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REVIEW PAPER

METAPROTEOMICS APPLIED TO THE IDENTIFICATION OF PATHOGENS IN FOODS: ADVANCES AND CHALLENGES IN SANITARY MONITORING

JOELLINGTON MARINHO DE ALMEIDA[1]*, RHAIRA RODRIGUES DALLA[1], VALTER OLIVEIRA DE SOUTO[2], MARGARETH SANTOS DA COSTA PENHA[3], JULIA BIBIANA MERCHAN-GAITAN[4], THALITA CRISTYNE DE OLIVEIRA ALVES[1], ZULENE LIMA DE OLIVEIRA[5], MERI BEATRIZ DA ROCHA ZANETTI[5], JULIENY ALINE DA SILVA NASCIMENO[6], KÊNIA VICTÓRIA PEREIRA ABDALA[3], JOÃO BOSCO CAVALCANTE DA SILVA SEGUNDO[7], ROGERIO SILVA DE ALMEIDA[7], JENNYFER SILVA CAMPOS[8], RODRIGO BARBOSA VIEIRA[8], ANDRÉ LUCAS DE SOUZA LOPES[8], LETÍCIA SOUZA DE OLIVEIRA RÊGO[9], CARLOS EDUARDO ASSIS DA SILVA[8]

[1] *Federal University of Espírito Santo, 514 Fernando Ferrari Avenue, 29075910 Vitória, Brazil*

[2] *Federal Institute of Santa Catatina, 225 Heitor Villa Lobos Street, 88506400 Lages, Brazil*

[3] *Federal University of Maranhão, 1966 Portugueses Avenue, 65085580 São Luís, Brazil*

[4] *Santo Tomás University, 2885 Héroes de la Concepción Avenue, 1100000 Iquique, Chile*

[5] *Federal University of Santa Catarina, Eng. Agronômico Andrei Cristian, 88040900 Florianopólis, Brazil*

[6] *Luiz de Queiroz College of Agriculture – University of São Paulo, 11 Pádua Dias Avenue, 13418900 Piracicaba, Brazil*

[7] *Federal University of Paraíba, Campus I, 58051900 Cidade Universitária, Brazil*

[8] *Darcy Ribeiro Noth Fluminense State University, 2000 Alberto Lamego Avenue, 28013602 Campos dos Goytacazes, Brazil*

[9] *Palmas College, 40 Quadra ACSU NS Avenue, 77021624 Tocantins, Brazil*

*Corresponding author: joellingtonalmeida@gmail.com

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Abstract

Metaproteomics has emerged as an innovative tool for the identification of foodborne pathogens, enabling the analysis of proteins and peptides from complex microbial communities. The present study aimed to examine how metaproteomics is associated with the microbiological control of pathogenic organisms in foods, acting in their detection and control. This approach allows the simultaneous detection of multiple pathogenic microorganisms, contributing to food safety and outbreak control. Peptide-based microbial biodiversity reveals the role of specific proteins in the virulence and resistance of pathogens such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Campylobacter jejuni*, making the development of effective detection methods essential. Proteomics plays a key role in microbiological quality control of foods, as it enables the identification of toxins and protein biomarkers indicative of contamination. Furthermore, mass spectrometry combined with bioinformatics allows accurate analysis

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of microbial proteins, supporting the implementation of preventive measures. The integration of metaproteomics with metagenomics and metabolomics broadens the understanding of microbial ecology and supports the development of strategies to reduce pathogen dissemination and antimicrobial resistance. Despite technical and standardization challenges, this approach offers new perspectives to improve food safety and prevent foodborne diseases.

Keywords: food safety, mass spectrometry, microbial communities, protein identification, sanitary control, systems biology

Introduction

Metaproteomics is a term used to describe the large-scale understanding of proteins and peptides from complex microbiotas at a given point in time (Wilmes and Bond, 2004; Cavalcante *et al*., 2025). In the food sector, this technique can assist in the simultaneous detection of multiple pathogens in products susceptible to the growth of microorganisms harmful to human health and responsible for foodborne outbreaks (Wilmes *et al*., 2015; Chen *et al*., 2017).

This technique belongs to the field of biotechnology dedicated to the comprehensive analysis of proteins and has established itself as a crucial tool for food quality assessment (Yang *et al*., 2025). The increasing demand for safe and high-quality products creates the need for analytical techniques that are more precise and sensitive. In this context, proteomics enables the discovery of protein biomarkers that indicate food authenticity, the presence of adulterating substances, and even contamination by microorganisms, thereby contributing to a safer food supply chain (Castro *et al*., 2023).

The genetic diversity of microorganisms present in foods directly influences their ability to adapt, their virulence, and their resistance to adverse conditions (Herschend *et al*., 2017; Heyer *et al*., 2025), in *Listeria monocytogenes*, for example, distinct serotypes and lineages differ in ecological fitness and pathogenic potential, with serotypes 1/2a, 1/2b, 1/2c, and 4b being most frequently associated with human listeriosis (Karakaya *et al*., 2025). Genomic plasticity also facilitates the acquisition of mobile genetic elements carrying antibiotic resistance genes (Moura *et al*., 2024). Additionally, lineage-dependent differences in biofilm formation enhance persistence in food-processing environments (Romeo *et al*., 2025). At the proteomic level, variability in protein sequences and differential protein accumulation allows pathogens such as *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* to develop specific strategies for colonization, survival, and evasion of the immune system (Staes *et al*., 2019; Johansson and Freitag, 2019; Meysman *et al*., 2013). Metaproteomic studies reveal that proteins involved in cell adhesion, biofilm formation, and antimicrobial resistance show significant variation among strains, highlighting the importance of proteomic analysis for more accurate food safety monitoring. Thus, the characterization of microbial protein diversity can provide valuable insights for the development of more effective control strategies (Ruiz *et al*., 2025).

