identified peptides included those with immunomodulatory effects (8%) and antioxidant properties (approximately 40%). Notably, the distribution of peptides exhibiting specific activities was comparable across both sample types (Pampanin et al., 2012).

Song et al. (2012) isolated a peptide fraction from half-fin anchovy (*Setipinna taty*) that exhibited inhibitory activity against *Escherichia coli*. This fraction, designated as HAHp2-3-I, was purified *via* RP-HPLC. Identification through UPLC-LTQ-Orbitrap MS revealed the fraction comprised three anionic peptides with extended strand and random coil structures, alongside five cationic peptides with alpha helices. Scanning electron microscopy evaluations demonstrated that HAHp2-3-I induced irreversible membrane damage in *E. coli*.

Peptidomics of fruits and vegetables

Fruits and vegetables, along with residues from their processing, have been the subject of extensive research as potential sources for isolating biologically active components, including BPs (Sosalagere et al., 2022). Moayedi et al. (2017) utilized peptidomics analysis to examine how amino acid composition and peptide molecular weight affect the antioxidant and ACE-inhibitory activities of protein fragments sourced from fermented tomato waste. Through MALDI-TOF MS, they identified that peptides with molecular weights ranging from 500 to 800 Da exhibited the highest ACE-inhibitory activity, while two distinct peptide groups (500–800 Da and 1200–1500 Da) displayed the most significant antioxidant effects. Additionally, bioactive peptides derived from fermented tomato seeds (fermented using *Bacillus subtilis* A14h) were identified using nano-LC-MS/MS, confirming both ACE-inhibitory and antioxidant activities.

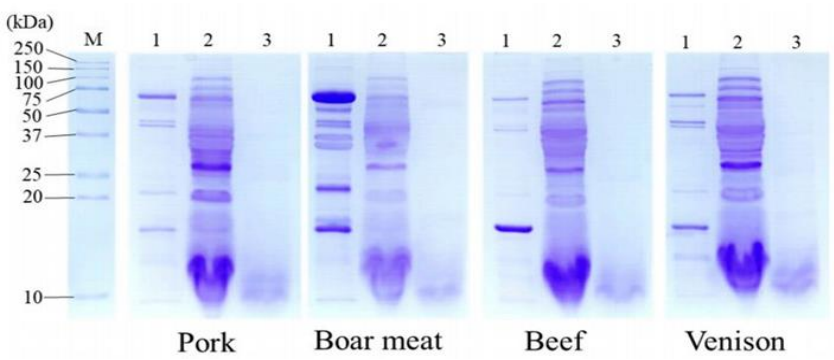

Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis profile of digested meats. Reprinted with permission from Takeda et al. (2020).

Table 2. Summary of proteomics and peptidomics analysis studies in fish and seafood products

