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*review*

# 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 receptors 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 α-helix (*33–35*). Some bioactive peptides are multifunctional. Bovine caseinomacropeptide – a C-terminal fragment of κ-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 β-casomorphin-7, which has opioid properties, including immunosuppression and could account for the relation between the consumption of β-casein $A^1$ 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*) |

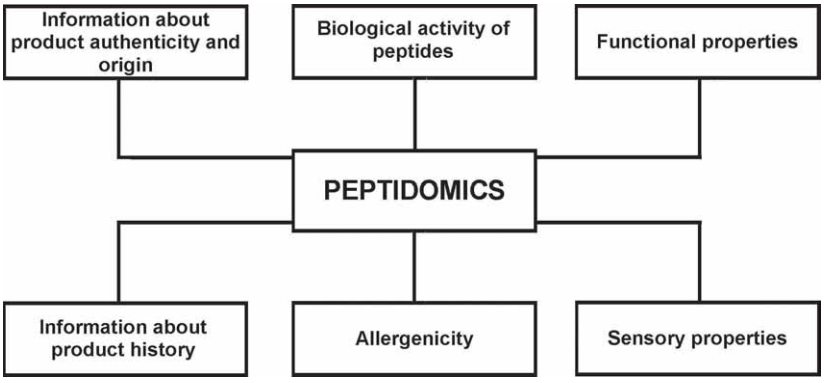


**Fig. 1.** Areas of interests in food peptidomics

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 subfractions 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*)

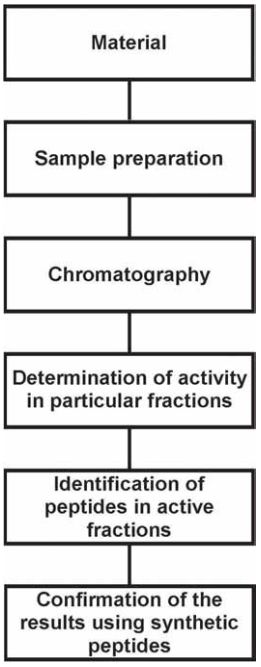


**Fig. 2.** Classical scheme of *in vitro* investigations on peptide bioactivity

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 β-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

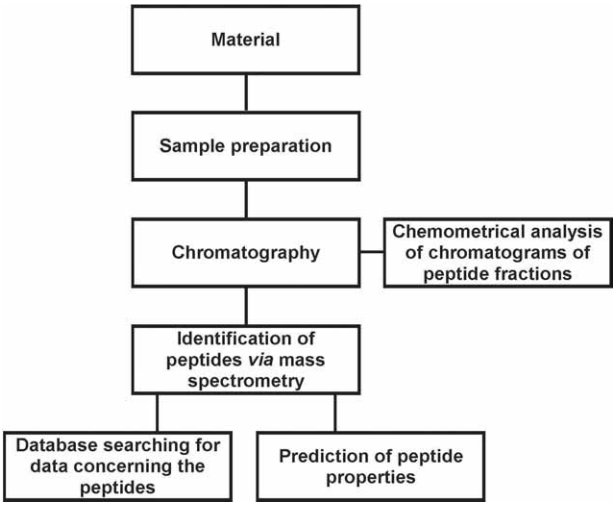


**Fig. 3.** Possible scheme of peptidomic research integrating experimental research, chemometrics and bioinformatic approach

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*). Vercruysse *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 specificity 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 bighead 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 physicochemical properties) methods enables rapid progress in this area. The increasing importance of data treatment and mining should be emphasized.