Microbiological quality control of foods depends on increasingly sensitive and specific methods for the detection of pathogenic microorganisms. The integration of mass spectrometry with bioinformatics enhances proteomic data analysis, enabling more robust interpretation and the construction of efficient predictive models (Heyer *et al*., 2019). Therefore, the aim of this study was to evaluate how metaproteomics can be used as a promising tool for the detection and control of pathogenic microorganisms capable of causing foodborne outbreaks and compromising food quality.

Biological diversity in food systems based on microbial effectors

The understanding of pathogenic microorganisms capable of developing in food products represents a major public health challenge (Karageorgos *et al*., 2016). Fresh foods are more susceptible to the proliferation of bacteria and fungi; however, processed products may also become conducive to the growth of undesirable microorganisms when failures occur during production or when they are improperly stored (Balan *et al*., 2024).

Foods are considered important routes of exposure to antimicrobial-resistant bacteria, which are capable of infecting humans. The genetic diversity based on proteins and peptides of pathogenic microorganisms present in foods is therefore a crucial field for food safety and for the control of pathogens that compromise public health (Banerjee *et al*., 2018). Pathogenic microorganisms such as *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter jejuni* are responsible for a large proportion of foodborne diseases (Lensch *et al*., 2024).

The ability of these pathogens to produce specific proteins and peptides that facilitate adhesion, invasion, and survival in hostile environments such as the human gastrointestinal tract or adverse conditions in foods is a determining factor in their pathogenicity. The genetic diversity of these pathogens, reflected in variations in their proteins and peptides, plays an essential role in virulence and resistance, making their control a continuous challenge (Andersen *et al*., 2001; De Wit *et al*., 2022).

Peptides and proteins produced by pathogenic microorganisms in foods can be classified into different types, such as enzymes, toxins, and adhesion factors. Toxins, for example, play a fundamental role in the pathogenicity of microorganisms such as *Clostridium botulinum* and *Staphylococcus aureus* and are responsible for severe foodborne intoxications (Andersen *et al*., 2001). The genetic diversity of these toxins is strongly influenced by environmental factors, such as temperature, pH, and food composition, which can affect gene expression and protein production (Guérin *et al*., 2016). Variations in toxin structure may influence their potency and their ability to cause damage to the host organism. Understanding the genetic diversity of pathogenic microorganisms and their bioactive products is therefore essential for the development of effective detection and control methods (Becker *et al*., 2003).

In addition to toxins, pathogenic microorganisms present in foods also produce adhesion and invasion proteins that are essential for host colonization and infection.

Surface proteins, such as fimbriae and membrane proteins, allow these bacteria to attach to intestinal cells, facilitating their persistence in food environments and within the host organism (Larsonneur *et al*., 2016). The genetic diversity of these adhesion proteins can result in different colonization strategies, depending on the bacterial lineage and the type of food. Furthermore, these microorganisms may secrete proteins that interfere with host immune responses, enabling them to evade detection or destruction by the immune system (Cremonesi *et al*., 2005).

Antimicrobial resistance, which is closely linked to the genetic diversity of proteins and peptides, is also an important factor in the persistence of pathogenic microorganisms in foods (De Wit *et al*., 2022). Resistance to antibiotics is often mediated by proteins that act as efflux pumps or enzymes that inactivate antimicrobial agents. This genetic diversity provides adaptive advantages to these pathogens, allowing them to survive in environments where antibiotics or other antimicrobial agents are used (Larsonneur *et al*., 2016).

Moreover, horizontal gene transfer of resistance determinants among different microorganisms present in foods can accelerate the spread of resistance, making microbiological control even more difficult (Farrukh *et al*., 2025). In this context, metaproteomics emerges as a promising tool for the study of complex contaminated food samples (Chen *et al*., 2017). Figure 1 illustrates a functional strategy ranging from sample preparation to mass spectrometry analysis for the identification of proteins and/or peptides produced by pathogenic microorganisms. In addition, the use of new technologies, such as genomics and proteomics, has been fundamental for investigating the genetic diversity of pathogenic microorganisms in foods. Through whole-genome sequencing, it is possible to identify genes involved in the production of proteins and peptides, including hydrolytic enzymes, toxins, and virulence factors (Fitzgerald *et al*., 2001).

Proteomics, in turn, enables the analysis of proteins naturally present and released by these pathogens, allowing the identification of key molecules involved in their survival and virulence processes. An example is the LIPI-1 pathogenicity island, which includes the gene *hly*, responsible for encoding listeriolysin O (LLO) in *Listeria monocytogenes* (Kaptchouang Tchatchouang *et al*., 2020; Seveau, 2014) and plays a central role in the disruption of the phagocytic vacuole and the progression of infection by the etiological agent. These advances not only improve the understanding of the mechanisms underlying food contamination, but also support the development of new methods for the detection, prevention, and treatment of foodborne infections (Braga *et al*., 2016).

A deeper understanding of the genetic profiles and the characteristics of proteins and peptides produced by these pathogens allows the development of more precise and rapid techniques for detecting contaminants in foods (Escobar *et al*., 2023). In addition, the identification of specific proteins may open new avenues for control strategies, such as the use of natural antimicrobials or antimicrobial enzymes, which can be applied directly to foods or during processing (Yang *et al*., 2020). Thus, the study of the genetic diversity of pathogenic microorganisms not only contributes to

food safety but also offers innovative approaches to reduce public health risks associated with food contamination (Flörl *et al*., 2025).