Purpose	Method	Bioactivity	Reference
Identification of bioactive peptides from fish muscle proteins digested by *trypsin (*in vitro*)*	▪ Nano HPLC-MS/MS	▪ Antimicrobial activity	(Capriotti et al., 2015)
Study the peptides generated from the sarcoplasmic and myofibrillar porcine proteins after hydrolysis by *Lactobacillus sakei* CRL1862 and *Lactobacillus curvatus* CRL7	▪ RP-HPLC	▪ ACE-inhibitory	(Castellano et al., 2013)
Comparison of bioactivity and peptidomic profile of cooked beef, pork, chicken, and turkey meat after in vitro gastro-intestinal digestion	▪ Nano LC-ESI-Q-TOF-MS/MS	▪ ACE-inhibitory ▪ DPP-IV inhibitory	(Martini et al., 2019)
The proteomics homology of antioxidant peptides obtained from dry-cured Xuanwei and Jinhua ham	▪ LC-ESI-Q-TOF-MS/MS	▪ Antioxidant activity	(Xing et al., 2018)
Investigating the amino acid composition and peptide profile of different European dry-fermented sausages	▪ SEC ▪ MALDI-TOF-MS ▪ Nano LC-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity	(Gallego et al., 2018)
Profile of bioactive peptides generated during the preparation of dry fermented camel sausages	▪ RP-HPLC-ESI-Q-TOF-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity	(Mejri et al., 2017)
Profile of antioxidant peptides formation in fermented sausage	▪ SDS-PAGE ▪ LC-MS/MS	▪ Antioxidant activity	(Luan et al., 2021)
Investigating the effect of sodium caseinate addition on the final peptide profile of dry-fermented sausages	▪ MALDI-TOF-MS ▪ Nano-LC-MS/MS	▪ ACE-inhibitory ▪ Antioxidant activity ▪ Immunomodulator ▪ DPP IV inhibitor	(Mora et al., 2015)
Bioactivity determination of proteins peptides extracted from tilapia (*Oreochromis spp.*) processing co-products	▪ SDS–PAGE ▪ LC–ESI–MS/MS	▪ ACE-inhibitory ▪ Antithrombotic activity ▪ Antiamnesic activity	(Huang et al., 2015)
Identification of putative bioactive peptides obtained from the mucus proteomes of various gastropods	▪ 1D SDS-PAGE ▪ Nano LC-MS/MS	▪ Antihypertensive ▪ Drug-delivering ▪ Antiparasitic	(Tachapuripunya et al., 2021)
Biofunctionality of enzymatically derived peptides from Codfish (*Gadus morhua*) frame	▪ 1D SDS-PAGE ▪ LC-MS/MS	▪ Antioxidant activity	(Jafarpour et al., 2020)
Comprehensive identification of native medium-sized and short bioactive peptides in sea bass muscle	▪ Nano HPLC-MS/MS ▪ UPLC- MS/MS	▪ Antioxidant activity ▪ ACE-inhibitory	(Cerrato et al., 2021)

Nano HPLC-MS/MS: Nanoscale high-performance liquid chromatography coupled to tandem mass spectrometry; RP-HPLC: Reversed-phase high-performance liquid chromatography; ACE: Angiotensin-converting-enzyme; Nano-LC-ESI-Q-TOF MS/MS: Nano-liquid chromatography–electrospray ionization–quadrupole time-of-flight tandem mass spectrometry; SEC: Size exclusion chromatography; MALDI-TOF-MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; RP-HPLC-ESI-Q-TOF-MS/MS: Reversed-Phase High-Performance Liquid Chromatography–Electrospray Ionization–Quadrupole Time-of-Flight Tandem Mass Spectrometry; 1D SDS-PAGE: One-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis; UPLC-MS/MS: Ultra-performance liquid chromatography–tandem mass spectrometry; DPP-IV: Dipeptidyl peptidase-IV.

In a different processing approach, Meshginfar et al. (2018) demonstrated that ultrafiltration fractionation (UF) of tomato seed protein hydrolysate yielded three fractions: UF1 (> 10 kDa), UF2 (3–10 kDa), and UF3 (< 3 kDa). Among these, the UF3 fraction displayed the strongest antioxidant activity. Further separation of the UF3 fraction by RP-HPLC produced sub-fractions F1 to F7, with F2 and F4 exhibiting the highest antioxidant effects. The superior activity of fraction F2 was elucidated through LC-ESI-MS/MS, suggesting its potency is attributed to a higher abundance of peptides rich in tyrosine and histidine residues.

Extending the scope beyond tomato by-products, González-García et al. (2014) hydrolyzed extracted proteins from plum (*Prunus domestica* L.) by-products using several commercial proteases, subsequently identifying the resulting peptides through RP-HPLC-MS/MS and SDS-PAGE. Among the hydrolysates, the alcalase-treated sample demonstrated the highest antioxidant and ACE-inhibitory capacities. Furthermore, analysis via RP-HPLC coupled with RP-HPLC–ESI–Q-TOF revealed that the alcalase extract was particularly enriched with short-chain and highly hydrophobic peptides favorable for bioactivity.

