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Advancement and prospects of bioinformatics analysis for studying bioactive peptides from food-derived protein: Sequence, structure, and functions

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ABSTRACT

Food-derived bioactive peptides, as potential ingredients in health-promoting functional foods targeting diet-related chronic diseases, have attracted increasing attention because of their high biological activities, low toxicity, and easy of metabolism in human body. However, conventional methods for analyzing the bioactive peptides are not only expensive but also time-consuming; these drawbacks limited detailed studies and rapid development of bioactive peptides. Emerging bioinformatics approaches may overcome these problems to enable bioactive peptide research. The aim of this review is to provide an overview of research progress in the bioinformatics methods used for identifying, characterizing, elaborating bioactive mechanisms of, and producing food-derived bioactive peptides, and also to present an effective workflow. The workflow has been integrated *in silico* and traditional methods to predict, validate, and modify bioactive peptides.

1. Introduction

1.1. Bioactive peptides from food-derived proteins

Food-derived bioactive peptides (BAPs) refers to various amino acids sequence fragments, normally comprising 3–20 amino acids [1], produced from food protein; BAPs show great benefits for controlling disease and promoting human health, such as the blood pressure-lowering, immunity-improving, anti-inflammation, cholesterol-lowering, lipid-lowering and anticoagulation effects. Over the past few decades, as the chemically synthesized drugs have been shown to trigger adverse side effects, the far-reaching significance of BAP_S has been recognized; thus, researchers begin to isolate and identify BAPs from various food proteins. Traditionally, BAP_S can be acquired using four methods: (i) conventional enzymatic hydrolysis by various enzymes, such as pepsin, trypsin, papain, neutrase, among others; (ii) microbial fermentation, such as by *lactobacillus*; (iii) a combination of the above two methods; (iv) food processing, such as heat- and high-pressure treatment. Among these methods, conventional enzymatic hydrolysis is the most convenient and widely used method for preparing BAP_S because of its relatively high efficiency and stability, low complexity, and ease of analyses compared to microbial fermentation, which may introduce bacteriocins, bacterial cells, polysaccharides, and other biomolecules [2]. Numerous bioactive peptides related to hypertension, thrombosis, type-2 diabetes, obesity, cancer, and osteoporosis *i.e.* have been accessed by enzymatic hydrolysis from milk, soybean, fish, and meat protein [2–4]. Classical approaches for identifying and producing BAPs involve four major steps: (i) identifying a suitable protein source; (ii) releasing peptide fragments with bioactivity by enzymatic hydrolysis; (iii) isolating bioactive peptides by membrane separation or chromatography methods; and (iv) validating the activities of the identified peptides [4]. However, these methods are both time-consuming and costly for researchers and manufacturers, and there is uncertainty regarding whether the proteins hydrolyzates are bioactive, which must be verified.

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1.2. Bioinformatics technology application

Bioinformatics develops methods and software tools for understanding biological mass data. This field combines computer science, biology, mathematics, and statistical techniques to analyze and interpret biological data. Hence, bioinformatics has been used for *in silico* analyses of biological queries and applied in the fields of biological science, particularly genomics and proteomics, to better understand the biological basis of disease. The association between disease/health and food ingredients has attracted increasing attentions. Therefore, the application of bioinformatics technology in foodomics is presently being wide examined; it involves the analysis of food and nutrition fields through the application and integration of proteomics, transcriptomics, and metabolomics using bioinformatics analysis techniques [5].

In some cases, some multifunctional peptides can induce more than one physiological activity if they have a unique primary structure that can resist proteolysis, and/or if they consist of one or more overlapping sequences, each of which triggers different biological responses [6]. Therefore, bioinformatics can minimize the number of tests that must be performed to prepare BAPs by determining how their structure relates to their activity.