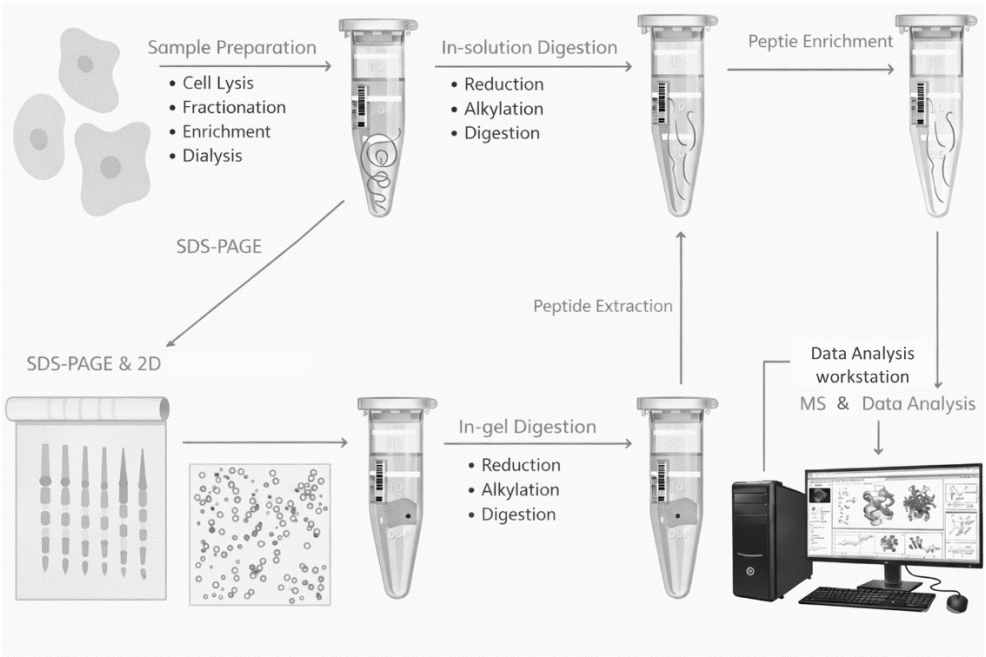

Figure 1. Mass spectrometry–based metaproteomic workflow.

The scheme outlines the analytical steps employed in the functional characterization of complex microbial communities, including sample preparation (cell lysis, fractionation, enrichment, and dialysis), protein separation by SDS-PAGE and two-dimensional electrophoresis, enzymatic digestion in solution and in gel (reduction, alkylation, and digestion), peptide extraction and enrichment, followed by mass spectrometry (MS) analysis and computational data processing. This integrated workflow enables the identification of proteins and peptides and the inference of metabolic functions, contributing to the understanding of the composition and metabolic activity of microbiomes associated with different biological matrices. Source: Authors.

Proteomics as a tool for microbiological quality control of food

Within the field of food safety, proteomics plays a crucial role in the identification of pathogens and toxins in food products (Flörl *et al*., 2025). The detection of pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* can be achieved through the analysis of proteins produced by these organisms. This approach enables rapid and accurate detection, allowing preventive actions to be implemented before contaminated products reach the final consumer (Valeriano *et al*., 2012).

In microbiological control, proteomics enables the identification and characterization of proteins expressed by microorganisms that are directly involved in virulence and pathogenicity. For example, hemolysins and enterotoxins can be detected and characterized using proteomic approaches. Enterotoxin A is a virulence

factor specifically associated with *Staphylococcus aureus*, whereas in *Escherichia coli*, the equivalent enterotoxin involved in pathogenic processes is enterotoxin 1 (Ménard and Dubreuil, 2008; Verbisck, 2017). This strategy supports the early detection of pathogenic and spoilage organisms, contributing to food safety. Furthermore, proteomic analysis makes it possible to assess the proteome and verify the presence of virulent strains in comparison with less or more hazardous variants, providing essential information for the adoption of preventive measures in the food industry (Medeiros *et al*., 2014).

Another important application of proteomics in microbiological control is the evaluation of the effectiveness of antimicrobial treatments and food preservation technologies (Sá *et al*., 2025). By analyzing the protein profiles of microorganisms subjected to different conditions, such as radiation, temperature, and pH, it is possible to understand their proliferation rates using predictive microbial growth models. This information is critical for optimizing industrial processes and ensuring that foods remain safe throughout the production and storage chain (Armengaud, 2023). Through protein analysis, it is also possible to evaluate microbial responses to various environmental conditions, including exposure to modified atmospheres, the use of natural antimicrobials, and the application of emerging technologies such as cold plasma and ultraviolet light. These approaches support the development of innovative strategies to extend food shelf life without compromising sensory and nutritional properties (Lisboa *et al*., 2024).

The evaluation of differentially expressed proteins in pathogenic microorganisms contributes to the understanding of resistance and adaptation mechanisms in bacteria, viruses, and fungi under different environmental conditions and antimicrobial agents, particularly in food processing contexts (Figure 2) (Bahule *et al*., 2022). Figure 2 also illustrates that the diversity of proteins identified through microbiological processes in food matrices is linked to structural and functional aspects and to their involvement in multiple metabolic pathways. The detection of proteins associated with biofilm formation enables the development or improvement of more effective strategies for controlling foodborne outbreaks and for surface decontamination in industrial facilities. In this way, proteomics supports the formulation of more efficient sanitation protocols and the rational use of preservatives in foods (Bergamo, 2020).

The integration of proteomics with other approaches, such as metagenomics and metabolomics, provides a comprehensive view of microbial ecology in foods (Holst-Jensen *et al*., 2003). This combined strategy not only enables the identification of the microorganisms present but also allows a better understanding of their roles within the food environment and their interactions with other microorganisms. As a result, it supports the development of more accurate methods for microbiological monitoring, reducing losses and ensuring the quality and safety of food products (Bodkhe *et al*., 2025).

