

THE PROTEIN PUZZLE: DECODING NUTRITION THROUGH PROTEOMICS INSIGHTS

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Summary

In the era of healthy and sustainable eating, foodomics brings a new perspective to nutrition, integrating proteomics to analyze the effects of foods on health. Proteomics, through advanced technologies such as HPLC and LC-MS, plays a crucial role in ensuring food quality and safety by identifying contaminants and allergens. This review prioritized studies investigating the potential utility of proteomics within the domains of nutrition and food safety, based on a comprehensive literature review spanning from 2010 to 2024, utilizing prominent electronic databases including Hinari, Pubmed, and Google Scholar. The application of proteomics in the field of nutrition has enabled the identification of allergens, the assessment of product freshness, and the detection of food fraud. Specifically, cow's milk allergy was discussed, where proteomics helps in identifying specific allergens and evaluating diagnostic methods. Additionally, proteomics' contributions to the early detection of diseases in the dairy industry, such as mastitis, and in combating food fraud were highlighted. In conclusion, proteomics provides valuable tools for advancing personalized nutrition and improving food safety. Through detailed analysis of the food proteome, new perspectives open up for the development of functional foods and for identifying the relationships between diet and health.

Keywords: proteomics, foodomics, nutrition, food safety, allergens, food fraud, biomarkers

Rezumat

Puzzle-ul proteinelor: decodificarea nutriției prin perspectivele proteomicii

În era alimentației sănătoase și sustenabile, foodomica aduce o perspectivă holistică asupra nutriției, integrând proteomica pentru a analiza efectele alimentelor asupra sănătății. Proteomica, cu ajutorul tehnologiilor avansate precum HPLC și LC-MS, contribuie esențial la asigurarea calității și siguranței alimentare, prin identificarea contaminanților și alergenilor. Articolul respectiv este o sinteză a literaturii de specialitate, publicată în perioada 2010-2024, fiind utilizate baze de date electronice precum Hinari, Pubmed și Google Scholar. Selecția s-a axat pe cercetări care abordează aplicabilitatea proteomicii în nutriție și siguranța alimentară. Aplicarea proteomicii în domeniul nutriției a permis identificarea alergenilor, evaluarea prospețimii produselor și detectarea falsificărilor. În particular, s-a discutat despre alergia la laptele de vacă, unde proteomica ajută la identificarea alergenilor specifici și la evaluarea metodelor de diagnostic. De asemenea, s-au evidențiat contribuțiile proteomicii în detectarea impurității a bolilor în industria laptelui, cum ar fi mastita, și în combaterea fraudelor alimentare. În concluzie, proteomica oferă instrumente valoroase pentru avansarea

nutriției personalizate și îmbunătățirea siguranței alimentare. Prin analiza detaliată a proteomului alimentar, se deschid noi perspective pentru dezvoltarea alimentelor funcționale și pentru identificarea interrelațiilor dintre dietă și sănătate.

Cuvinte-cheie: proteomica, foodomics, nutriție, siguranța alimentară, alergeni, falsificări alimentare, biomarkeri

Резюме

Белковая головоломка: расшифровка питания с помощью протеомических данных

В эпоху здорового и устойчивого питания, фудомика предлагает целостный взгляд на питание, интегрируя протеомику для анализа влияния продуктов питания на здоровье. Протеомика, используя передовые технологии, такие как ВЭЖХ и ЖХ-МС, играет ключевую роль в обеспечении качества и безопасности продуктов питания, идентифицируя загрязнители и аллергены. Данный обзор отдавал приоритет исследованиям, изучающим потенциальную полезность протеомики в областях питания и безопасности продуктов питания, на основе всестороннего обзора литературы за период с 2010 по 2024 год, используя известные электронные базы данных, включая Hinari, Pubmed и Google Scholar. Применение протеомики в области питания позволило идентифицировать аллергены, оценить свежесть продуктов и обнаружить фальсификацию продуктов питания. В частности, обсуждалась аллергия на коровье молоко, где протеомика помогает в идентификации конкретных аллергенов и оценке диагностических методов. Кроме того, был подчеркнут вклад протеомики в раннее обнаружение заболеваний в молочной промышленности, таких как мастит, и в борьбе с фальсификацией продуктов питания. Протеомика предоставляет ценные инструменты для продвижения персонализированного питания и улучшения безопасности продуктов питания. Благодаря подробному анализу протеома пищи открываются новые перспективы для разработки функциональных продуктов питания и идентификации взаимосвязей между диетой и здоровьем.