In addition to conventional enzymatic hydrolysis, emerging physical technologies have been explored to enhance peptide recovery. Calleja-Gómez et al. (2026) assessed the application of pulsed electric fields (PEF) (50 kJ/kg, 2.5 kV/cm, 6 h) for improving the extraction of functional proteins and peptides from several mushroom species, including *Agaricus bisporus*, *A. brunnescens*, *Lentinula edodes*, *Pleurotus ostreatus*, and *Lactarius deliciosus*, in comparison to conventional maceration. Protein content and peptide profiles were analyzed using LC-TOF-MS/MS, while protein integrity before and after

in vitro digestion was evaluated by SDS-PAGE. Results indicated that both protein and peptide profiles were species-dependent, with PEF treatment significantly increasing total protein content (ranging from 8.7 to 22.6 g/100 g dry weight) and enhancing peptide recovery (by 3.35–65.84%). Notably, PEF also enriched the extracts with peptides known for anti-inflammatory, immunomodulatory, and anticancer activity, highlighting specific sequences such as VPP, YG, EAE, PY, and IPP, particularly in *P. ostreatus*, which yielded 249 potential bioactive peptides. SDS-PAGE analysis confirmed the preservation of protein integrity during PEF treatment and effective hydrolysis following in vitro digestion, with peptide fragments predominantly below 37 kDa.

These findings affirm that fruits/vegetables and their by-products are valuable, low-cost sources of bioactive peptides. Their functionality is significantly influenced by peptide size, amino acid composition, and the chosen extraction methodology. While enzymatic hydrolysis remains a validated technique, innovative physical technologies such as PEF offer improved recovery and broader bioactivity profiles. The integration of advanced peptidomics tools is crucial for sequence identification and elucidation of structure-activity relationships, ultimately enhancing the valorization of agro-industrial residues within a sustainable circular economy, provided future efforts focus on scale-up, in vivo efficacy, and optimization of green processing.

Peptidomics of cereals and legumes

Cereals are recognized as a vital source of food, supplying approximately 50% of the recommended daily intake (RDI) of carbohydrates and 33% of the RDI for proteins for the global population (Alves et al., 2019; Ortiz-Martinez et al., 2014). Recent advancements in proteomics have effectively facilitated the detection and identification of bioactive proteins and peptides in cereal and legume-based products. Zheng et al. (2019) demonstrated through proteomic and in vitro analyses that quinoa bran albumin, when hydrolyzed with trypsin and alcalase, yields antioxidant and ACE inhibitory peptides, which maintain their bioactivity after gastrointestinal digestion.

In a separate study, Tamam et al. (2019) investigated BPs in tempeh produced under varying sanitation conditions. They employed ultrafiltration (< 3 kDa), GFC, and RP-HPLC for peptide separation followed by LC-MS. Their results indicated that tempeh producers with good sanitation levels had a higher content of BPs (58%) compared to those with moderate (43%) or poor (35%) sanitation. The tempeh products demonstrated antioxidant, antihypertensive, antidiabetic, and antitumor peptide activities.

Similarly, an LC-MS/MS analysis of soybean fermented with various *Bacillus* species revealed that while some peptides were common across the different fermented products, a notable number of unique peptides were produced. The peptide SEDDVFVIPAAYPF was shown to interact with myeloperoxidase, an enzyme associated with elevated oxidative stress, indicating that the fermented soybean contained specific peptides with relevant health benefits (Sanjukta et al., 2021).

Additionally, Heymich et al. (2021) utilized micro-LC–ESI–Q-TOF-MS/MS to analyze the peptide profiles of chickpeas. They identified twenty-one peptide sequences with potential antimicrobial properties, notably Leg1 (RIKTVTSFDLPALRFLKL) and Leg2 (RIKTVTSFDLPALRWLKL), exhibiting antimicrobial activity at 10 to 1,000 times more potent than conventional food preservatives.