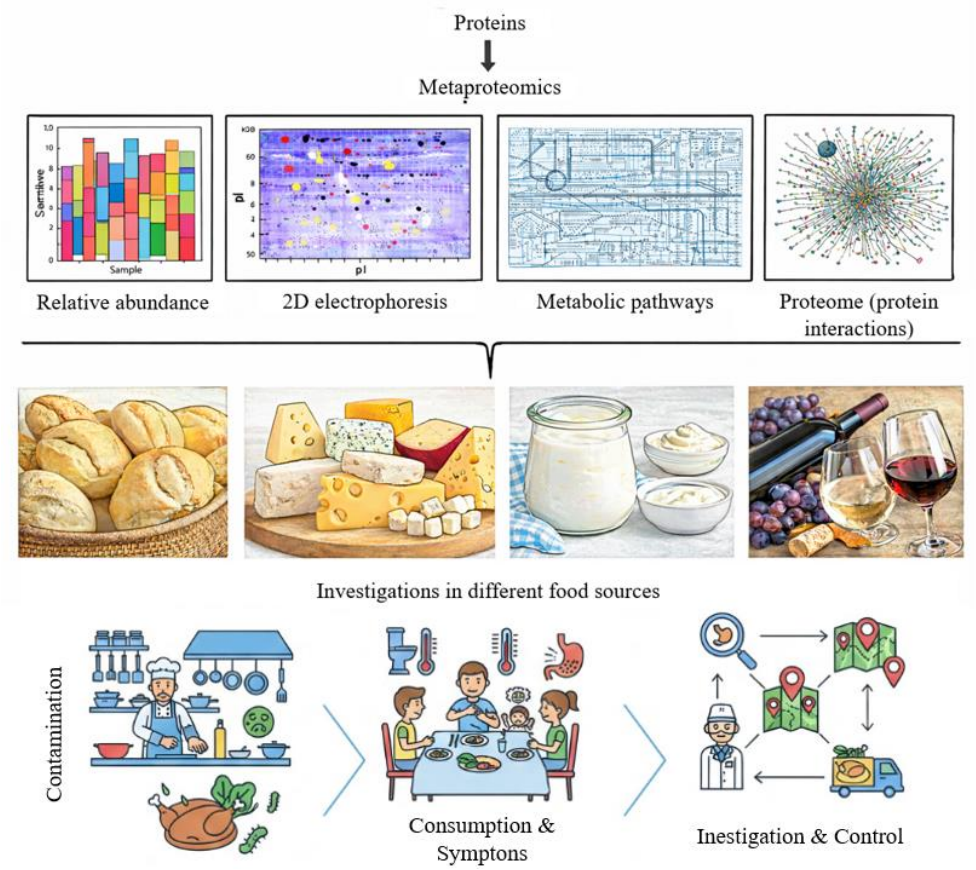

Figure 2. Metaproteomics as an integrative approach for investigating foodborne outbreaks.

By enabling the analysis of relative protein abundance, protein separation by two-dimensional electrophoresis, reconstruction of metabolic pathways, and protein–protein interaction networks, metaproteomics provides functional insights into complex microbial communities associated with different food matrices. Applied across diverse food sources, this approach supports the identification of contamination events, links microbial activity to consumption-related symptoms, and contributes to outbreak investigation, traceability, and control strategies within food safety systems. Source: Authors.

Metaproteomics as a multifunctional approach for food authentication and pathogen identification

The increasing complexity of the food supply chain demands faster, more accurate, and high-throughput analytical methods. Omics technologies (genomics, proteomics, metabolomics, and lipidomics) have emerged as integrative sciences in conjunction with biostatistics, chemometrics, and bioinformatics (Zhang *et al*., 2024). In addition, traditional techniques are becoming increasingly costly and time-consuming. The integration of different omics platforms overcomes the limitations of isolated analyses, providing a deeper understanding of complex biological mechanisms and ensuring more robust authentication, enabling traceability and the

detection of biological hazards and product originality. Food fraud is driven by economic motivations, involving the intentional addition of ingredients (e.g., starch in cheese) (Lima *et al*., 2025), substitution with cheaper varieties (e.g., wines) (Miele, 2012), falsification of records, and misleading labeling of geographical origin or production methods (e.g., champagne) (Valente *et al*., 2012). Food authenticity is defined by three main attributes: quality (intrinsic and nutritional characteristics), origin (geographical and biological traceability), and declaration (truthfulness of label information) (Zhang *et al*., 2024).

Furthermore, metaproteomics expands omics-based analysis to complex microbial communities, such as biofilms, which are relevant both to food contamination and industrial processes. Herschend *et al*. (2017) identified that the species *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans*, and *Paenibacillus amylolyticus* demonstrated that co-cultivation increases biofilm formation by up to fivefold. This phenomenon becomes possible due to the presence of microbial effectors responsible for promoting, inhibiting, or eliminating the growth of other microorganisms through the synthesis of natural antibiotics (Heyer *et al*., 2025). Additionally, the presence of toxins and antimicrobial peptides (AMPs), also known as ribosomally synthesized peptides, as well as non-ribosomal peptides (NRPs), are spontaneously produced as defense mechanisms against viruses, fungi, and competing bacteria. In this context, metaproteomics can identify the presence of diverse effectors within microbial communities present in samples, influencing the dynamics of host-related responses (Heyer *et al*., 2025).

Protein labeling (protein stable isotope probing – protein-SIP, through the incorporation of ^{2}H, ^{13}C, ^{15}N, or ^{18}O) (Von Bergen *et al*., 2013; Kleiner *et al*., 2020; Starke *et al*., 2020), and click chemistry approaches (bioorthogonal non-canonical amino acid tagging – BONCAT, via the incorporation of non-canonical amino acids such as 1-homopropargylglycine (HPG) and 1-azidohomoalanine (AHA)) (Van Kasteren and Rozen, 2023) are auxiliary techniques for the identification and quantification of effectors involved in microbial metabolic activity (Heyer *et al*., 2025). In parallel, the addition of taxonomic analysis contributes to the identification of organisms present in the sample, linking proteins to their respective taxonomic origins (Bossche *et al*., 2025). This technological integration enhances food product authenticity and ensures its microbiological quality.