Ключевые слова: протеомика, фудомика, питание, безопасность продуктов питания, аллергены, фальсификация продуктов, биомаркеры

Introduction

In light of a global transition towards health-conscious and sustainable dietary practices, the notion of "foodomics" has gained escalating significance. Foodomics integrates proteomics and other omics technologies to analyze foods and their

effects on human health, providing a holistic vision of nutrition [1].

This approach focuses on the chemical makeup of foods and their metabolic effects on the body, thereby facilitating the advancement of personalized dietary regimens and sophisticated nutritional interventions. However, food safety and quality remain a major global challenge, with millions of people affected annually by food-related illnesses.

Proteomic technologies, such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS or LC-MS), play a crucial role in detecting contaminants, including toxins and allergens, contributing to better monitoring and control of food safety [2]. This article aims to explore the contribution of proteomics in advancing the fields of nutrition and food technology, highlighting how it facilitates the development of innovative solutions for improving the quality and safety of food products.

The article highlights the application of proteomics in identifying nutritional markers, assessing the impact of food processing on protein quality, and developing functional foods that meet the specific health and nutrition needs of the population. Starting from the hypothesis that a detailed analysis of the food proteome can reveal complex relationships between diet and health, proteomics offers a promising path towards personalized nutrition and sustainable improvement of food quality. By decoding food proteomes, proteomics has the potential to transform the food industry, aligning production with the nutritional and health requirements of modern society.

The **aim of the study** was to emphasize the importance of applied proteomics in nutrition.

Material and methods

For the gathering and examination of pertinent data, the following electronic databases were utilized: Hinari, PubMed, Scopus, Google Scholar, ScienceDirect, and NCBI. The literature retrieval process encompassed 50 sources but centered on 41 publications spanning from 2010 to 2024, with a specific emphasis on works released within the past decade.

The inclusion criteria encompassed observational studies, clinical trials, systematic reviews, meta-analyses, and scholarly articles available in both English and Romanian, addressing the analyzed topic and with open access. The analysis was strictly confined to data from peer-reviewed scientific journals recognized in the respective specialty field and no older than 2010.

Results

In the field of nutrition, the use of proteomics has revolutionized how allergens are identified, the quality and freshness of products are evaluated, product forgeries are detected, and pathogenic microorganisms in food are identified. The first area of interest and public health concern is allergies.

Cow's milk allergy is commonly encountered, especially among children, with an estimated prevalence of between 0.5 and 3% in Europe and a trend of decreasing from north to south [3]. It consists of an IgE-mediated adverse reaction against one or more proteins from cow's milk, which normally should not be harmful to individuals who are not allergic. Among the approximately 200 proteins present in cow's milk, glycoproteins, including allergens [4], can be found.

The main allergens of cow's milk are caseins, β -lactoglobulin, and α -lactalbumin [6]. However, in 75% of cases, individuals develop sensitization to multiple proteins, not just to the main allergens [7], because the milk proteome is very heterogeneous due to the presence of numerous protein isoforms [8] that result from alternative mRNA splicing, unique point mutations, and post-translation modifications [9].

In this context, traditional tests are still capable of measuring specific IgE (sIgE) for a limited number of milk allergens (such as casein, β -lactoglobulin, and α -lactalbumin). However, there are cases where patients with acute manifestations of cow's milk allergy do not show elevated sIgE levels for these evaluated proteins. In such situations, the diagnosis of cow's milk allergy can be confirmed through an oral food challenge, considered the gold standard test for diagnosing food allergies. The presence of proteins in very small quantities but with high immunoreactivity requires the use of testing methods with high sensitivity.