In a peptidomic analysis by Zhao et al. (2025) using LC-MS/MS on dynamically digested samples, distinct peptide profiles were observed among oat, soy, almond, flax, and cow milks. Soy milk (2,495 peptides) and cow milk (1,597 peptides) displayed the highest peptide diversity, whereas flax milk had the fewest (53 peptides). During gastric digestion, the majority of peptides fell within the 752–1814 Da range, with oat milk showing the highest proportion (95.69%). After gastrointestinal digestion, trypsin hydrolysis shifted the peptide profile towards smaller peptides, with oat and flax milk peptides exclusively within the 762–1814 Da range, while cow and soy milk retained detectable high-molecular-weight peptides (1814–5000 Da). Bioactive peptide prediction using PeptideRanker indicated that cow milk gastric digests contained the highest proportion of potentially active peptides (23.73%), followed by soy milk (19.17%). In gastrointestinal digests, almond and flax milk exhibited 100% bioactive peptide content. Database matching against BIOPEP-UWM further confirmed the presence of multifunctional peptides with ACE-inhibitory and DPP-IV inhibitory activities across all milk types, particularly highlighting soy milk, almond milk, and cow milk as promising sources for bioactive peptide supplementation. This research underscores the efficacy of peptidomics as a valuable tool for assessing the nutritional and functional quality of plant-based milk alternatives.

Peptidomics of nuts

Nuts are nutrient-dense foods that serve as an excellent source of health-promoting bioactive compounds. Regular consumption of nuts is widely recommended to fully harness the benefits of their nutrients, antioxidants, and bioactive substances. These compounds may work synergistically to reduce the risk of cardiometabolic and other non-communicable diseases through various mechanisms (Alasalvar et al., 2020). Nut and nut-based products are rich in potential BPs, which have prompted the application of proteomic methods to identify specific peptide sequences derived from nut proteins.

For instance, hydrolysis of wild almond proteins using multiple enzymes including pepsin, trypsin, chymotrypsin, alcalase, and flavourzyme yielded bioactive hydrolysates exhibiting ACE inhibitory activity. Among these, the alcalase-derived hydrolysate demonstrated the highest bioactivity, followed by those from chymotrypsin, trypsin/pepsin, and flavourzyme. Further separation of alcalase hydrolysate through sequential ultrafiltration (cut-off at 3 kDa and 10 kDa) revealed that the most active fraction (< 3 kDa) underwent additional separation via RP-HPLC. The identification of peptides was conducted using LC-ESI-MS/MS, resulting in the discovery of over 40 potential ACE-inhibitory sequences (Mirzapour et al., 2017).

Additionally, the peptide composition of defatted walnut meal hydrolysates was characterized using HPLC-FTMS analysis, identifying eight novel peptides that exhibited significant in vitro antioxidant activity on hydrogen peroxide-injured nerve cells (Sheng et al., 2019). A new ACE-inhibitory peptide, EPNGLLLPQY, was successfully identified from walnut protein hydrolysate using UPLC-ESI-Q-TOF-MS/MS (Wang et al., 2018). Moreover, Liu et al. (2016) discovered novel ACE-inhibitory peptides (< 1.5 kDa) from almond protein, utilizing a combination of ultrafiltration, GFC, and reversed-phase preparative chromatography. The identified peptide sequences were Met-His-Thr-Asp-Asp and Gln-His-Thr-Asp-Asp, indicating that almond proteins are a rich source of potential antihypertensive peptides suitable for incorporation into functional foods and beverages (R.-L. Liu et al., 2016).

