

## Food Peptidomics

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### Summary

The aim of this review is to discuss the definition of food peptidomics and highlight the role of this approach in food and nutrition sciences. Similar to living organisms, food peptidome may be defined as the whole peptide pool present in a food product or raw material. This definition also covers peptides obtained during technological processes and/or storage. The area of interest of food peptidomics covers research concerning the origin of peptidome, its dynamic changes during processing and/or storage, the influence of its presence, the composition and changes in the pool of peptides on the properties of food products or raw materials as well as the methods applied in research into this group of compounds. The area of interests of food peptidomics would include biological activity, functional properties, allergenicity, sensory properties and information on the product or resource authenticity and origin as well as its history and relationships. Research methods applied in food peptidomics, with special emphasis on computational methods, are also summarized.

*Key words:* food, biologically active peptides, peptidomics, functional properties, bioinformatics, proteolysis

### Introduction

The so-called ‘-omics’ approaches in food science are of emerging significance. Genomics, transcriptomics, proteomics, glycomics, metabolomics and nutrigenomics are examples of the implementations of a holistic point of view on the role of individual groups of constituents of living organisms and/or foods, as well as the interactions of food constituents with the human body. The initiative of the milk genomic consortium (1) or a new area of nutrition science named ‘nutrigenomics’ may serve as examples of such approaches (2). The role of proteomics in the biology of living organisms treated as integrated systems has been highlighted by Souchelnytskiy (3).

Peptides, together with proteins, are one of the major groups of food components. They affect both biological as well as functional properties of food products. The same interactions govern the biological activity of

compounds and their behaviour during technological processes as highlighted by Tolstoguzov (4). This finding may be extended to peptides. The view that all proteins are potential precursors of peptides exhibiting various kinds of biological activity (5), both as endogenous constituents of living organisms, as well as exogenous compounds of food origin, peptidomics may be considered to be an important part of the holistic approach in food science postulated by Eads (6). Food properties depend on the properties of all components and their interactions as well as the technological processes applied and storage conditions (6).

Peptidome is defined as a pool of all peptides of an organism, tissue or cell. Peptidomics may be defined as the area of science focused on composition, interactions and properties of peptidome, as well as research methods applied for peptidome analysis (7–11). In the case of

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food products, we can define peptidome as the whole peptide pool present in food products or raw material. This definition also covers peptides obtained during technological processes and storage. The area of interest of food peptidomics covers research concerning the origin of peptidome, its dynamic changes during processing and/or storage, influence of its presence, composition and changes in the pool of peptides on the properties of food products or raw material as well as the methods applied in research into this group of compounds. The peptides found in fish (12) may serve as an example. Bauchart *et al.* (12) also describe changes in peptide content during ice storage and cooking. Another example of changes of food product peptidome is proteolysis during cheese ripening (13). Although proteolysis introduced as a step of analytical procedure does not belong strictly to peptidomics, but is more closely related to the so-called shotgun proteomics, it may be reviewed together with the above-mentioned area due to the fact that the same analytical methods are applied in both approaches. The detection and determination of the composition of milk and soybean preparations involving proteolysis followed by chromatography and chemometrics (14) may serve as an example. Peptidomics partially covers the same approaches of research and uses similar methods as proteomics. The proteomic approach in food and nutrition science has been reviewed in many articles (15–21). Various aspects concerning peptides in milk, such as biological activity, functional properties, allergenicity and the taste of peptides have been reviewed by Kilara and Panyam (22).

The aim of this review is to present the current status of food peptidomics, *i.e.* areas of interest and contemporary research methods.

### Areas of Interests in Food Peptidomics

The areas of interests in food peptidomics are presented in Fig. 1. Apart from those defined and reviewed by Kilara and Panyam (22), peptidome has also been included as a source of information about product authenticity and history.