Currently, in proteomics, two approaches are used: bottom-up and top-down [10]. The bottom-up technique involves two-dimensional electrophoresis (2DE) and mass spectrometry (MS), ideal for detailed analysis of highly complex samples. It uses one or more proteases to fragment proteins into peptides, which are then analyzed by MS [11]. In contrast, the top-down strategy, based exclusively on MS, focuses on the analysis of intact proteins, without preliminary digestion, providing data on the total mass of proteins and their amino acid sequence [12].

The use of immunological methods in tandem with mass spectrometry techniques for protein identification has allowed the identification of two new allergenic molecules in cow's milk, lactoperoxidase

and the FAM13A protein, which had not been previously reported [13].

The analysis of extracted caseins is very important for understanding milk allergy. Since epitopes do not have an ordered secondary and tertiary structure, they rely more on primary sequence than on conformation. The most allergenic region in α -casein is the N-terminal section 1-25, rich in E in a poly-Glu motif. This peptide is heat-stable but is affected by the presence of phosphate, necessary for the protein's calcium-binding function. These proteins are highly resistant to high temperatures, so they can constitute the main part of the protein content in cooked foods. Also, due to their structure and micellar composition, caseins do not degrade when heated to high temperatures and can be isolated in complex and thermally processed foods [14].

This observation may explain why patients with severe allergies, who do not tolerate thermally processed milk *in vivo*, show high levels of IgE for casein epitopes. Such examples show us that proteomics is capable of tracking post-translational changes and is also a valuable tool for detecting the Maillard reaction (MR), which is a spontaneous reaction between amino acids or proteins and reducing sugars, like the saccharides that occur during the thermal processing of foods. [14, 15].

Food allergens can be as harmful as foodborne pathogens, highlighting the importance of rapid and detailed food safety assessment. The use of proteomic methods for the accurate identification of allergens plays a crucial role in detecting and assessing the risks associated with exposure to allergic consumers [16]. This process improves the food industry's ability to provide safer products for people with allergies, both in traditional and new foods. Proteomics is particularly valuable in discovering allergens not highlighted in complex compositions, given that some individuals may react even to minimal amounts of allergens. For example, the accurate detection of traces of casein or egg proteins, such as lysozyme and ovalbumin, frequently used in wine clarification, minimizes risks for consumers susceptible to allergies [14]. Compared to immunochemical tests, the advantage of proteomic methods lies in the ability to identify proteins even when their structure has been chemically or physically altered.

Currently, the only standard treatment approved for cow's milk allergy is the elimination diet [17]. In the search for suitable alternatives for allergic children, extensively hydrolyzed formulas are often recommended as a top option. However, these formulas have disadvantages, including high costs, unpleasant taste, and the potential to trigger allergic reactions in individuals with increased sensitivity due

to the presence of residual allergenic epitopes. Therefore, milk from other mammals has been proposed as a viable alternative.

Studies based on proteomic techniques on various types of mammalian milk have shown that camel milk, due to the absence of β -lactoglobulin - a major allergen in cow's milk - could be a valuable protein source for people allergic to β -LG in cow's milk [14,18]. In the clinical context, it has been observed that some children with IgE-mediated cow's milk allergy can consume cow's milk that has been subjected to intensive heating processes, in the form of thermally processed foods, without problems.

The benefit of introducing this type of milk into children's diets lies not only in diversifying their food but also in promoting tolerance to raw milk through controlled exposure to thermally treated dairy products. On the other hand, there are cases where the consumption of thermally processed milk triggers more intense allergic reactions, including risks of anaphylaxis and long-term allergic manifestations [14].

The perfect cow's milk substitute should be hypoallergenic, nutritionally suitable, pleasant to taste, and accessible [19]. As a top solution in this regard, hypoallergenic formulas that use extensively hydrolyzed proteins position themselves as the first option in the therapeutic approach. Hydrolyzed formulas consist of enzymatically degraded proteins, devoid of typical IgE-binding allergenic epitopes, to avoid the onset of allergy symptoms. Recently, there is increasing evidence regarding the presence of bioactive peptides in cow's milk hydrolysates, with possible immunomodulatory properties [20].