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## References

1. J.B. German, F.L. Schanbacher, B. Lönnerdal, J.F. Medrano, M.A. McGuire, J.L. McManaman, D.M. Roche, T.P. Smith, M.C. Neville, P. Donnelly, M. Lange, R. Ward, International milk genomics consortium, *Trends Food Sci. Technol. 17* (2006) 656–661.
2. E.C.M. Mariman, Nutrigenomics and nutrigenetics: The 'omics' revolution in nutritional science, *Biotechnol. Appl. Biochem. 44* (2006) 119–128.
3. S. Souchelnytskiy, Bridging proteomics and systems biology: What are the roads to be traveled?, *Proteomics, 5* (2005) 4123–4137.
4. V. Tolstoguzov, Phase behaviour of macromolecular components in biological and food systems, *Nahrung*, *44* (2000) 299–308.
5. A.A. Karelin, E.Y. Blischenko, V.T. Ivanov, A novel system of peptidergic regulation, *FEBS Lett. 428* (1998) 7–12.
6. T.M. Eads, Molecular basis of structure and functionality in foods, *Trends Food Sci. Technol. 5* (1994) 147–159.
7. M. Schrader, P. Schulz-Knappe, Peptidomics technologies for human body fluids, *Trends Biotechnol*. (Suppl.), *19* (2001) 55–60.
8. G. Baggerman, P. Verleyen, E. Clynen, J. Huybrechts, A. De Loof, L. Schoofs, Peptidomics, *J. Chromatogr. B*, *803* (2004) 3–16.
9. L. Paulson, R. Persson, G. Karlsson, J. Silberring, A. Bierczynska-Krzysik, R. Ekman, A. Westman-Brinkmalm, Proteomics and peptidomics in neuroscience. Experience of capabilities and limitations in a neurochemical laboratory, *J. Mass Spectrom. 40* (2005) 202–213.
10. M. Soloviev, P. Finch, Peptidomics: Bridging the gap between proteome and metabolome, *Proteomics*, *6* (2006) 744–747.
11. V.T. Ivanov, O.N. Yatskin, O.V. Sazonova, A.G. Tolmazova, K.V. Leontiev, M.M. Philippova, A.A. Karelin, E.Y. Blischenko, Peptidomics: A modern approach to biodiversity of peptides, *Pure Appl. Chem. 78* (2006) 963–975.
12. C. Bauchart, C. Chambon, P.P. Mirand, I. Savary-Auzeloux, D. Remond, M. Morzel, Peptides in rainbow trout (*Oncorhynchus mykiss*) muscle subjected to ice storage and cooking, *Food Chem. 100* (2007) 1566–1572.
13. S. Visser, Proteolytic enzymes and their relation to cheese ripening and flavor: An overview, *J. Dairy Sci. 76* (1993) 329–350.
14. J. Dziuba, D. Nałęcz, P. Minkiewicz, B. Dziuba, Identification and determination of milk and soybean protein preparations using enzymatic hydrolysis followed by chromatography and chemometrical data analysis, *Anal. Chim. Acta, 521* (*2004*) 17–24.
15. F. Kvasnička, Proteomics: General strategies and application to nutritionally relevant proteins, *J. Chromatogr. B*, *787* (2003) 77–89.
16. M. Carbonaro, Proteomics: Present and future in food quality evaluation, *Trends Food Sci. Technol. 15* (2004) 209–216.
17. M.A. Manso, J. Léonil, G. Jan, V. Gagnaire, Application of proteomics to characterization of milk and dairy products, *Int. Dairy J. 15* (2005) 845–855.
18. E. Bendixen, The use of proteomics in meat science, *Meat Sci. 71* (2005) 138–149.
19. D.J. Skylas, D. Van Dyk, C.W. Wrigley, Proteomics of wheat grain, *J. Cereal Sci. 41* (2005) 165–179.
20. D. Fuchs, I. Winkelman, I.T. Johnson, E.C.M. Mariman, U. Wenzel, H. Daniel, Proteomics in nutrition research: Principles, technologies and applications, *Br. J. Nutr. 94* (2005) 302–314.
21. J.J. Wang, D.F. Li, L.J. Dangott, G.Y. Wu, Proteomics and its role in nutrition research, *J. Nutr. 136* (2006) 1759–1762.
22. A. Kilara, D. Panyam, Peptides from milk proteins and their properties, *Crit. Rev. Food Sci. Nutr. 43* (2003) 607–633.
23. J. Dziuba, P. Minkiewicz, D. Nałęcz, Biologically active peptides from plant and animal proteins, *Pol. J. Food Nutr. Sci. 8/49* (1999) 3–16.
24. R.J. FitzGerald, B.A. Murray, Bioactive peptide and lactic fermentation, *Int. J. Dairy Technol. 59* (2006) 118–125.
25. E. Schlimme, H. Meisel, Bioactive peptides derived from milk proteins. Structural, physiological and analytical aspects, *Nahrung*, *39* (1995) 1–20.
26. H. Korhonen, A. Pihlanto-Leppälä, P. Rantamäki, T. Tupasela, Impact of processing on bioactive proteins and peptides, *Trends Food Sci. Technol. 9* (1998) 307–319.
27. J. Dziuba, P. Minkiewicz, D. Nałęcz, A. Iwaniak, Database of biologically active peptide sequences, *Nahrung*, *43* (1999) 190–195.
28. M. Gobbetti, L. Stepaniak, M. De Angelis, A. Corsetti, R. Di Cagno, Latent bioactive peptides in milk proteins: Proteolytic activation and significance in dairy processing, *Crit. Rev. Food Sci. Nutr. 42* (2002) 223–240.
29. H. Meisel, Biochemical properties of peptides encrypted in bovine milk proteins, *Curr. Med. Chem. 12* (2005) 1905–1919.
30. V. Vermeirssen, J. Van Camp, W. Verstraete, Bioavailability of angiotensin I-converting enzyme inhibitory peptides, *Br. J. Nutr. 92* (2004) 357–366.
31. J. Dziuba, P. Minkiewicz, K. Plitnik, Chicken meat proteins as potential precursors of bioactive peptides, *Pol. J. Food Nutr. Sci. 5/46* (1996) 85–96.
32. M.K. Schmidl, S. Taylor, J.A. Nordlee, Use of hydrolysate-based products in special medical diets, *Food Technol. 48* (1994) 77–85.
33. D.A. Clare, G.L. Catignani, H.L. Swaisgood, Biodefense properties of milk: The role of antimicrobial proteins and peptides, *Curr. Pharm. Des. 9* (2003) 1239–1255.
34. B. Bechinger, Structure and function of membrane lytic peptides, *Crit. Rev. Plant Sci. 23* (2004) 271–292.
35. A.H. Pripp, T. Isakson, L. Stepaniak, T. Sørhaug, Y. Ardö, Quantitative structure activity relationship modelling of peptides and proteins as a tool in food science, *Trends Food Sci. Technol. 16* (2005) 484–494.
36. J. Dziuba, P. Minkiewicz, Influence of glycosylation on micelle-stabilizing ability and biological properties of C-terminal fragments of cow's κ-casein, *Int. Dairy J. 6* (1996) 1017–1044.
37. C. Thomä-Worringer, J. Sørensen, R. López-Fandiño, Health effects and technological features of caseinomacropeptide, *Int. Dairy J. 16* (2006) 1324–1333.