Bioinformatic infrastructure for the analysis and interpretation of metaproteomic data

Metaproteomics generates large volumes of highly complex data, mostly derived from liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), requiring a robust bioinformatic infrastructure to enable accurate and reproducible data preprocessing, analysis, and interpretation. The analysis of metaproteomic data follows a multi-step workflow that includes raw spectra conversion, quality control, peptide and protein identification, relative or absolute quantification, and functional and taxonomic annotation (Liao *et al*., 2018).

Chen *et al*. (2020), in their review of the main bioinformatics methods applied to mass spectrometry–based proteomic data analysis, emphasize that the selection of

appropriate bioinformatics tools and databases is critical for the success of these steps, since high microbial diversity and sequence redundancy can hinder protein identification and reduce analytical sensitivity (Arıkan and Atabay, 2024). To address these challenges, platforms such as iMetaLab and Galaxy have been developed to automate much of the data processing, promoting greater standardization and comparability among complex datasets (Heyer *et al*., 2017).

As an example, Cheng *et al*. (2017) developed MetaLab, a platform capable of integrating sample-specific database generation, peptide and protein identification and quantification, as well as taxonomic profiling directly from raw mass spectrometry data, all within a single automated workflow. The development of this pipeline significantly accelerated the identification of redundant spectra and the construction of a reduced database, decreasing processing time without loss of analytical sensitivity, while also enabling the estimation of the relative abundance of taxa in complex microbiomes, such as mouse gut microbiome samples.

Another crucial component of metaproteomic bioinformatic infrastructure is the construction of representative protein databases. Because microbial communities typically comprise thousands of species, the coverage of reference sequences directly influences protein identification rates and subsequent functional interpretation. To overcome this limitation, studies have increasingly built protein databases from cultured or metagenomic references, which serve as tailored reference sequence sets for peptide identification in mass spectrometry analyses. For example, Gao *et al*. (2025) constructed a protein database based on cultured genomes of microorganisms present in the human gut (CGR2), including more than 3,000 sequenced genomes of isolated bacteria, so that the database more comprehensively represented common microbial groups and increased peptide identification rates, including those from low-abundance species. At the same time, this approach provided more accurate taxonomic and functional annotations compared with traditional databases derived solely from metagenomes or isolated public repositories. This strategy demonstrated that it is possible to generate a protein sequence "standard" that more closely reflects the actual composition of a specific microbiota, thereby increasing both the sensitivity and taxonomic resolution of the analytical pipeline.

In addition to computational requirements and representative databases, the biological interpretation of metaproteomic profiles poses an additional challenge. This step involves not only the in silico functional assignment of identified proteins, but also their integration into the biological and ecological context of the system under study. Studies addressing best practices in metaproteomics highlight that effective interpretation requires the integration of multifactorial approaches, such as metatranscriptomics and metabolomics, to validate protein expression and link it to specific biological processes (Van *et al*., 2025). Integrated analytical strategies help overcome the limitations imposed by data complexity and incomplete functional information in public databases. Despite recent advances in metaproteomic bioinformatics, significant technical and methodological challenges remain, including workflow standardization, robust statistical analysis, and the development

of scalable tools capable of handling large volumes of heterogeneous data (Heyer *et al*., 2017).

In the context of public food safety, this bioinformatic infrastructure has supported the understanding not only of the presence but also of the actual functional activity of microbial communities associated with foods, processing surfaces, and industrial production environments. Studies such as those by Yang *et al*. (2020) and Tarbeeva *et al*. (2023), which applied metaproteomics to fermented beverages and meat products, have demonstrated that the integration of automated pipelines, representative databases, and functional annotations enables the identification of proteins related to desirable fermentation, spoilage, and potential risk factors, such as enzymes associated with toxin production or biofilm formation. Thus, bioinformatics combined with metaproteomics has emerged as a strategic tool for food safety, as it allows a more accurate functional assessment of food systems, going beyond traditional analyses based solely on taxonomic detection.

Advantage of proteomics over classical methods for the analysis of microorganisms in food

Microbiological analysis of food represents a fundamental component of food safety and sanitary inspection, particularly due to the increasing complexity of production chains and the diversity of microorganisms present in current food matrices (Pedro *et al*., 2024). Although these methods have played a crucial role in the advancement of food microbiology, their limitations are becoming increasingly evident in light of contemporary demands for analyses that are rapid, precise, and capable of providing functional information (Álvarez *et al*., 2023).

Traditional methods are based on the growth of microorganisms under laboratory conditions, limiting identification to a fraction of the microbiota present in foods. Viable microorganisms that cannot be cultured may remain undetected, and the prolonged analysis time reduces the effectiveness of these approaches in contexts that require rapid responses, such as foodborne outbreaks and industrial control decisions (Justé *et al*., 2008).

Molecular biology methods, such as PCR and qPCR, have increased sensitivity and precision in the detection of microorganisms without the need for cultivation. However, they present relevant limitations regarding the functional interpretation of risk, since the mere presence of genes associated with virulence or resistance does not guarantee their active expression or actual impact on food safety (Elizaquível *et al*., 2014).

In this context, proteomics emerges as a more advanced methodological approach, as it allows direct access to microbial functionality through the identification and quantification of proteins that are effectively expressed under real processing, preservation, and storage conditions. This feature enables a more accurate understanding of metabolic activity, environmental adaptation, microbial interactions, and pathogenic potential, representing a conceptual advancement over traditional and molecular approaches (Shiny Matilda *et al*., 2020; Hettich *et al*., 2012). Given these methodological differences and the distinct levels of information

generated by classical, molecular, and omics approaches, Table 1 presents an integrated comparative summary of the main analytical criteria applied to the analysis and control of microorganisms in food.