Recently, facing the challenges that the classical approaches for discovering BAPs encountered, bioinformatics has been rapidly used to evaluate bioactive peptides in proteins [7]. Bioinformatics, also known as *in silico* analysis, involves computational methods applied to manage, curate, and interpret information related to biological systems [4]. These *in silico* methods include kinds of databases, online tools, and software, some of which are listed in Table 1. Notably, among them, one tool may possess multiple functions. For example, BIOPEP (developed at the University of Warmia and Mazury in Poland) is a tool that not only interlinks databases of protein sequences, bioactive peptides, and sensory peptides, but also is an inbuilt program that aids in the prediction of proteolytic hydrolyzates and allergenic peptides [8]. Protein databases, such as UniProtKB, NCBI, and BIOPEP contain various protein sequences that can be used to analyze the amino acid profiles of precursor proteins. To predict theoretical bioactive profiles, the online tools BIOPEP and ExPASy-PeptideCutter have always been adopted by selecting the specific enzymes and proteins [9,10].

After the results of *in silico* hydrolysis are acquired, the released peptides can be compared with the bioactive peptides reported in the literature and databases, such as BIOPEP, Pepbank, PeptideDB, and BitterDB. As shown in Fig. 1, there are approximately 50 types of bioactivities reported in ScienceDirect database and other specific bioinformatics databases, such as BIOPEP, with the total number of bioactive peptides at 3566 peptides (accessed December, 2017). The number of bioactive peptides varies with different activities. The peptides with angiotensin-converting enzyme inhibitory (ACEI) activity showed the largest amount (903 peptides), followed by antioxidative activity (569 peptides), antibacterial activity (464 peptides), and other bioactivities. These bioactive peptides are generally derived from bovine casein, soybean proteins, walnut proteins, and marine proteins. Many novel peptides without activity reports can be accessed from proteins, and PeptideRanker then exerts its function to evaluate the likelihood of peptide activity by assigning scores from 0 to 1, where “1” and “0” present the most and least likely to be BAPs, respectively [11]. Molecular docking, as well as Quantitative structure-activity relationship (QSAR) also have been used to screen and predict the potential BAPs [12,13]. *In silico* methods can also predict the toxicity and allergenicity of the peptides by ToxinPred (available at http://crdd.osdd.net/raghava//toxinpred/) and AlgPred (available at http://crdd.osdd.net/raghava//algpred/), respectively.

1.3. General strategies

Compared with traditional drugs, BAPs have two advantages, namely, low toxicity and few side effects in humans. In recent years, the advent of proteomics technologies, based on mass spectrometric methods, has provided simple and effective approaches for discovering and identifying BAPs, in which a complex protein mixture is specifically enzymatically hydrolyzed into peptides; the peptides are then evaluated by a combination of high-performance liquid chromatography and mass spectrometric (MS) [5,14,15], such as MALDI-TOF MS, CE-TOF MS, and UPLC-Q/TOF MS [16]. Generally, as the technologies used in genome projects, proteomics, foodomics, and computational methods have undergone great development, a novel protocol combining bioinformatics, mass spectrometry, omics (proteomics and/or peptidomics), and *in vitro* enzymatic experiments has become particularly useful for analyzing and identifying functional peptides from a targeted protein. In this review, we summarize reported novel bioinformatics approaches for identifying and processing bioactive peptides, as shown in Fig. 2. Seven steps form the core of this approach: (i) selecting the suitable enzyme and protein source; (ii) enzymatic hydrolysis; (iii) identifying and predicting BAPs; (iv) validating the activities of peptides *in vivo* and/or *in vitro*; (v) exploring the mechanisms of BAPs; (vi) predicting the properties of peptides; and (vii) modifying the BAPs. The detailed contents will be discussed in the following sections.