Biological activity can be interpreted as any beneficial or negative influence on an organism (23). Peptides are considered as bioactive when they possess a hormone, or drug-like, activity which modulates physiological functions through binding interaction to specific recep-

tors on target cells, leading to the induction of physiological responses (24). The main biological activities of food peptides (22,23,25–29) are presented in Table 1. Peptides may reveal biological activity *in vivo* in the gut lumen through the receptors on the intestinal cell walls or after absorption from the digestive tract. The short peptides, containing two or three amino acid residues, are usually absorbed easily as compared to longer ones and are thus especially interesting for food scientists and nutritionists (30). Such fragments occur in protein sequences with relatively high probability (31). Regardless of the bioactivity, peptide chain length is an important factor in the design of hydrolysate-based medical diets (32). Peptides exerting activity without being absorbed from the digestive tract, such as antimicrobial peptides, are longer. The typical structure of antimicrobial peptides is a cationic  $\alpha$ -helix (33–35). Some bioactive peptides are multifunctional. Bovine caseinomacropeptide – a C-terminal fragment of  $\kappa$ -casein – may serve as an example. It displays activities, for example such as regulating the action of digestive tract along with antibacterial, antiviral and toxin-binding abilities. Caseinomacropeptide may be considered as an example of a natural combinatorial library due to the fact that its activity strongly depends on the number and location of posttranslational modifications such as glycosylation and phosphorylation (36,37). In some cases, peptides exert both beneficial and negative effects, such as antibacterial peptides, which are often toxic for mammalian cells (38), or  $\beta$ -casomorphin-7, which has opioid properties, including immunosuppression and could account for the relation between the consumption of  $\beta$ -casein A<sup>1</sup> and diabetes incidence (39).

Table 1. Main biological activities of peptides of food origin

Activity	References
Antihypertensive inhibitors of angiotensin-converting enzyme [EC 3.4.15.1]	(22,23,25–29)
Opioid agonists or antagonists	(22,23,25–29)
Antithrombotic	(22,23,25–29)
Antimicrobial	(22,23,25–29)
Immunomodulating (immunostimulating or immunosuppressing)	(22,23,25–29)
Mineral carriers	(22,23,25–29)
Celiac-allergenic (celiac-toxic)	(23,27)

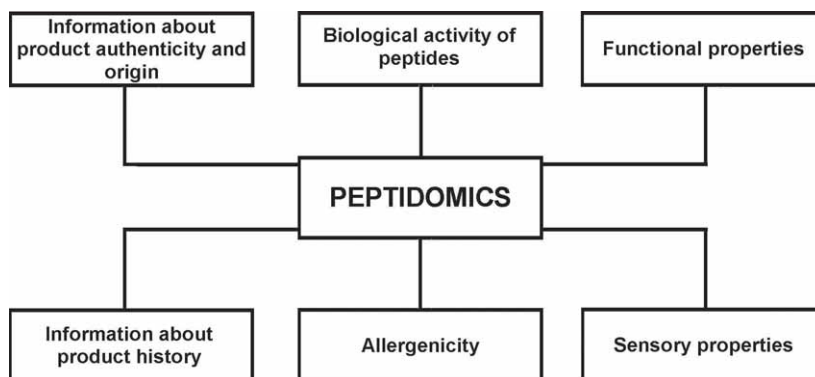


Fig. 1. Areas of interests in food peptidomics

Details of the influence of peptides on the functional properties of food have been reviewed based on the examples of peptides from milk (22,40,41). Hydrolysis of proteins is considered a valuable tool in improving their functional properties. Functional properties are understood as physical and chemical properties of food components (*e.g.* proteins and peptides) which influence their behaviour in food products during manufacturing and consumption (42). Functional properties include solubility, emulsifying and foaming properties as well as gelation (22).