The peptidomics study conducted by Zheng et al. (2025) examined the structural evolution and bioactive peptide generation of two walnut-derived peptides, LPLLR (LP5) and TWLLPR (TW7), following in vitro simulated gastrointestinal digestion. LC-MS/MS analysis revealed six digestion-derived peptides, i.e., LPLL, PLLR, WLPLPR, TWLLPLP, TWLLPL, and LPLPR, with molecular weights ranging from 454 to 780 Da. Both parent peptides underwent substantial hydrolysis during intestinal digestion, with residual concentrations decreasing to 4.1 μg/mL for LP5 and 8.8 μg/mL for TW7. Concurrent physicochemical characterization indicated significant structural reorganization, including an increase in β-turn and random coil content, enhanced UV absorption indicating aromatic residue exposure, reduced particle size, and modified hydrogen-bonding environments. These changes corresponded with markedly improved antioxidant activity in ABTS and DPPH assays. In silico bioinformatics screening, utilizing PeptideRanker and ADMET predictions, identified TWLLPLP as the most promising anti-inflammatory candidate, achieving high bioactivity scores (0.86), favorable blood-brain barrier permeability potential (0.412), and no predicted toxicity. Molecular docking studies demonstrated strong binding affinities of TWLLPLP to inflammatory targets iNOS (−9.9 kcal/mol) and NLRP3 (−10.2 kcal/mol), with binding stability facilitated by hydrogen bonds (involving residues PHE706, GLU861, ARG697, and LYS885) and hydrophobic interactions. Molecular dynamics simulations over a 100 ns period confirmed the dynamic stability of the TWLLPLP-NLRP3 complex, showing root mean square deviation equilibration after 20,000 ps and a binding free energy of −45.69 kcal/mol, thus supporting its potential as a stable anti-inflammatory agent. This integrated peptidomics and computational approach effectively connected digestive processing to structure-activity relationships, providing a complete framework for screening and designing peptide-based functional food ingredients optimized for digestive resilience and bioactivity.

Another peptidomics study by Zhang et al. (2025b) examined the impact of walnut kernel pellicle on the composition, properties, and bioactivity of enzymatic hydrolysates derived from walnut meal. Utilizing LC-MS/MS-based peptidomics, a total of 3,423 peptide sequences were identified across peeled walnut protein hydrolysates (PWPH) and unpeeled walnut protein hydrolysates (UWPH). The presence of walnut kernel pellicle significantly influenced alkaline protease cleavage patterns on walnut precursor proteins, thereby affecting both the quantity and size of released peptides. Principal component analysis confirmed distinct compositional differences between PWPH and UWPH, while bioinformatics predictions suggested that peeling enhances peptide release, increases the formation of bioactive peptides, reduces potential allergenicity, and improves water solubility. Notably, seven peptides with acetylcholinesterase (AChE) inhibitory activity were identified, with the octapeptide Val-Gly-Ala-Pro-Phe-Asp-Gly-Ala (VGAPFDGA) demonstrating the strongest inhibitory effect, with an IC_{50} of 0.38 ± 0.01 mg/mL. This research highlights walnut kernel peeling as a crucial processing step that fundamentally alters the peptidomic profile and functional characteristics of walnut protein hydrolysates, reporting seven novel AChE-inhibitory peptides with potential applications in the management of neurodegenerative diseases.

CURRENT CHALLENGES IN PEPTIDOMICS

Bioinformatics and peptidomics are two computational and analytical methodologies that have the potential to accelerate the development of BPs from laboratory research to market applications. The structure–activity relationships identified in peptides serve as the foundation for bioinformatics and the in silico prediction of bioactive sequences inherent in food proteins. Conversely, peptidomics utilizes hyphenated techniques, specifically LC coupled with mass spectrometry, for the detection, profiling, and quantification of peptides. Collectively, the integration of bioinformatics and peptidomics provides a cost-effective and efficient approach for predicting, profiling, and screening bioactive protein hydrolysates and peptides derived from food sources (Agyei et al., 2018).

The discovery and analysis of BPs derived from food sources present significant challenges; however, the integration of bioinformatics and peptidomics offers promising solutions. Each approach carries inherent limitations, yet recent advancements are facilitating more efficient and comprehensive peptide research.