2. Controlling enzymatic hydrolysis

2.1. In silico prediction tools

The first step in producing BAPs is enzymatic hydrolysis, which facilitates the cleavage of bonds in molecules through the addition of water and plays an important role in food digestion. Several factors affect the bioactive properties of enzymatic hydrolyzates and peptides, including the specificity of the enzymes used for hydrolysis, processing conditions, degree of hydrolysis, and structural properties of the resulting peptides, such as molecular size, hydrophobicity, and amino acid composition [3]. Moreover, the specific enzymatic substrate is also important for the bioactivity of hydrolyzates. To choose suitable enzymes and protein substrates, numerous parallel experiments must be conducted to compare the results obtained on using traditional BAPs production approaches. Fortunately, the emergence of *in silico* (computer simulation) methods can gradually overcome these problems.

Several servers, including BIOPEP, PeptideCutter, and EnzymePredictor, have been used to predict possible cleavage sites. Furthermore, databases, such as BIOPEP, PeptideDB, CAMP, APD2, and PepBank, which catalog BAPs, are becoming more efficient for identification and characterization of new peptides [17]. BIOPEP (“enzyme action” module, http://www.uwm.edu.pl/biochemia/index.php/en/biopep) and ExPASy-PeptideCutter (http://web.expasy.org/peptide_cutter) are popular online tools for predicting amino acids or peptides release from specific protein substrates, based on the knowledge of cleavage specificity of specific enzymes. Furthermore, using virtual digestion in combination with the bioactive peptides database (such as BIOPEP), several novel controlled enzymatic hydrolysis methods have been explored by (a) identifying the number of reported peptides with a specific activity among the released peptides *in silico* [18,19]; (b) calculating the frequency of occurrence of BAPs in a protein chain [20]; and (c) predicting the potential biological activity of the protein [21]. *In silico* analysis can not only simulate the single enzyme hydrolysis, but also predict the hydrolyzates produced by multiple enzymes.

Table 1
In silico methods contains some database, online tools, and software.

Category	Name	Website	Function
Protein database	NCBI Protein database	https://www.ncbi.nlm.nih.gov/	Basic sequence information for proteins
	UniProtKB	http://www.uniprot.org/	Basic sequence and structural information for proteins
	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Protein sequence database
In silico digestion tools	PeptideCutter	http://web.expasy.org/peptide_cutter/	Server for predicting potential cleavage sites cleaved by proteases or chemicals in a given protein sequence
	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Server for predicting potential cleavage sites cleaved by proteases in a given protein sequence
	Enzyme Predictor	http://bioware.ucd.ie/~enzpred/Enzpred.php	Tool to evaluate the evidence for which enzymes are most likely to have cleaved a sample containing peptides from hydrolyzed proteins.
Bioactive peptide database	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Bioactive peptide database
	BitterDB	http://bitterdb.agri.huji.ac.il/bitterdb/	Bitter compounds database
	EROP-Moscow database	http://erop.inbi.ras.ru	Database of biologically active peptides
	APD	http://aps.unmc.edu/AP/main.html	Several kinds of bioactive peptide database mainly focus on antimicrobial peptides
	PeptideDB	http://www.peptides.be/	Biologically active peptide database
	PepBank	http://pepbank.mgh.harvard.edu/	Biologically active peptide database providing searching program for fragments with sequence similar to the peptides in the database
	AHTPDB	http://crdd.osdd.net/raghava/ahtpdb/	Antihypertensive peptide database
Potential bioactivity prediction	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Tool for the evaluation of proteins as the precursors of bioactive peptides
	PeptideRanker	http://bioware.ucd.ie/~compass/biowareweb/Server_pages/peptideranker.php	Server for the prediction of bioactive peptides.
	AntiBP2	http://crdd.osdd.net/raghava//antibp2/	Predicting the antibacterial peptides in a protein sequence
Allergenicity/toxicity prediction/analyzing	AlgPred	http://crdd.osdd.net/raghava//algpred/	Predicting allergenic proteins and peptides
	BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	Allergenic protein database
	ToxinPred	http://crdd.osdd.net/raghava//toxinpred/	Predicting toxicity of peptides
Physicochemical characteristics prediction	Expasy-Compute pI/Mw	http://web.expasy.org/compute_pi/	Tool to compute the theoretical pI (isoelectric point) and Mw (molecular weight)
	ProtParam	http://web.expasy.org/protparam/	Tool to compute grand average of hydropathicity (GRAVY) and Instability index
	PepDraw	http://www.tulane.edu/~biochem/WW/PepDraw/	Tool to compute net charge and hydrophobicity
Peptide Structure Prediction Server	Pep-Fold	http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/	Tool to predict peptide structures from amino acid sequences
	PEPstrMOD	http://osddlinux.osdd.net/raghava/pepstrmod	Server to predict the tertiary structure of small peptides
Protein Structure Prediction Server	I-TASSER	https://zhanglab.ccmb.med.umich.edu/I-TASSER/	Protein structure and function prediction
Mainly Software for the study of protein-ligand interactions	Discovery studio	/	/
	Sybly	/	/
	Autodock vina	/	/
	Schrödinger	/	/
	Dock	/	/
	FlexX	/	/
	ICM-Docking	/	/
	GOLD	/	/