Proteins and their fragments are one of the major groups of food allergens (22,43,44). The approaches considered in studies on food allergy are: reduction of allergenicity *via* enzymatic hydrolysis (45–47), problems of resistance of allergens to proteolysis by gastrointestinal enzymes (48–52), and absorption of allergens from the digestive tract (53). Another problem is gluten intolerance, also known as ‘celiac disease’ or ‘non-tropical sprue’ (54–56). A crucial factor in this disease is the reaction of organisms caused by peptides mainly of wheat gluten origin (known as ‘celiac toxic’ or ‘celiac allergenic’). Such peptides are classified as examples of bioactive peptides exerting a negative influence on organisms.

Peptides belong to a group of components affecting the product taste. The bitter peptides, *e.g.* from milk, have been the most extensively studied to date (57–59). Peptides may reveal all kinds of taste. An example of such a situation has been recently described by Lioe *et al.* (60). They separated peptide fractions of soy sauces using size-exclusion chromatography and isolated sub-fractions with sweet, sour, salty, bitter and umami tastes.

Peptidome has recently been considered as a source of information on product authenticity, origin and history. Chemometrical interpretation of data from peptidome analysis has recently been used in experiments in the above-mentioned approach (61). Reversed-phase high-performance liquid chromatography (RP-HPLC), as well as capillary electrophoresis (CE) are the basic analytical methods used for chemometrical analysis of peptidome (62). Chromatographic and chemometrical analyses of peptide fractions may be a source of information on *e.g.* the origin of cheese (63), the history of milk used for cheesemaking (64) and cheese maturity (65). Proteolysis followed by RP-HPLC and chemometrical data analysis has been used as a tool for identification and determination of soybean and milk proteins in commercial protein preparations (14). The chemometrical analysis performed in that experiment was used for the selection of peaks as markers of the presence and content of proteins from both sources. Van der Ven *et al.* (66) reported on Fourier transform infrared spectra in combination with multivariate data analysis as a valuable tool in hydrolysate fingerprinting and their use as an alternative for laborious peptide functionality measurements.

### Experimental Strategies Used in Peptidomics

The general strategy used in peptidomic experiments concerning living organisms has been discussed in a number of works (9,67,68). A commonly-applied *in vitro*

experiment aimed at searching for novel biologically active peptides of food origin is presented in Fig. 2. Most biologically-active peptides from foods (23,25,26,28,29)

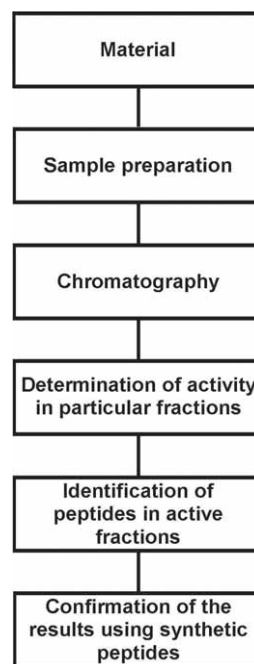


Fig. 2. Classical scheme of *in vitro* investigations on peptide bioactivity

have been discovered within experiments performed according to this or similar schemes. Sample preparation involves isolation of a peptide fraction from a product or resource. Many experiments involve enzymatic hydrolysis of proteins from food resources (milk, plant resources, meat, fish or eggs). The following step is chromatographic separation. The typical sequence of methods used for chromatographic separations is size-exclusion chromatography (SEC) followed by reversed-phase high-performance liquid chromatography (RP-HPLC). Fractions from RP-HPLC are collected and screened for activity. Peptides from active fractions are identified. Mass spectrometry has recently become the most efficient tool for peptide identification (69–71). The usual last step is the measurement of the activity of synthetic peptides with amino acid sequences identical to those identified in active fractions resulting from chromatographic separation. Recent examples of experiments performed according to classic or related schemes have been published, *e.g.* by Motoi and Kodama (72), Marczak *et al.* (73), Sørensen *et al.* (74), Gómez-Ruiz *et al.* (75), Hernández-Ledesma *et al.* (76), Mallikarjun Gouda *et al.* (77), Ma *et al.* (78) and López-Expósito *et al.* (79). All of the above-mentioned studies are concerned with identification of novel biologically active peptides (mainly angiotensin-converting enzyme inhibitors). The peptides were obtained from various sources such as wheat gliadin hydrolysate (72), rapeseed (73), fish muscles (74), cheese (74,75), hydrolysate of bovine  $\beta$ -lactoglobulin (76), hydrolysate of soybean glycinin (77), buckwheat seeds (78) or ovine  $\alpha_{s2}$ -casein (79). In all cases protein hydrolysates or