Another avenue facilitated by proteomics is the timely identification of diseases that pose potential threats to the entire dairy production chain, such as mastitis. This ailment detrimentally impacts milk quality, inducing significant shifts in its nutritional profile. Ogola and colleagues observed notable alterations including elevated levels of non-casein fractions, sodium, chloride, and free fatty acids, concomitant with diminished concentrations of casein and lactose. Mastitis is signaled by an abundance of somatic cells in milk, which also correlates with proteomic variations, encompassing changes in vital compounds like fatty acids and lactose. Noteworthy biomarkers indicative of this infection, such as serotransferrin, fibrinogen β chain, and cathelicidin, have been discerned, suggesting potential avenues for subclinical diagnosis [21].

Milk is considered sterile when secreted from the nipple and, due to its composition rich in amino acids, fatty acids, and lactose, is a perfect medium for microbial growth.

From this stage onward, milk becomes host to a diverse microbiota, originating from the skin of the nipple and the epithelial mucosa of the milk duct. Additionally, certain microorganisms may migrate from milking equipment, the animal's feeding area, or bedding material [23].

To ensure the health of the milk and to increase its shelf life, it is imperative to perform thermal treatment under conditions that reduce the bacterial load. Some of these treatments, such as pasteurization and sterilization, have proven to be effective. However, a heating process can cause a decrease in food quality by affecting the color, aroma, or nutritional value [24].

It has been reported that heat could cause chemical changes in proteins such as Alpha-lactalbumin and β -lactoglobulin, such as glycation, oxidation, denaturation, and aggregation, affecting the bioavailability of amino acids and their functionality [24].

Ebner and colleagues [25] discovered 16 peptides present in consumer milk that could serve as indicators of abnormal thermal treatment. Thus, the relative amount of these peptides' changes with the increase in thermal load. Particularly sensitive to temperature variations was the peptide β -casein 196-209 (m/z 1460.9 Da), which proved to be useful as a marker for monitoring the processing conditions of milk.

Liu et al. [25] used proteomics to identify milk degraded by excessive heat application. Upon subjecting cow's milk to a temperature of 85°C for 5 minutes, a significant degradation of serum proteins, including lactoferrin, immunoglobulin, and lactoperoxidase, was observed. In contrast, non-thermal treatments such as ultraviolet-C irradiation and thermo-ultrasonication resulted in these proteins remaining largely intact.

In addition, complement proteins, xanthine dehydrogenase/oxidase, and fatty acid-binding protein significantly reduced their concentration [9]. Other classical procedures such as freezing, heating, drying, fermenting, salting, and the use of chemicals are the most common, which can also cause changes. Milk and dairy products are subjected to various types of thermal treatments, from pasteurization (72°C for 15 seconds) to ultra-high temperature (UHT; 135-150°C for 2-6 seconds) sterilization. These procedures lead to the Maillard reaction, which represents the non-enzymatic glycation of amino groups (mainly lysine residues in milk proteins) by reducing sugars (lactose is the main reducing sugar in milk). The products of this complex reaction can be different depending on the duration of heating. In milk, lactulosyllysine (bound to several milk proteins) is the main product

in the early stage of thermal treatment, and in the advanced stage (longer thermal treatment) of the Maillard reaction, many other reaction products are formed [26]. Thus, Siciliano et al. [21] using demonstrated that, like β -Lg, alpha-lactalbumin (α -La) undergoes lactosylation, preferentially at Lys98, during thermal treatment. The degree of lactosylation for both proteins was proportional to the thermal treatment used (sterilization>UHT>pasteurization). Moreover, the authors reported that, during thermal treatment, the strong denaturation of β -Lg caused the formation of aggregates with caseins. This led to the depletion of whey protein from milk and a further reduction in the nutritional value of thermally treated foods, due to the limited bioavailability of proteins and amino acids [21].