2.2. Single enzymatic hydrolysis

To acquire peptides with high bioactivities, a number of factors must be taken into consideration. Maximum biological activities and limited generation of bitter flavor may be achieved by the appropriate selection of enzymes for proteolysis. Therefore, enzymatic hydrolysis of proteins under controlled conditions, according to the application of bioinformatics prior to the digestion, can greatly improve the efficiency of experiments.

Single enzyme hydrolysis has been used to release peptides with various biological activities, such as dipeptidyl peptidase IV (DPP-IV) inhibitory peptides, angiotensin-converting enzyme inhibitory peptides, antithrombotic peptides, anti-inflammatory peptides, and immunomodulating peptides [22–25]. *In silico* proteolysis also has been adopted to analyze peptides in hydrolyzates digested by single enzymes. For instance, Rani et al. [26] compared the number of ACEI peptides potentially released from goat milk proteins digested with pepsin or chymotrypsin A using BIOPEP. The results showed that pepsin is more suitable for releasing ACEI peptides. Similarly, Udenigwe performed *in silico* proteolysis by using pepsin and thermolysin digested oryzacystatin. The results showed that several BAPs will be released, which will facilitate efficient production of BAPs from rice bran for value-added use of the by-product in functional food formulations. Hsieh et al. [27]

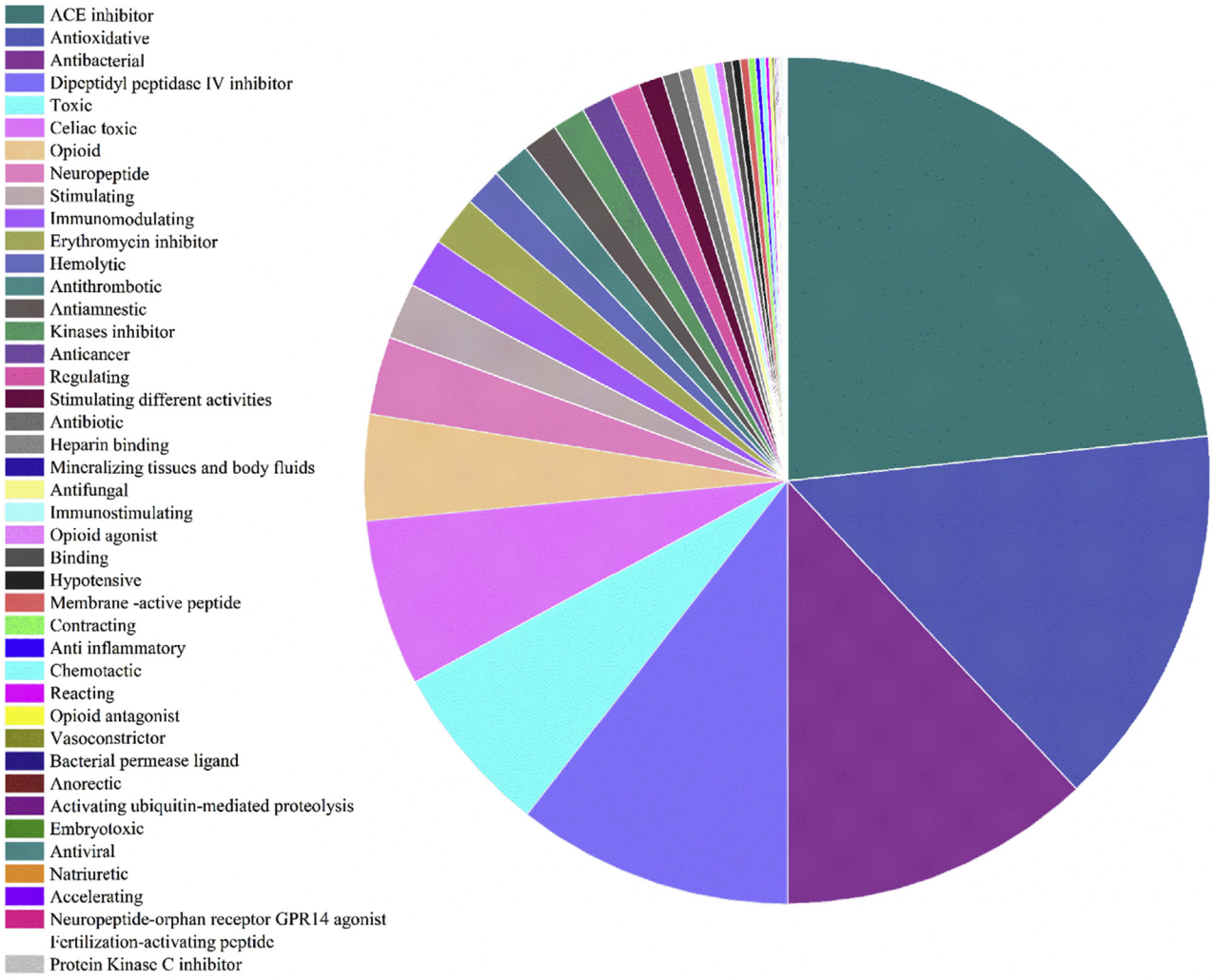

Fig. 1. Number of bioactive peptides relevant to human health promotion and disease prevention. Accessed from the BIOPEP database in December 2017.