*38.* E. Glukhov, M. Stark, L.L. Burrows, C.M. Deber, Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes, *J. Biol. Chem. 280* (2005) 33960–33968.

*39.* B.H. Birgisdottir, J.P. Hill, A.V. Thorsson, I. Thorsdottir, Lower consumption of cow milk protein A1 β-casein at 2 years of age, rather than consumption among 11- to 14--year-old adolescents, may explain the lower incidence of type I diabetes in Iceland than in Scandinavia, *Ann. Nutr. Metab. 50* (2005) 177–183.

*40.* M. Darewicz, J. Dziuba, Functional properties of proteins and peptides in the context of their molecular characteristics, *Natur. Sci. 6* (2000) 7–23.

*41.* M. Darewicz, J. Dziuba, M. Dziuba, Functional properties and biological activities of bovine casein proteins and peptides, *Pol. J. Food Nutr. Sci.* (Suppl. 1) *15/56* (2006) 79–86.

*42.* J.E. Kinsella, Physical properties of food and milk components. Research needs to expand uses, *J. Dairy Sci. 70* (1987) 2419–2429.

*43.* E.N. Clare Mills, J.A. Jenkins, M.J.C. Alcocer, P.R. Shewry, Structural, biological and evolutionary relationships of plant food allergens sensitizing *via* the gastrointestinal tract, *Crit. Rev. Food Sci. Nutr. 44* (2004) 379–407.

*44.* S.K. Sathe, H.H. Kshirsagar, K.H. Roux, Advances in seed protein research: A perspective on seed allergens, *J. Food Sci. 70* (2005) R93–R120.

*45.* A.D. Siemensma, W.J. Weijer, H.J. Bak, The importance of peptide lengths in hypoallergenic infant formulae, *Trends Food Sci. Technol. 4* (1993) 16–21.

*46.* C.T. Cordle, Control of food allergies using protein hydrolysates, *Food Technol. 48* (1994) 72–76.

*47.* S.L. Taylor, S.L. Hefle, Allergen control, *Food Technol. 59* (2005) 40–43.

*48.* T.J. Fu, Digestion stability as a criterion for protein allergenicity assessment, *Ann. NY Acad. Sci. 964* (2002) 99–110.

*49.* T.J. Fu, U.R. Abbott, C. Hatzos, Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – A comparative study, *J. Agric. Food Chem. 50* (2002) 7154–7160.

*50.* T.J. Fu, U.R. Abbott, C. Hatzos, Rebuttal on digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – A comparative study, *J. Agric. Food Chem. 51* (2003) 5185–5187.

*51.* S.L. Taylor, Comment on digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – A comparative study, *J. Agric. Food Chem. 51* (2003) 5183–5184.

*52.* F.J. Moreno, F.A. Mellon, M.S.J. Wickham, A.R. Bottrill, E.N. Clare Mills, Stability of the major allergen Brazil nut 2S albumin (Ber e 1) to physiologically relevant *in vitro* gastrointestinal digestion, *FEBS J. 272* (2005) 341–352.

*53.* S.J. Chambers, M.S.J. Wickham, M. Regoli, E. Bertelli, P.A. Gunning, C. Nicoletti, Rapid *in vivo* transport of proteins from digested allergen across pre-sensitized gut, *Biochem. Biophys. Res. Commun. 325* (2004) 1258–1263.

*54.* L.M. Sollid, Celiac disease: Dissecting a complex inflammatory disorder, *Nature Rev. Immunol. 2* (2002) 647–655.

*55.* D. Dewar, S.P. Pereira, P.J. Ciclitira, The pathogenesis of celiac disease, *Int. J. Biochem. Cell Biol. 36* (2004) 17–24.

*56.* J. Waga, Structure and allergenicity of wheat gluten proteins – A review, *Pol. J. Food Nutr. Sci. 13/54* (2004) 327–338.