peptide pools isolated from products were fractionated using chromatography. Particular fractions were tested for biological activity such as inhibition of angiotensin-converting enzyme (72,73,75–78), inhibition of prolyl endopeptidase (74) or antibacterial activity (79). Sequencing of peptides in active fractions and confirmation of activity using synthetic peptides were the final steps of experiments. The strategy of including mass spectrometric identification of previously known bioactive peptides has also been introduced (80–82). This strategy was applied to dairy products such as fermented milk (80,81) or cheese (82). The concept utilizes the fact that milk proteins are most extensively studied precursors of biologically active peptides among food proteins (22, 26–29). High number of known biologically active sequences present in milk protein chains provides high probability that some of them will be released in a given product. Recently, affinity-based methods of isolation of peptides revealing a given activity have been proposed (83). In this case immobilized angiotensin-converting enzyme served as a medium for isolation of antihypertensive peptides. Another possibility is the measurement of the activity of a whole hydrolysate or a peptide fraction of a product without isolation and identification of individual peptides (84–88). Such strategy was applied for angiotensin-converting enzyme inhibitory activity of peptidic fractions of dairy products (84), soy protein hydrolysates (86) and sorghum kafirin hydrolysates (88) as well as antioxidant activity of capelin protein hydrolysates (85) and bighead carp muscle protein hydrolysates (87).

Challenges provided by whole peptidome analysis have led to the design of multidimensional analytical platforms, including various chromatographic or capillary electrophoresis methods combined with mass spectrometry (69,89–91). Analytical methodology is the same as that used in so-called ‘shotgun’ proteomics, *i.e.* analysis involving enzymatic hydrolysis, subsequent separation and identification of peptides as well as database searching to identify proteins as the precursors of peptides. Systems including ion-exchange chromatography followed by reversed-phase chromatography or reversed-phase chromatography followed by capillary electrophoresis have been developed. The application of two-dimensional chromatography for the separation of peptides from cheese has been described by Lagerwerf *et al.* (92).

Recently, new possibilities in science concerning food peptides have occurred due to the implementation of the bioinformatic method. The place for bioinformatics in peptide science has been discussed by Dziuba *et al.* (93) based on the BIOPEP program. A possible scheme for a peptidomic experiment combining chemometrics and bioinformatics – including elements published earlier (9,93) – is presented in Fig. 3. The first steps, including sample preparation, separation and identification of peptides, are the same as in the ‘classic’ scheme. Chromatography, mainly RP-HPLC, may be replaced by capillary electrophoresis. Chemometrical analysis may be sufficient to extract information about the properties of products, correlated with a chromatographic, electrophoretic or mass spectrometric profile. It could also help to find markers of a particular property (*e.g.* adulteration). The most sufficient method of identification of