combined the virtual hydrolysis developed "frequency (A)", ratio of the number of the peptides with Pro and Ala as the penultimate N-terminal residues to the total number of total peptide fragments released by proteases, and *in vitro* experiments to analyze the dipeptidyl peptidase IV inhibitory activity and antidiabetic effect of a sodium caseinate hydrolyzate; their results indicated that the novel *in silico* method has the potential as a screening tool to predict dietary proteins to produce DPP-IV inhibitory and antidiabetic peptides. Lafarga et al. [28] utilized *in silico* methodologies, peptide databases, and software, including ProtParam (http://web.expasy.org/protparam/), Basic Local Alignment Tool (BLAST), ExPASy-PeptideCutter (https://web.expasy.org/peptide_cutter/), and BIOPEP to assess the release of potentially bioactive DPP-IV, renin, and ACE inhibitory peptides from bovine and porcine meat proteins, and confirmed the bioactivities of the peptides by using chemical synthesis and *in vitro* bioassays. Dziuba and Dziuba [29] searched for new milk protein-derived peptides with potential antimicrobial activity through analyzing the peptides released from major milk proteins by *in silico* hydrolysis with 28 kinds of enzymes. Tulipano et al. [30] applied *in silico* methods to predict the release of DPP-IV inhibitors during the gastrointestinal digestion of β-lactoglobulin and α-lactalbumin. Furthermore, *in vitro* and *in vivo* studies should be carried out to confirm the obtained results.