*57.* L. Lemieux, R.E. Simard, Bitter flavour in dairy products. I. A review of the factors likely to influence its development, mainly in cheese manufacture, *Lait, 71* (1991) 599–636.

*58.* L. Lemieux, R.E. Simard, Bitter flavour in dairy products. II. A review of bitter peptides from caseins: Their formation, isolation and identification, structure masking and inhibition, *Lait, 72* (1992) 335–382.

*59.* R.J. FitzGerald, G. O'Cuinn, Enzymatic debittering of food protein hydrolysates, *Biotechnol. Adv. 24* (2006) 234–237.

*60.* H.N. Lioe, K. Wada, T. Aoki, M. Yasuda, Chemical and sensory characteristics of low molecular weight fractions obtained from three types of Japanese soy sauce (shoyu) – Koikuchi, tamari and shiro shoyu, *Food Chem. 100* (2007) 1669–1677.

*61.* A.H. Pripp, L. Stepaniak, T. Sørhaug, Chemometrical analysis of proteolytic profiles during cheese ripening, *Int. Dairy J. 10* (2000) 249–253.

*62.* C.J. Coker, R.A. Crawford, K.A. Johnston, H. Singh, L.K. Creamer, Towards the classification of cheese variety and maturity on the basis on statistical analysis of proteolysis data – A review, *Int. Dairy J. 15* (2005) 631–643.

*63.* J. Sørensen, C. Benfeldt, Comparison of ripening characteristics of Danbo cheeses from two dairies, *Int. Dairy J. 11* (2001) 355–362.

*64.* C. Benfeldt, J. Sørensen, Heat treatment of cheese milk – Effect on proteolysis during cheese ripening, *Int. Dairy J. 11* (2001) 567–574.

*65.* A.A. Hayaloglu, M. Guven, P.F. Fox, P.L.H. McSweeney, Influence of starters on chemical, biochemical and sensory changes in Turkish white-brined cheese during ripening, *J. Dairy Sci. 88* (2005) 3460–3474.

*66.* C. van der Ven, S. Muresan, H. Gruppen, D.B.A. de Bont, K. Merck, A.G.J. Voragen, FTIR spectra of whey and casein hydrolysates in relation to their functional properties, *J. Agric. Food Chem. 50* (2002) 6943–6950.

*67.* P. Verhaert, S. Uttenweiler-Joseph, M. de Vries, A. Loboda, W. Ens, K.G. Standing, Matrix-assisted laser desorption/ionization quadrupole time-of-flight mass spectrometry: An elegant tool for peptidomics, *Proteomics, 1* (2001) 118–131.

*68.* M. Svensson, K. Sköld, P. Svenningsson, P.E. Andren, Peptidomics-based discovery of novel neuropeptides, *J. Proteome Res. 2* (2003) 213–219.

*69.* Y. Shi, R. Xiang, C. Horváth, J.A. Wilkins, The role of liquid chromatography in proteomics, *J. Chromatogr. A, 1053* (2004) 27–36.

*70.* R.S. Johnson, M.T. Davis, J.A. Taylor, S.D. Patterson, Informatics for protein identification by mass spectrometry, *Methods, 35* (2005) 223–236.

*71.* P. Hernandez, M. Müller, R.D. Appel, Automated protein identification by tandem mass spectrometry: Issues and strategies, *Mass Spectrom. Rev. 25* (2006) 235–254.

*72.* H. Motoi, T. Kodama, Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides from wheat gliadin hydrolysate, *Nahrung, 47* (2003) 354–358.

*73.* E.D. Marczak, H. Usui, H. Fujita, Y. Yang, M. Yokoo, A.W. Lipkowski, M. Yoshikawa, New antihypertensive peptides isolated from rapeseed, *Peptides, 24* (2003) 791–798.

*74.* R. Sørensen, E. Kildal, L. Stepaniak, A.H. Pripp, T. Sørhaug, Screening for peptides from fish and cheese inhibitory to prolyl endopeptidase, *Nahrung, 48* (2004) 53–56.

*75.* J.A. Gómez-Ruiz, M. Ramos, I. Recio, Identification and formation of angiotensin-converting enzyme-inhibitory peptides in Manchego cheese by high-performance liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A, 1054* (2004) 269–277.

*76.* B. Hernández-Ledesma, M. Ramos, I. Recio, L. Amigo, Effect of β-lactoglobulin hydrolysis with thermolysin under denaturing temperatures on the release of bioactive peptides, *J. Chromatogr. A, 1116* (2006) 31–37.

77. K.G. Mallikarjun Gouda, L.R. Gowda, A.G. Appu Rao, V. Prakash, Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11S globulin of soybean (*Glycine max*), *J. Agric. Food Chem. 54* (2006) 4568–4573.

78. M.S. Ma, I.Y. Bae, H.G. Lee, C.B. Yang, Purification and identification of angiotensin-converting enzyme inhibitory peptide from buckwheat (*Fagopyrum esculentum* Moench), *Food Chem. 96* (2006) 36–42.