Table 1. Integrated comparison between classical methods, PCR/qPCR, and proteomics in the analysis and control of microorganisms in food.

Criterion/ Evaluated aspect	Classical methods	PCR/qPCR	Proteomics
Level of information	Phenotypic	Genetic	Functional
Dependence on cultivation	Yes	No	No
Response time	Days	Hours	Hours to 1 day
Detection of VBNC[1] microorganisms	No	Partial	Yes
Evaluation of metabolic activity	No	No	Yes
Strain-level resolution	Low	Moderate	High
Suitability for complex matrices	Limited	Moderate	High
Pathogen identification	Yes	Yes	Yes
Functional risk assessment	No	No	Yes
Direct toxin detection	Limited	Indirect	Direct
Antimicrobial resistence analysis	Phenotypic	Genetic	Functional
Multi-species analysis	Limited	Limited	Broad
Intergration with omics approaches	No	Partial	High
Support for sanitary decision-making	Moderated	High	Very high

[1]VBNC (Viable But Non-Culturable) it refers to viable microorganisms with detectable metabolic activity that do not form colonies on conventional culture media, but may retain pathogenic potential and recover culturability under favorable conditions. Source: Authors.

The integrated comparison shows that proteomics should not be understood as a substitute for classical or molecular methods, but rather as a higher-level complementary approach capable of filling critical gaps related to microbial functionality. By enabling the direct identification of proteins associated with virulence, antimicrobial resistance, biofilm formation, and adaptation to technological stresses, proteomics shifts the analytical focus from mere detection to the understanding of the mechanisms that effectively determine microbiological risk in foods (Shiny Matilda *et al*., 2020; Duracova *et al*., 2018).

Furthermore, its ability to simultaneously analyze complex microbial communities supports a systemic and predictive approach, contributing to the improvement of industrial control, sanitary surveillance, and regulatory decision-making. Thus, the incorporation of proteomics into microbiological monitoring systems represents a paradigmatic advancement in food microbiology, strengthening preventive strategies and food safety.

Use of mass spectrometry and bioinformatics in food control

Food safety is a global concern, and the rapid and accurate identification of harmful microorganisms in foods is essential to protect public health (Cavalcante *et al*., 2025). In this context, metaproteomics together with mass spectrometry (MS) and bioinformatics techniques emerges as an effective tool for the identification and quantification of microbial proteins directly in food samples (Bandeira et al., 2008).

The application of mass spectrometry enables the detection of proteins with high sensitivity and accuracy (Braga *et al*., 2016). In this process, complex bacterial communities (Figure 3) are subjected to protein extraction followed by enzymatic digestion, resulting in the generation of peptides. These peptides are then ionized and analyzed by mass spectrometry, in which the MS1 stage allows the detection and selection of precursor ions based on their mass-to-charge ratio (m/z). Subsequently, the selected precursor ions undergo collision-induced dissociation, generating MS2 spectra.

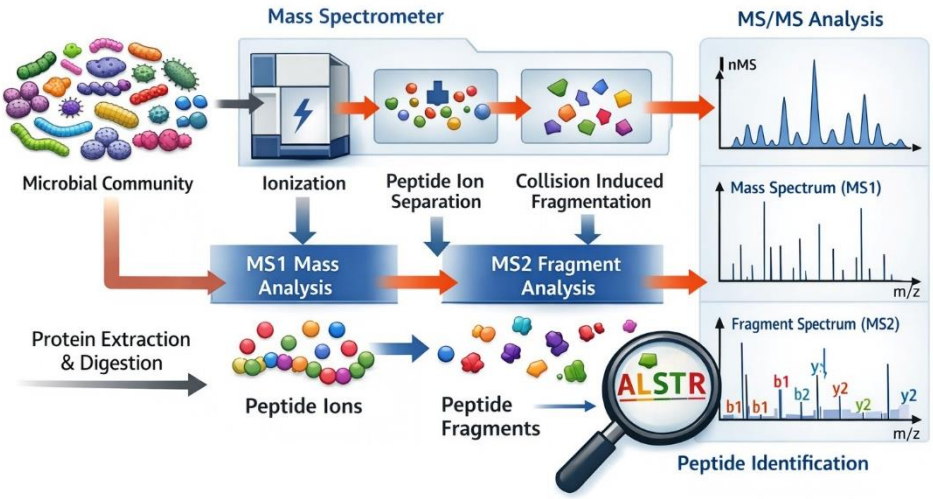

Figure 3. Schematic representation of the analytical workflow of MS/MS-based metaproteomics for the identification of proteins and peptides derived from a complex microbial community.

The bacterial pool is subjected to protein extraction followed by enzymatic digestion, resulting in the generation of peptides. These peptides are ionized and analyzed by mass spectrometry, in which the MS1 stage enables the detection and selection of precursor ions based on their mass-to-charge ratio (m/z). Subsequently, the selected precursor ions undergo collision-induced dissociation, generating MS2 spectra. The interpretation of fragmentation patterns allows peptide sequence identification and protein inference, providing functional insights into the composition and metabolic activity of the microbial community. Source: Authors.

The interpretation of fragmentation patterns enables the identification of peptide sequences and protein inference, providing functional insights into the composition and metabolic activity of the microbial community. In this context, bioinformatics

plays a central role in the analysis of MS data, as it allows the comparison of the obtained spectra with protein databases, enabling the reliable identification of microbial proteins. In addition, bioinformatics tools support the integration of this information for the reconstruction of microbial metabolic pathways, thereby enhancing the understanding of the underlying biological processes and the overall functioning of the microbiota (Jung, 2025).