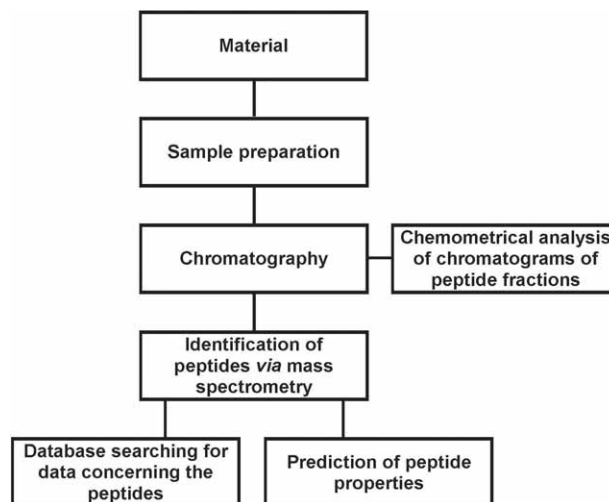


Fig. 3. Possible scheme of peptidomic research integrating experimental research, chemometrics and bioinformatic approach

peptides is mass spectrometry. Molecular mass may serve for preliminary searching in databases. Peptide sequence databases such as BIOPEP (94) or SwePep (95) enable searching according to molecular mass. The last step of identification is sequencing *via* tandem or multi-stage mass spectrometry. Sequence may be used for database searching to find known activity of the peptide identified. Sequence may also serve as a basis for prediction of the biological or functional properties of peptides using Quantitative Structure-Activity Relationship (QSAR) or another computational approach.

### Computational Methods Applied in Research on Peptides in Foods

Computer-aided methods such as databases or programs are commonly used in research on proteins (96, 97). Theoretical predictions and simulations are considered as an emerging tool in peptide science. Theoretical methods have been applied to date in the following approaches: prediction of the biological activity of products of protein hydrolysis, simulation of protein hydrolysis by enzymes, finding relationships between the sequence and activity of peptides, prediction of the functional properties of peptides, chemometrical analysis of the results of peptidome analysis, development of methods sufficient for high-throughput peptide identification.

Classification of proteins as potential precursors of bioactive peptides can be performed using the BIOPEP database. Detailed description of the database has been published elsewhere (94). The BIOPEP database and program have been applied to construct profiles of the potential biological activity of protein fragments (26,31, 93,98–100), classification of proteins as precursors of bioactive peptides using designed quantitative parameters (93,94,99,101) and for predictions of the release of bioactive peptides by endopeptidases (93,102–104). Ver-cruysse *et al.* (105) searched for bioactive fragments in consensus sequences among various proteins. They used the Clustal W program, designed by Thompson *et al.* (106). Potential biological activity of protein fragments



may be predicted *via* searching for sequence similarity between peptides known to be bioactive and proteins from internet databases. The BLAST (107,108) or FASTA (109) programs have been applied in research aimed at finding new, potentially celiac-toxic, peptides (110–113). Programs designed for interpretation of the results of peptide and protein analysis obtained using mass spectrometry such as PeptideSearch (114) or MS BLAST (115, 116) are also sufficient for protein database screening for fragments with amino acid sequences identical or similar to biologically active peptides (117,118). Bioinformatic methods are widely used for prediction of potential allergenicity of proteins and peptides from their sequences. Progress in this area has been reviewed by Korber *et al.* (119). *In silico* methods were also applied in investigations aimed at searching for endogenous neuropeptides (120–122). Programs MEME (123) and Pratt (124) were used for construction of motifs corresponding to neuropeptides. Protein sequence databases were then screened for such motifs using the MAST program (125). Databases of biologically active peptide sequences are now available (94,95,126). The BIOPEP database (94) utilizes literature resources mainly from the area of food science. Other databases, like SwePep (95) and EROP-Moscow (126), are oriented towards endogenous peptides from various living organisms. Apart from the above-mentioned general peptide databases, more specialized databases oriented towards single activity are developed. The databases of antimicrobial peptides such as ANTIMIC (127) or APD (128) may serve as examples of such kind of bioinformatic tools.