79. I. López-Expósito, J.A. Gómez-Ruiz, L. Amigo, I. Recio, Identification of antibacterial peptides from ovine $\alpha_{s2}$-casein, *Int. Dairy J. 16* (2006) 1072–1080.

80. T. Rokka, E.L. Syväoja, J. Tuominen, H. Korhonen, Release of bioactive peptides by enzymatic proteolysis of *Lactobacillus* GG UHT milk, *Milchwissenschaft*, *52* (1997) 675–678.

81. B. Hernández-Ledesma, B. Miralles, L. Amigo, M. Ramos, I. Recio, Identification of antioxidant and ACE-inhibitory peptides in fermented milk, *J. Sci. Food Agric. 85* (2005) 1041–1048.

82. J.A. Gómez-Ruiz, G. Taborda, L. Amigo, I. Recio, M. Ramos, Identification of ACE-inhibitory peptides in different Spanish cheeses by tandem mass spectrometry, *Eur. Food Res. Technol. 223* (2006) 595–602.

83. C. Megías, J. Pedroche, M. del Mar Yust, M. Alaiz, J. Girón-Calle, F. Millán, J. Vioque, Affinity purification of angiotensin-converting enzyme inhibitory peptides using immobilized ACE, *J. Agric. Food Chem. 54* (2006) 7120–7124.

84. H. Meisel, A. Goepfert, S. Günther, ACE-inhibitory activities in milk products, *Milchwissenschaft*, *52* (1997) 307–311.

85. R. Amarowicz, F. Shahidi, Antioxidant activity of peptide fractions of capelin protein hydrolysates, *Food Chem. 58* (1997) 355–359.

86. W.M.Y. Lo, E.C.Y. Li-Chan, Angiotensin I converting enzyme inhibitory peptides from *in vitro* pepsin-pancreatin digestion of soy proteins, *J. Agric. Food Chem. 53* (2005) 3369–3376.

87. L. Li, J. Wang, M. Zhao, C. Cui, Y. Jiang, Artificial neural network for production of antioxidant peptides derived from bighead carp muscles with alcalase, *Food Technol. Biotechnol. 44* (2006) 441–448.

88. V. Kamath, S. Niketh, A. Chandrasekhar, P.S. Rajini, Chymotryptic hydrolysates of α-kafirin, the storage protein of sorghum (*Sorghum bicolor*) exhibited angiotensin converting enzyme inhibitory activity, *Food Chem. 100* (2007) 306–311.

89. G. Mitulović, C. Stingl, M. Smoluch, R. Swart, J.P. Chervet, I. Steinmacher, C. Gerner, K. Mechtler, Automated on-line two-dimensional nano liquid chromatography tandem mass spectrometry for rapid analysis of complex protein digests, *Proteomics*, *4* (2004) 2545–2557.

90. J. Flensburg, A. Tangen, M. Prieto, U. Hellman, H. Wadensten, Chemically-assisted fragmentation combined with multi-dimensional liquid chromatography and matrix-assisted laser desorption/ionization post source decay, matrix-assisted laser desorption/ionization tandem time-of--flight or matrix-assisted laser desorption/ionization tandem mass spectrometry for improved sequencing of tryptic peptides, *Eur. J. Mass Spectrom. 11* (2005) 169–179.

91. D.C. Simpson, S. Ahn, L. Paša-Tolić, B. Bogdanov, H.M. Motton, A.N. Vilkov, G.A. Anderson, M.S. Lipton, R.D. Smith, Using size-exclusion chromatography-RPLC and RPLC-CIEF as two-dimensional separation strategies for protein profiling, *Electrophoresis*, *27* (2006) 2722–2733.

92. F.M. Lagerwerf, P.D. van Wassenaar, J. Haverkamp, Comparison of Cheddar cheeses produced with recombinant chymosin and with calf rennet using two dimensional high-performance liquid chromatography, *J. Dairy Res. 62* (1995) 673–679.

93. M. Dziuba, J. Dziuba, P. Minkiewicz, Design of food protein proteolysis with a view to obtaining bioactive peptides, *Pol. J. Natur. Sci. 21* (2006) 999–1020.

94. J. Dziuba, A. Iwaniak: Database of Protein and Bioactive Peptide Sequences. In: *Nutraceutical Proteins and Peptides in Health and Disease,* Y. Mine, F. Shahidi (Eds.), CRC – Taylor & Francis, Boca Raton, FL, USA (2006) pp. 543–563.

95. M. Fälth, K. Sköld, M. Norrman, M. Svensson, D. Fenyö, P.E. Andren, SwePep, a database designed for endogenous peptides and mass spectrometry, *Mol. Cell. Proteomics*, *5* (2006) 998–1005.

96. D. Jelić, D. Toth, D. Verbanac, Macromolecular databases – A background of bioinformatics, *Food Technol. Biotechnol. 41* (2003) 269–286.