In metaproteomics applied to food control, mass spectrometry and bioinformatics techniques are used to detect and verify proteins from harmful microorganisms, such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*, present in a wide range of foods, including meat, dairy products, fruits, and vegetables. This methodology enables the detection and characterization of microbial proteins in food matrices, supporting the assessment of microbial presence and associated pathogenic traits (Xiao *et al*., 2025).

Metaproteomics can also be applied to investigate microorganism–food interactions by identifying microbial proteins involved in the degradation of food components and the formation of metabolites. This approach contributes to a better understanding of food spoilage processes and supports the development of strategies to extend product shelf life (Gu *et al*., 2024). The simultaneous analysis of endogenous food proteins and microbial proteins enables the elucidation of the molecular mechanisms involved in spoilage and pathogenicity processes (Martín-Miguélez *et al*., 2025). Gu *et al*. (2024) observed an abundance of glycolytic enzymes and proteins related to the production of biogenic amines, compounds associated with food spoilage, during the refrigeration period of pork meat, resulting from the activity of microbial enzymes from *Pseudomonas* and *Clostridium*. In addition, metaproteomics also allowed the detection of high levels of ribosomal proteins, suggesting an increased transcriptional and translational capacity of these microorganisms, thereby promoting the synthesis of degradative enzymes and accelerating the spoilage process (Gu *et al*., 2024).

Bioinformatics assumes a fundamental role in the interpretation of metaproteomic data through the development of tools and algorithms aimed at microbial protein identification, protein quantification, and metabolic pathway reconstruction (Simopoulos *et al*., 2022). Furthermore, bioinformatics is essential for the construction and curation of microbial protein databases, which facilitate the identification of microorganisms present in food samples (Yohannis *et al*., 2025).

Nevertheless, several challenges remain, including the complexity of food matrices, the need for standardized protocols, and the handling of large volumes of data (Bandeira *et al*., 2008). The development of new technologies and bioinformatics methods is therefore essential to overcome these limitations and to expand the application of metaproteomics in food monitoring and control (Yohannis *et al*., 2025). Table 2 presents examples of proteins and peptides from pathogenic microorganisms that can be detected in foods using MS-based techniques, thus supporting food quality control.

Table 2. Protein- and peptide-based identification of foodborne pathogens.

Microbial effectors	Microorganism	Indication	Metaprote-omics tools	Reference
Listeriolysin O*	*Listeria monocytogenes*	Confirms the presence of Listeria monocytogenes, a pathogen associated with contaminated foods.	NI	Kaptchouang Tchatchouang *et al*. (2020)
Staphylococcal enterotoxin A	*Staphylococcus aureus*	Indicative of contamination by Staphylococcus aureus in foods, which may cause food intoxication.	Q-Orbitrap LC-MS/MS	Aveilla *et al*. (2024)
Botulinum toxin type A	*Clostridium botulinum*	Confirms the presence of Clostridium botulinum, a producer of the toxin responsible for foodborne botulism.	Endopep-MS	Kalb *et al*. (2015)
Hemolysin*	*Escherichia coli*	Indicative of pathogenic strains of Escherichia coli in foods, associated with intestinal infections.	NI	Schwidder *et al*. (2019)
GroEL protein	*Campylobacter jejuni*	Indicative of contamination by Campylobacter jejuni in foods, associated with gastrointestinal infections.	nano-LC-MS/MS1	Kalmokoff *et al*. (2006)
Flagellins (pseudaminic acid-modified), urease α and β subunits, 125 candidate glycoproteins	*Helicobacter pylori*	Confirms the presence of Helicobacter pylori, which may indicate contamination in foods.	MOE (Ac4GlcNAz), Staudinger ligation Phos-FLAG, Western blotting, MudPIT LC-MS/MS, HPLC-Chip/Q-TOF	Champasa *et al.* (2013)
Termolysin*	*Bacillus cereus*	Indicative of contamination by Bacillus cereus in foods, associated with food intoxication outbreaks.	NI	Soares *et al*. (2008)

IpadC and IpaD protein (classical virulence factors); Hmp, YkfE, AePA, MobC, MetK, LuxS, and OsmY (newly associated	*Shigella flexneri*	Confirms the presence of Shigella flexneri, associated with foodborne infection outbreaks.	LC-MS/MS	Epler *et al*. (2012); Batalha *et al*. (2021)
TcpA protein (TCP pilin); overlapping peptides generated by pepsin digestion	*Vibrio cholerae*	Indicative of contamination by Vibrio cholerae in foods, the causative agent of cholera.	LC-MS/MS	Boyd and Waldor (2002); Li *et al*. (2008)
CPE toxin; peptides EILDLAAATER (quantification) and EVFLISEDLK (confirmation)	*Clostridium perfringens*	Confirms the presence of Clostridium perfringens, associated with food intoxication outbreaks.	LC-MS/MS (MRM)	Lindsay (1996); Koike *et al*. (2023)
OmpX protein*	*Enterobacter cloacae*	Indicative of contamination by Enterobacter cloacae in foods.	NI	Mezzatesta, Gona, Stefani (2012)
Hsp60 protein*	*Mycobacterium avium*	Confirms the presence of Mycobacterium avium, a pathogen that can contaminate foods and cause infections in humans.	NI	Nagabhushanam *et al*. (2001)
AcrA, TolC, OmpF protein	*Salmonella enterica Serovar Typhimurium*	Indicative of contamination by Salmonella Typhi in foods, the causative agent of typhoid fever.	2D HPLC-MS/MS, SEQUEST	Coldham and Woodward (2004)

[1]Nano-liquid chromatography-tandem mass spectrometry; NI: Not identified; *Proteins identified from studies conducted using molecular biology or non-proteomic biochemical approaches. Selected as potential targets for implementation of the technique