Secondary structures, as well as hydrophobicity, serve as descriptors to characterize bioactive peptides. Many algorithms for secondary structure prediction are available *via* the websites such as ExPASy (129), EVA (130) or Pôle Bioinformatique Lyonnais (131). Among the hydrophobicity scales, the most common one is the hydropathy index (132). The hydrophobicity scales and other physicochemical parameters of amino acids are available in the AAIndex database (133). A program connecting sequence similarity searching and comparing hydrophobicity patterns has been developed by Nakai *et al.* (134,135). It has been applied both for description of antimicrobial activity (134) as well as emulsifying properties (135) of peptides. An example of the relationship between the structure expressed *via* angles between particular bonds and the anti-aggregative activity of peptides has been described by Mel'nik *et al.* (136).

Proteolysis simulation and design appear to be an important approach in theoretical work on biologically active peptides. The simplest tools for such simulation are programs searching for single bonds susceptible to endopeptidases such as PeptideCutter (129). This program has been successfully used to find enzymes releasing the antimicrobial lactoferrampin domain from the lactoferrin chain (137). The BIOPEP program utilizes an algorithm to search for the so-called recognition sequences containing few amino acid residues (27,94,98). Recently, more sophisticated strategies for proteolysis simulation have been developed using protein unfolding, sequence similarity and physicochemical properties of amino acids surrounding the bond hydrolyzed by an enzyme (138–141). Theoretical work on proteolytic enzyme spec-

ificity is concentrated on endopeptidases. The annotation of exopeptidase specificity, using peptide bonds resistant to the enzyme action, has been proposed by O'Connor and Warwicker (142).

The Quantitative Structure-Activity Relationship (QSAR) approach in research into food peptides has recently been reviewed by Nakai *et al.* (143) and Pripp *et al.* (35). More recent works have been concerned with the description of the common structural properties of antihypertensive (144) and prolyl endopeptidase inhibitory (145) peptides. QSAR has recently been used for designing novel antiviral peptides (146).

Another approach is the prediction of the biological activity of protein hydrolysates. To date, forecasts have covered the prediction of the release of known biologically-active peptides by endopeptidases (147) or the prediction of the biological activity of peptides potentially released by proteolytic enzymes with known specificity using the QSAR strategy (148). In both cases, the PeptideCutter program (129) was used for simulation of proteolysis.

The analytical approach in peptidomics covers the development of computational tools for identification of peptides and their protein precursors, including genetic variants and chemical as well as enzymatic modifications by mass spectrometry. This area has been recently reviewed by Johnson *et al.* (70). Recent standards used in proteomic investigations, which may also be sufficient for peptidomics, have been reviewed by Hogan *et al.* (149) and Droit *et al.* (150). Algorithms for the prediction of retention times of peptides separated using reversed-phase high-performance liquid chromatography (151–154) or migration times of peptides separated using capillary electrophoresis (155) have also been developed. The latter was successfully applied in an experiment with rapeseed peptides (156). Kim *et al.* (157) have found a correlation between the retention time and the activity of a set of antibacterial peptides.

Chemometrical analysis patterns obtained *via* analysis of peptidomes using chromatography or capillary electrophoresis have been reviewed based on the example of cheeses by Coker *et al.* (62). This strategy involves principal component analysis (PCA), discriminant analysis, multiple linear regression and partial least squares regression or cluster analysis. The latter method enabled, among others, the identification of peaks as markers of adulteration of milk protein preparations by soybean proteins or soybean protein preparation by milk proteins (14). Chemometric methods, such as artificial neural networks, may also be applied in the optimization of the process of protein hydrolysis aimed at obtaining bioactive peptides, such as antioxidative peptides from big-head carp muscles (87).

## Final Remarks

The biological activity of peptides has recently become a 'hot' topic. The influence of peptides on taste, allergenicity and functional properties, interactions with compounds such as proteins, sugars, lipids, and other food components as well as dynamic changes of peptidome during technological processes and storage should

all be studied further. Recent development of both experimental (such as chromatography, electrophoresis, mass spectrometry and biological activity tests) and computational (databases and programs processing peptide sequences as well as predicting structure and physico-chemical properties) methods enables rapid progress in this area. The increasing importance of data treatment and mining should be emphasized.

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