97. P.K. Akalin, Introduction to bioinformatics, *Mol. Nutr. Food Res. 50* (2006) 610–619.

98. J. Dziuba, P. Minkiewicz, K. Puszka, S. Dąbrowski, Plant seed storage proteins as potential precursors of bioactive peptides, *Pol. J. Food Nutr. Sci. 4/45* (1995) 31–42.

99. J. Dziuba, A. Iwaniak, P. Minkiewicz, Computer-aided characteristics of proteins as potential precursors of bioactive peptides, *Polimery* (*Warsaw*), *48* (2003) 50–53.

100. W.Y. Wang, E. Gonzalez de Meija, A new frontier in soy bioactive peptides that may prevent age-related chronic diseases, *Compr. Rev. Food Sci. Food Saf. 4* (2005) 63–78.

101. A. Iwaniak, J. Dziuba, M. Niklewicz, The BIOPEP database – A tool for the *in silico* method for classification of food proteins as the source of peptides with antihypertensive activity, *Acta Aliment. 34* (2005) 417–425.

102. J. Dziuba, A. Iwaniak, M. Darewicz, M. Niklewicz, Computer simulation of animal proteins proteolysis in the aspects of bioactive peptide obtaining, *Pol. J. Natur. Sci. 10* (2002) 209–222.

103. J. Dziuba, M. Niklewicz, A. Iwaniak, M. Darewicz, P. Minkiewicz, Bioinformatic-aided prediction for release possibilities of bioactive peptides from plant proteins, *Acta Aliment. 33* (2004) 227–235.

104. J. Dziuba, M. Niklewicz, A. Iwaniak, M. Darewicz, P. Minkiewicz, Structural properties of proteolytic-accessible fragments of selected animal proteins, *Polimery* (*Warsaw*), *50* (2005) 424–428.

105. L. Vercruysse, J. Van Camp, G. Smagghe, ACE inhibitory peptides derived from hydrolysates of animal muscle proteins, *J. Agric. Food Chem. 53* (2005) 8106–8115.

106. J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: Improving the sensitivity of progressive multiple alignment through sequence weighting position-specific gap penalties and weight matrix choice, *Nucleic Acids Res. 22* (1994) 4673–4680.

107. S.F. Altschul, W. Gish, W. Miller, E.W. Meyers, D.J. Lipman, Basic local alignment search tool, *J. Mol. Biol. 215* (1990) 403–410.

108. S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI--BLAST: A new generation of protein database search programs, *Nucleic Acids Res. 25* (1997) 3389–3402.

109. W.R. Pearson, Flexible sequence similarity searching with the FASTA3 program package, *Methods Mol. Biol. 132* (2000) 185–219.

110. L. Shan, Ø. Molberg, I. Parrot, F. Hausch, F. Filiz, G.M. Gray, L.M. Sollid, C. Khosla, Structural basis for gluten intolerance in celiac sprue, *Science*, *297* (2002) 2275–2279.

111. L. Shan, S.W. Quiao, H. Arentz-Hansen, Ø. Molberg, G.M. Gray, L.M. Sollid, C. Khosla, Identification and analysis of multivalent proteolytically resistant peptides from gluten: Implications for celiac sprue, *J. Proteome Res. 4* (2005) 1732–1741.

*112.* L.W. Vader, D.T. Stepniak, E.M. Bunnick, Y.M.C. Kooy, W. De Haan, J.W. Drijfhout, P.A. Van Weelen, F. Koning, Characterization of cereal toxicity for celiac disease patients based on protein homology in grains, *Gastroenterology*, *125* (2003) 1105–1113.

*113.* G. Zanoni, R. Navone, C. Lunardi, G. Tridente, C. Bason, S. Sivori, R. Beri, M. Dolcino, E. Valletta, R. Corrocher, A. Puccetti, In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes, *PLoS Med. 3* (2006) 1637–1653.

*114.* M. Mann, M. Wilm, Error-tolerant identification of peptides in sequence databases by peptide sequence tags, *Anal. Chem. 66* (1994) 4390–4399.

*115.* A. Shevchenko, S. Sunyaev, A. Loboda, A. Shevchenko, P. Bork, W. Ens, K.G. Standing, Charting the proteomes of organisms with unsequenced genomes by MALDI-quadrupole time-of-flight mass spectrometry and BLAST homology searching, *Anal. Chem. 73* (2001) 1917–1926.

*116.* N. Wielsch, H. Thomas, V. Surendranath, P. Waridel, A. Frank, P. Pevzner, A. Shevchenko, Rapid validation of protein identifications with the borderline statistical confidence via *de novo* sequencing and MS BLAST searches, *J. Proteome Res. 5* (2006) 2448–2456.

*117.* L.W. Vader, A. de Ru, Y. van der Wal, Y.M.C. Kooy, W. Benckhuijsen, M.L. Mearin, J.W. Drijfhout, P. van Veelen, F. Koning, Specificity of tissue transglutaminase explains cereal toxicity in celiac disease, *J. Exp. Med. 195* (2002) 643–649.