A primary limitation of bioinformatics is its reliance on predicting bioactivity solely from primary amino acid sequences, neglecting the three-dimensional conformation of proteins. The tertiary structure of proteins influences protease accessibility, leading to a discrepancy where in silico digestion predicts a higher yield of peptides than what is obtained experimentally. Furthermore, the presence of bioactive motifs alone may not reliably forecast actual activity, as various experimental conditions including pH, temperature, and ionic strength can affect outcomes. To enhance prediction accuracy, future bioinformatics tools should incorporate protein structures and specific reaction conditions into their models (Agyei et al., 2018).

Another challenge lies in the failure of current tools to account for post-translational modifications (PTMs), such as oxidation, methylation, and acetylation. These modifications frequently occur during food processing methods, including heating and drying. To mitigate this issue, integrative tools such as iPTMnet, STRAP PTM,

and iEcho have been developed to study PTMs and can be incorporated into workflows for food peptide analysis. Additionally, the selection of protein precursors is often restricted to those with sequences available in open-access databases, potentially excluding minor or alternative-source proteins. Advances in protein sequencing technologies targeting sustainable sources such as single-cell proteins, marine organisms, and edible insects are necessary to broaden the database (Rajendran et al., 2016; Spencer et al., 2013; Wu et al., 2017; Zhao et al., 2015).

Peptidomics encounters difficulties in the separation and identification of peptides within complex food matrices. Co-elution in LC complicates the detection of less concentrated peptides. Moreover, many analyses focus on multiply charged ions (with charge states ranging from 2+ to 5+), often overlooking peptides smaller than 800 Da, which are frequently the most potent and bioavailable. To address co-elution effects, tandem fragmentation techniques such as collision-induced dissociation (CID), high-energy collision-induced dissociation (HCD), and electron transfer dissociation (ETD) can be effectively combined, as these methods have demonstrated success in comprehensive peptide profiling using LC-FT MS/MS (Shen et al., 2011).

Technological progress in biomolecular sciences can be applied to food peptidomics. For instance, imaging mass spectrometry allows for spatial visualization of compounds, ion mobility mass spectrometry provides rapid separation and detection capabilities, and direct tissue/cell MALDI-TOF analysis facilitates profiling in intact samples. Though these innovative techniques are not yet commonly employed in the study of food peptides, they offer significant potential for future research (Dallas et al., 2015).

CONCLUSION

Research on bioactive peptides has grown considerably over the past two decades, driven by their potential application in functional food and nutraceutical formulations. However, the complexity of these compounds necessitates the use of advanced analytical methods for their study. The production processes and sources of BPs significantly influence their application in food. Consequently, the effective integration of proteomics and peptidomics in BP research and engineering is essential for identifying peptides of interest, optimizing production, controlling variables, assessing product quality and safety, and understanding receptor-BP interaction mechanisms. Maintaining control over BP stability during processing and storage is crucial due to the potential interactions between peptides and other food components. This control helps prevent the formation of toxic or allergenic substances and ensures that the desired dosage is preserved for the optimal utilization of these valuable resources.

Technological advancements are central to innovative peptidomics methodologies. Continuous enhancements in mass spectrometry, particularly through increased sensitivity, accuracy, and resolution, are pivotal. A notable trend is the transition from data-dependent acquisition to data-independent acquisition, which enhances peptide identification coverage and reproducibility. The introduction of the Orbitrap mass analyzer marks a significant advancement, offering improved resolution and speed that facilitates the rapid identification of thousands of peptides and enable de novo sequencing.

Looking into the future, the emergence of artificial intelligence (AI) presents a potential paradigm shift in the field of MS/MS sequencing. AI has the capability to analyze extensive mass spectral datasets more efficiently than existing database-dependent software, allowing it to identify complex patterns and potentially enhance the accuracy of post-translational modification identification. Although still in the early stages of development, alternative technologies, such as single-molecule protein sequencing via biological nanopores, can turn into exciting future possibilities for achieving single-molecule sensitivity, despite not yet being implemented widely.

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DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

The authors declare that they have not done any experiment on humans or animals in this research.

DATA AVAILABILITY

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