During the storage of milk, a series of changes occur that affect the quality of the milk. The proteolytic activity of enzymes naturally present, could cause changes in the peptidome, hence, the peptide profile could again be suitable for evaluating the product's condition both during storage and during processing [25]. Throughout the storage of commercial UHT milk, it has been found that up to 22 peptides significantly increased due to proteolytic activity attributed to different mechanisms (e.g., endogenous proteases and microbial proteases). Ten peptides were selected as potential markers to measure milk quality, highlighting the peptide β -casein 196-206 (m/z 1668.9) as the most suitable for differentiating UHT milk from that which is not suitable for consumption [27].

Cheese, a widely consumed dairy product, is generally considered safe. Nonetheless, it can become a conducive environment for dangerous microorganisms, such as *Listeria monocytogenes*, *S. aureus*, and *Escherichia coli*, which threaten consumer health. Proteomic techniques offer promising solutions for the identification and combat of these pathogens. For example, the application of Western Blot tests, using the mAb-3F8 and anti-InlA mAb-2D12 antibodies, has proven the ability to distinguish between different types of bacteria present in artificially contaminated cheeses [28].

Extremely relevant are the proteomics advances in evaluating milk quality for cheese manufacturing. Hinz and colleagues (2012) described, using 2D electrophoresis, how different proteolysis of various lactation phases could influence the quality of cheddar cheese [29]. Another very important quality of milk for the production of high-quality cheeses is related to its coagulation strength. Milk with reduced coagulation strength usually represents a burden for cheese quality. Each cheese proteome is unique and can be used as a digital fingerprint that character-

izes the product and differentiates it from the rest. In this line, Silva et al. [30] worked on characterizing the proteome of Coalho cheese, a Brazilian artisanal cheese, confirming 32 proteins successfully identified (11 from α S1-casein, three from α S2-casein, 15 from β -casein, and three from κ -casein). The peptide profile could be used as a unique biomarker for this type of cheese and could serve to guarantee the quality of the final product.

There are multiple applications of proteomics regarding food quality, a relevant example being the evaluation of meat quality, as meat is an animal-origin food with a high protein content. Proteomic studies for evaluating meat quality have been applied to various types of meat: beef, pork, lamb, and chicken. Another study on sheep muscles highlighted quantitative trait loci for muscular hypertrophy, showing the overexpression of proteins involved in glycolytic metabolism and chaperone proteins [21]. Animal welfare could influence differences in meat quality. Compensatory growth in pigs, associated with meat fineness, consists of an increased growth rate after a period of food restriction. It was found that, after slaughter, pigs that underwent a period of compensatory growth had a faster tenderization of the meat.

This trait was investigated proteomically by Lametsch and colleagues [31]. More recently, birth weight in chicks can influence meat quality. Liu and colleagues demonstrated that low birth weight in response to a high-fat diet produces changes in the expression of stress proteins in the muscle [32]. The quality of pork was investigated using 2DE coupled with MS. This study identified 27 candidate proteins that changed coincident with meat quality traits during aging. Most of these proteins included cytoskeletal and metabolic proteins and relative degradation products [21]. Di Luca et al., using a gel-based approach (2D-DIGE and WB) on pork meat exudates, highlighted the lower abundance of stress response proteins in fresh pork meat with higher drip and water losses [33].

The stress before slaughter and slaughtering procedures influence meat quality. Franco and colleagues [33] described differential protein expression in the longissimus thoracis muscle of cattle of proteins involved in structural-contractile and metabolic functions. Interestingly, the authors grouped these proteins with a commonly used qualitative parameter in beef (DARK FIRM DRY). The color of beef is also important, as it is a parameter associated with the animal's health. A positive correlation of glycolytic enzymes (phosphoglucosmutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) with redness and color stability

was described [34, 35]. The pork was analyzed from 0 to 48 hours after slaughter to highlight changes in the proteome pattern. Proteins such as actin, myosin heavy chain, and troponin T were found to be differentially expressed [21]. Another important proteomic experiment for meat tenderness is the study of calpain-dependent myofibrillar degradation. The phosphoproteome also changes within 24 hours postmortem, suggesting that glycolysis may play a key role in the meat maturation process [36]. A recent approach showed that glutamate, serine, and arginine could serve as good predictors of final meat quality parameters [37].