*118.* M. Darewicz, J. Dziuba, P. Minkiewicz, Bioinformatic--aided characteristics and identification of peptides for *in silico* detection of potentially celiac-toxic proteins, *Food Sci. Technol. Int. 13* (2007) 125–133.

*119.* B. Korber, M. LaBute, K. Yusim, Immunoinformatics comes of age, *PLoS Comput. Biol. 2* (2006) 484–492.

*120.* G. Baggerman, F. Liu, G. Wets, L. Schoofs, Bioinformatic analysis of peptide precursor proteins, *Ann. NY Acad. Sci. 1040* (2005) 59–65.

*121.* F. Liu, G. Baggerman, W. D'Hertog, P. Verleyen, L. Schoofs, G. Wets, *In silico* identification of new secretory peptide genes in *Drosophila melanogaster*, *Mol. Cell. Proteomics, 5* (2006) 510–522.

*122.* F. Liu, G. Baggerman, L. Schoofs, G. Wets, Uncovering conserved patterns in bioactive peptides in *Metazoa*, *Peptides*, *27* (2006) 3137–3153.

*123.* T.L. Bailey, C. Elkan, Fitting a mixture model by expectation maximization to discover motifs in biopolymers, *Proceedings of the Second Conference on Intelligent Systems for Molecular Biology*, AAAI Press, Menlo Park, CA, USA (1994) pp. 28–36.

*124.* I. Jonassen, J.F. Collins, D.G. Higgins, Finding flexible patterns in unaligned protein sequences, *Protein Sci. 4* (1995) 1587–1595.

*125.* T.L. Bailey, M. Gribskov, Combining evidence using p-values: Application to sequence homology searches, *Bioinformatics*, *14* (1998) 48–54.

*126.* A.A. Zamyatnin, A.S. Borchikov, M.G. Vladimirov, O.L. Voronina, The EROP-Moscow oligopeptide database, *Nucleic Acids Res. 34* (2006) D261–D266.

*127.* M. Brahmachary, S.P.T. Krishnan, J.L.Y. Koh, A.M. Khan, S.H. Seah, T.W. Tan, V. Brusic, A.V. Bajic, ANTIMIC: A database of antimicrobial sequences, *Nucleic Acids Res. 32* (2004) D586–D589.

*128.* Z. Wang, G. Wang, APD: The antimicrobial peptide database, *Nucleic Acids Res. 32* (2004) D590–D592.

*129.* E. Gasteiger, C. Hoogland, A. Gattiker, S. Duvaud, M.R. Wilkins, R.D. Appel, A. Bairoch: Protein Identification and Analysis Tools on the ExPASy Server. In: *The Proteomics Protocols Handbook*, J.M. Walker (Ed.), Humana Press Inc., Totowa, NJ, USA (2005) pp. 571–607.

*130.* I.Y.Y. Koh, V.A. Eyrich, M.A. Marti-Renom, D. Przybylski, M.S. Madhusudhan, E. Narayanan, O. Graña, F. Pazos, A. Valencia, A. Sali, B. Rost, EVA: Evaluation of protein structure prediction servers, *Nucleic Acids Res. 31* (2003) 3311–3315.

*131.* Pôle Bioinformatique Lyonnais (*http://npsa-pbil.icpb.fr*).

*132.* J. Kyte, R.F. Doolittle, A simple method for displaying the hydropathic character of a protein, *J. Mol. Biol. 157* (1982) 105–132.

*133.* S. Kawashima, M. Kanehisa, AAIndex: Amino acid index database, *Nucleic Acids Res. 28* (2000) 374.

*134.* S. Nakai, J.C.K. Chan, E.C.Y. Li-Chan, J. Dou, M. Ogawa, Homology similarity analysis of sequences of lactoferricin and its derivatives, *J. Agric. Food Chem. 51* (2003) 1215–1223.

*135.* S. Nakai, N. Alizadeh-Pasdar, J. Dou, R. Buttinov, D. Roussou, A. Poulson, Pattern similarity analysis of amino acid sequences for peptide emulsification, *J. Agric. Food Chem. 52* (2004) 927–935.

*136.* O.V. Mel'nik, V.P. Martinovich, V.P. Golubovich, The structure-antiaggregative activity relationship in a series of Arg-Gly-Asp analogues, *Russ. J. Bioorg. Chem. 32* (2006) 122–128.

*137.* J.G.M. Bolscher, M.I.A. van der Kraan, K. Nazmi, H. Kalay, C.H. Grün, W. van't Hof, E.C.I. Veerman, A.V.A. Nieuw Amerongen, A one-enzyme strategy to release an antimicrobial peptide from LFampin-domain of bovine lactoferrin, *Peptides*, *27* (2006) 1–9.

*138.* M.M. Vorob'ev, I.A. Goncharova, Computer simulation of proteolysis. Peptic hydrolysis of partially demasked β-lactoglobulin, *Nahrung, 42* (1998) 61–67.