2.3. Combined enzymatic hydrolysis

Generally speaking, enzymatic digestion by a single enzyme exhibits a low degree of hydrolysis. The degree of hydrolysis is closely related to the characteristics of hydrolyzates as the functionality and bioactivity of peptides rely on the size, type, and amino acid sequence of the hydrolyzates [31]. Protein hydrolyzates with a low hydrolysis degree (DH < 10%) can serve as food texture enhancers, whereas extensive hydrolysis is essential to obtain BAPs for dietary and medical applications [6,32]. However, over-hydrolysis may release peptides with no functional or bioactive properties as reported by Klompong et al. [33], Rodríguez Patino et al. [34] and Wu et al. [35]. *In silico* analysis can also predict the peptides released by multiple enzyme digestion. Experiments conducted by Majumder and Wu [36] showed that compared to ovotransferrin hydrolyzates obtained on digestion with individual enzymes (pepsin or thermolysin), the hydrolyzates obtained on digestion with a combination of enzymes (thermolysin + pepsin) exhibited the lowest ACE inhibitory IC_{50} value (198.0 ± 1.21 μg mL^{-1}). *Salmo salar* collagen alpha-1(VII) chain-like isoform X5 was *in silico* digested by both pepsin and trypsin, using ExPASy-PeptideCutter, with pepsin and trypsin; Finally, 692 fragments were produced, and with further *in silico* an *in vitro* analysis, tetrapeptides PGAR and IGPR were identified as potent ACE inhibitors [37].