Adulteration in the meat industry is not limited to the improper mixing of different types of meat; often, soy proteins are introduced into meat preparations as emulsifiers to improve functionalities, taking advantage of their lower cost to use them in unauthorized quantities. In 2006, Leitner applied the 2D-LC-MS/MS/MS technique to identify five predominant types of glycinin and all three forms of alpha-conglycinin, highlighting them as specific indicators of the presence of soy in processed meat [38].

Using proteomic techniques, valuable data can be obtained about the health of fish in aquaculture. Benchmark studies have compared the proteomic profile between aquaculture and wild fish, highlighting significant variations in the muscle proteome of species such as seabass, cod, and *Sparus aurata* [21]. In addition, storage conditions, such as keeping fish on ice, affect its quality, altering the texture and taste of the muscle. Proteomic analyses conducted over 8 days of ice storage have revealed multiple changes, demonstrating the involvement of various biochemical processes in the postmortem protein evolution [21].

β -Parvalbumins, which are found in large quantities in the sarcoplasmic fraction of the white muscle of fish, are the main fish allergens [39]. Regarding other fish allergens, fructose biphosphate aldolase (39.54 kDa), which is involved in gluconeogenesis, glycolysis, and the Calvin cycle, is also considered a fish allergen in species of cod, salmon, and tuna. Enolase (β isoform; 47-50 kDa) is an enzyme responsible for the penultimate step of glycolysis and is also considered a potential fish allergen in species of cod, salmon, and tuna. Crustacean allergens include tropomyosin, arginine kinase, the sarcoplasmic Ca^{2+} binding protein, myosin light chains 1 and 2, troponin C, and triose phosphate isomerase. Among these, tropomyosin has been considered the main allergen found in all edible parts of either crustacean species (such as shrimp, crabs, and lobsters) or mollusks (including mussels, oysters, clams, and squid) [40, 41].

Discussions

Proteomics serves as a fundamental tool in unravelling the complexities of nutrition and food technology, offering new perspectives on the intricate interaction between diet and health. Its diverse applications, ranging from allergen detection to food quality assessment and the elucidation of biochemical mechanisms underlying food spoilage and processing, underscore its significant potential.

For example, in evaluating food quality, proteomics can identify specific proteins associated with the freshness and quality of food products, thereby helping producers maintain high-quality standards. In the case of food spoilage, proteomics can detect protein modifications caused by microorganisms, providing rapid and precise methods for identifying contamination and ensuring food safety.

The discourse stemming from these investigations emphasizes the ongoing need to integrate proteomics into the formulation of food safety protocols and personalized nutrition approaches. Through the proteomic analysis of foods, strategies can be developed to prevent allergic reactions by identifying and eliminating allergens from food products.

Furthermore, exploring proteomics in the realms of food allergies and fraudulent practices presents new avenues for innovative solutions within the food industry. Proteomics can detect specific proteins used in counterfeit products, thus helping to combat food fraud and protect consumers.

Therefore, proteomics proves to be an essential tool in adapting food production to meet the dietary and health demands of contemporary society, ultimately fostering a culture of wholesome and sustainable dietary practices.

Through its applicability in various aspects of food science and technology, proteomics significantly contributes to improving quality of life and developing innovative solutions for current challenges in the food industry.

Conclusions

In summary, *omics* technologies, particularly proteomics, significantly contribute to the advancement of nutrition and food technology. By identifying allergens, assessing quality, and detecting contaminants, these technologies enhance food safety standards. This progress facilitates the emergence of personalized nutrition approaches and the creation of innovative food products tailored to individual health requirements. Consequently, proteomics plays a pivotal role in promoting healthy and sustainable dietary practices, ultimately bolstering food security and public health initiatives.

Declaration of interests

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