Despite the costs associated with its implementation, the application of mass spectrometry (MS) combined with bioinformatics enables the detection and quantification of microbial proteins with remarkable precision and sensitivity, providing valuable information on the presence and activity of microorganisms in food products (Assis *et al*., 2011). Quantitative proteomics based on MS allows not only the identification of pathogens present in samples but also the quantification of proteins accumulated under different environmental conditions (Carbonelle *et al*., 2011). Carbonelle *et al*. (2011) evaluated that the MALDI-TOF technique associated with mass spectrometry (MS) can become more feasible when properly applied for bacterial identification compared to conventional phenotypic techniques or molecular biology methods. Methods such as label-free quantification (LFQ), isobaric tags for relative and absolute quantification (iTRAQ), and tandem mass tags

(TMT) enable comparative analyses of proteins from different production batches, supporting the early detection of microbiological changes and reducing the risk of foodborne outbreaks. In this way, metaproteomics-based quality control emerges as a complementary approach to traditional methods, offering greater sensitivity and specificity for pathogen detection (Carvalho *et al*., 2008; Carvalho *et al*., 2012).

Bioinformatics plays a fundamental role in metaproteomics applied to food safety by enabling the analysis and interpretation of large volumes of proteomic data generated by mass spectrometry (Williams, Hathout, Fenselau, 2002). Tools such as MaxQuant, Proteome Discoverer, and MSFragger are widely used for microbial protein identification against databases, allowing metabolic pathway reconstruction and the prediction of biological functions of biomolecules present in food samples (Carvalho *et al*., 2012; Becker *et al*., 2003). In addition, machine learning and artificial intelligence algorithms implemented in platforms such as DeepMS and MetaProteomeAnalyzer have been incorporated to improve pattern recognition in proteomic profiles, making data analysis more accurate and efficient (Lay Jr, 2000). The integration of bioinformatics with metaproteomics enables not only the identification of foodborne pathogens but also the differentiation between virulent and non-virulent strains, supporting more comprehensive food safety monitoring (Becker *et al*., 2003).

The use of specialized databases for proteomic identification represents one of the major advances enabled by bioinformatics, allowing the comparison of protein sequences from different pathogenic microorganisms. Databases such as UniProt, NCBI Protein, the Metaproteomics Gateway, and PRIDE (Proteomics Identifications Database) support the curation of protein sequences and the association of identified peptides with known proteins (Carvalho *et al*., 2012). Software tools such as PeptideShaker and X!Tandem assist in mass spectrum assembly, facilitating the detection of contamination biomarkers. Furthermore, structural modeling tools, including AlphaFold and Swiss-Model, enable the prediction of molecular interactions and contribute to the development of new strategies for pathogen control in foods (Carvalho *et al*., 2008). Nevertheless, challenges such as the need for improved database standardization, protein sequence curation, and result interpretation remain and must be addressed to expand the applicability of metaproteomics in the food sector.

Future perspectives and challenges of metaproteomics in food safety

Future perspectives of metaproteomics in food safety point to its increasing integration into routine sanitary monitoring systems. Advances in mass spectrometry, bioinformatics, and data integration are expected to expand the applicability of this approach beyond research settings, enabling faster, more sensitive, and functionally informative detection of foodborne pathogens.

The large volume of data generated by the application of omics techniques requires the integration of new analytical tools based on Artificial Intelligence, capable of enhancing the processing, interpretation, and integration of complex information. In

this context, the incorporation of metaproteomics into sanitary monitoring systems represents a strategic advance, as it enables faster, more sensitive, and functionally informative analyses of microbial communities associated with foods.

This approach may indirectly contribute to global goals related to food security and public health by supporting preventive pathogen control strategies, reducing the occurrence of foodborne outbreaks, and minimizing losses along the production chain. Such advances align with international objectives focused on responsible food production and health promotion, reinforcing the role of advanced analytical technologies in the development of safer, more resilient, and sustainable food systems.

Conclusions

Beyond pathogen detection, metaproteomics contributes significantly to the understanding of virulence mechanisms and antimicrobial resistance in microorganisms present in foods. Detailed analyses of proteins and peptides enable the characterization of adhesion, invasion, and survival factors of pathogens in food environments and within the human host. Thus, this approach not only enhances microbiological monitoring capabilities but also provides essential data for the development of effective strategies to control and mitigate contamination. Overall, the present work reinforces the need for continuous improvement of proteomic analysis techniques to ensure food safety and minimize public health impacts. The implementation of metaproteomics as a monitoring tool can contribute to the early detection of pathogens, waste reduction, and improved food quality. By combining technological innovation with microbiological control strategies, metaproteomics is consolidated as a promising approach for ensuring safer and more reliable foods for society.

In summary, metaproteomics stands out as a strategic tool for food safety by going beyond the mere detection of pathogens and enabling an in-depth understanding of molecular mechanisms and key biological processes enriched during microbial virulence and antimicrobial resistance in food matrices. The detailed characterization of proteins and peptides associated with microbial adhesion, invasion, and survival provides crucial insights into pathogen adaptation to both food environments and the human host.

Consequently, the application of metaproteomics expands microbiological monitoring capabilities and supports the development of more efficient strategies for contamination control and mitigation throughout the food production chain. Moreover, the continuous improvement of proteomic techniques is essential to enhance analytical sensitivity, robustness, and reproducibility, thereby strengthening their application in sanitary surveillance programs. The incorporation of metaproteomics as a monitoring tool contributes to early pathogen detection, reduction of losses, and improvement of food quality, positively impacting public health. Thus, by integrating technological innovation with microbiological control

strategies, metaproteomics is consolidated as a promising approach for the production of safer, more reliable foods aligned with sustainable production policies.

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