*139.* I. Callebaut, F. Schoentgen, K. Prat, J.P. Mornon, P. Jollès, Characterization and study of a κ-casein-like chymosin--sensitive linkage, *Biochim. Biophys. Acta, 1749* (2005) 75–80.

*140.* A. Amare, A.B. Hummon, B.R. Southey, T.A. Zimmerman, S.L. Rodriguez-Zas, J.V. Sweedler, Bridging neuropeptidomics and genomics with bioinformatics: Prediction of mammalian neuropeptide prohormone processing, *J. Proteome Res. 5* (2006) 1162–1167.

*141.* N. Ozer, T. Haliloglu, C.A. Schiffer, Substrate specificity in HIV-1 protease by a biased sequence search method, *Proteins, 64* (2006) 444–456.

*142.* J. O'Connor, J. Warwicker, Prediction of peptides in the fermented cocoa storage protein with reference to the modelled 3D structure, *J. Sci. Food Agric. 77* (1998) 109–114.

*143.* S. Nakai, M. Ogawa, S. Nakamura, J. Dou, K. Funane, A computer-aided strategy for structure-function relation study of food proteins using unsupervised data mining, *Int. J. Food Prop. 6* (2003) 25–47.

*144.* J. Wu, R.E. Aluko, S. Nakai, Structural requirements of angiotensin I-converting enzyme inhibitory activity: Quantitative structure-activity relationship study of di- and tripeptides, *J. Agric. Food Chem. 54* (2006) 732–738.

*145.* A.H. Pripp, Quantitative structure-activity relationship of prolyl oligopeptidase inhibitory peptides derived from β-casein using simple amino acid descriptors, *J. Agric. Food Chem. 54* (2006) 224–228.

*146.* H. Jenssen, T.J. Gutteberg, O. Rekdal, T. Lejon, Prediction of activity, synthesis and biological testing of anti-HSV active peptides, *Chem. Biol. Drug Design, 68* (2006) 58–66.

*147.* V. Vermeirssen, A. van den Bent, J. Van Camp, A. van Amerongen, W. Verstraete, A quantitative in silico analysis calculates the angiotensin I converting enzyme (ACE) inhibitory activity in pea and whey protein digests, *Biochimie, 86* (2004) 231–239.

*148.* A.H. Pripp, Initial proteolysis of milk proteins and its effect on formation of ACE-inhibitory peptides during gastrointestinal proteolysis: A bioinformatic approach, *Eur. Food Res. Technol. 221* (2005) 712–716.

*149.* J.M. Hogan, R. Higdon, E. Kolker, Experimental standards for high-throughput proteomics, *OMICS, 10* (2006) 152–157.

*150.* A. Droit, J. Fillon, J. Morissette, G.G. Poirier, Bioinformatic standards for proteomics-oriented mass spectrometry, *Curr. Proteom. 3* (2006) 119–128.

*151.* M. Palmblad, M. Ramström, K.E. Markides, P. Håkansson, J. Bergquist, Prediction of chromatographic retention and protein identification in liquid chromatography/mass spectrometry, *Anal. Chem. 74* (2002) 5826–5830.

*152.* T. Bączek, P. Wiczling, M. Marszałł, Y. Vander Heyden, R. Kaliszan, Prediction of peptide retention at different HPLC conditions from multiple linear regression models, *J. Proteome Res. 4* (2005) 555–563.

*153.* R. Put, M. Daszykowski, T. Bączek, Y. Vander Heyden, Retention prediction of peptides based on uninformative variable elimination by partial least squares, *J. Proteome Res. 5* (2006) 1618–1625.

*154.* K. Petritis, L.J. Kangas, B. Yan, M.E. Monroe, E.F. Strittmatter, W.J. Qian, J.N. Adkins, R.J. Moore, Y. Xu, M.S. Lipton, D.G. Camp II, R.D. Smith, Improved peptide elution time prediction for reversed-phase liquid chromatography--MS by incorporating peptide sequence information, *Anal. Chem. 78* (2006) 5026–5039.

*155.* B. Tessier, F. Blanchard, R. Vanderesse, C. Harscoat, I. Marc, Applicability of predictive models to the peptide mobility analysis by capillary electrophoresis-electrospray mass spectrometry, *J. Chromatogr. A, 1024* (2004) 255–266.

*156.* B. Tessier, M. Schweizer, F. Fournier, X. Framboisier, I. Chevalot, R. Vanderesse, C. Harscoat, I. Marc, Prediction of the amino acid composition of small peptides contained in a plant protein hydrolysate by LC-MS and CE-MS, *Food Res. Int. 38* (2005) 577–584.

*157.* S. Kim, S.S. Kim, B.J. Lee, Correlation between the activities of $\alpha$-helical antimicrobial peptides and hydrophobicities represented as RP HPLC retention times, *Peptides, 26* (2005) 2050–2056.