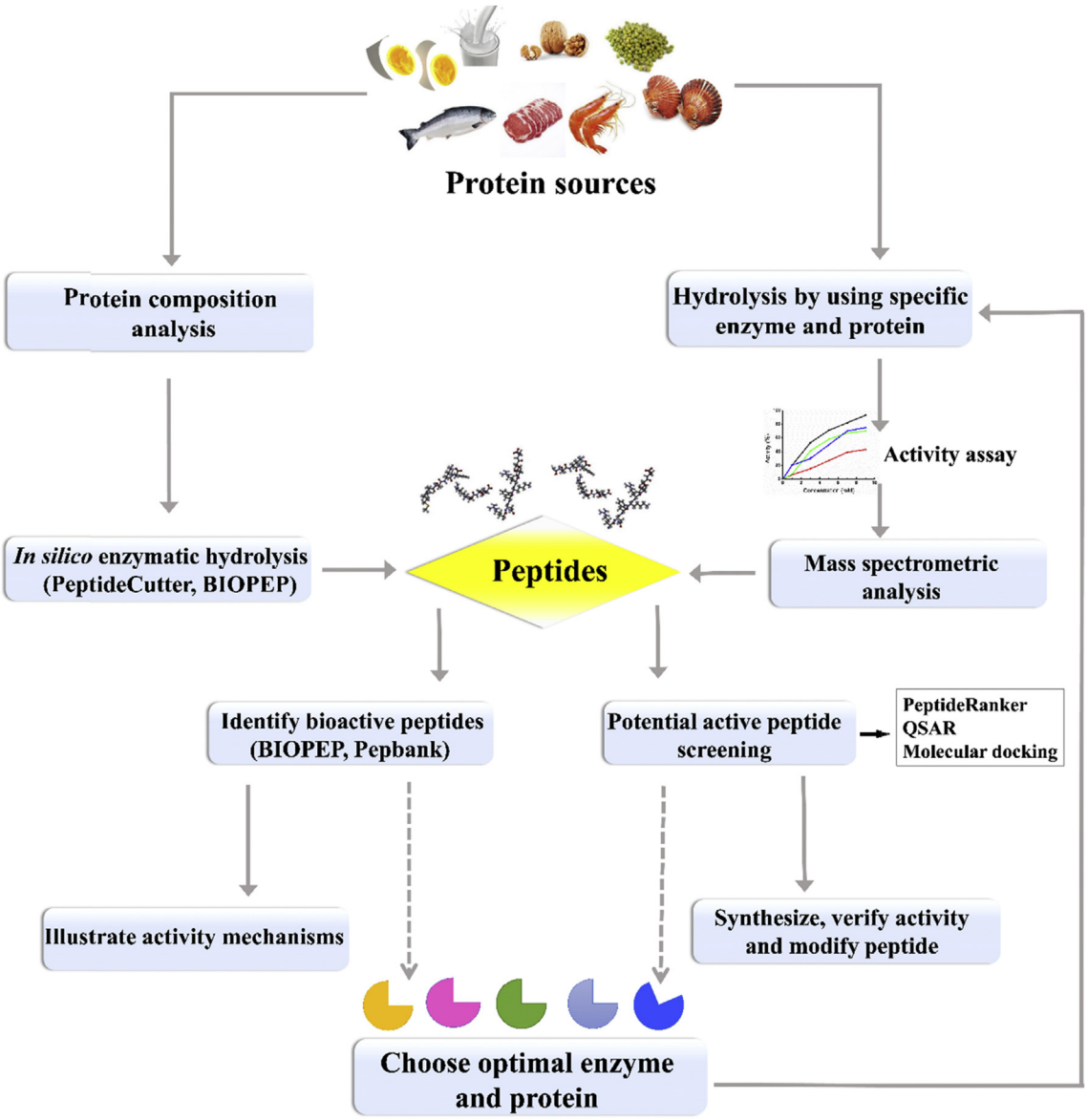

Fig. 2. Novel bioinformatics approaches for identifying and validating food protein-derived bioactive peptides (BAPs).

Similarly, ExPASy-PeptideCutter was used to *in silico* digest fifteen common food proteins by single enzyme (thermolysin) and multiple enzymes (thermolysin + pepsin; thermolysin + pepsin + trypsin); In combination of QSAR model to predict ACE inhibitory activity of *in silico* digested peptides (expressed as IC_{50} values), the results showed that meat proteins from pork, beef and chicken had the highest number of potent peptides, followed by egg, soybean and canola, while fish (with the exception of salmon) and cereals (oat and barley) had the lowest number of potent ACE-inhibiting peptides [38]. One major limitation of BAPs is whether they can retain their activities after undergoing the gastrointestinal (GI) tract digestion process, as the many enzymes in the GI tract may degrade BAPs into several fragments. To answer this question, researchers predicted the stability of BAPs after GI tract digestion using *in silico* tools such as PeptideCutter and BIOPEP. Enzymes used in this process always contain pepsin (pH 1.3 and pH > 2), trypsin and chymotrypsin [10].

Although *in silico* hydrolysis has been widely used for screening and identifying the BAPs, the consistency between the outcomes of *in silico* analysis and *in vitro* and *in vivo* experimental results should be taken into consideration. Several studies of hydrolyzates of pea, egg, and bovine whey proteins confirmed *in silico* digestion by *in vitro* digested hydrolyzates, as reviewed by Nongonierma and Fitzgerald [39]. Meanwhile, for *in vitro* digestion, in a previous study showing that the peptide bonds of β-lactoglobulin are cleaved selectively by B*acillus licheniformis* protease at different pH (7.0–9.0) [40]; Inouye et al. [41] confirmed that thermal treatment of proteins can enhance enzymatic hydrolysis possibly by unfolding the proteins, increasing enzyme and protein interactions. Therefore, the difference may exist in *in vitro* hydrolyzates compared to the *in silico* simulation. Briefly, the difference in the number and types of peptides generated from *in vitro* and *in silico* hydrolysis may contribute to the following factors: (i) the purity of enzyme activity, which may affect hydrolysis *in vitro*; (ii) *in silico* methods limitly consider hydrolysis conditions (such as pH, temperature, hydrolysis time, and enzyme-substrate ratios), (iii) *in silico* hydrolysis software assume that the enzyme will access and hydrolyze all cleavable peptide bonds; (iv) post-translational modifications may

occur during processing and storage modifications, as this was not taken into account during *in silico* digestion. (v) pretreatment of the protein also affects the hydrolyzates produced. (vi) *in silico* digestion tools cannot be used for the samples whose protein sequences have not been reported and for enzymes with no knowledge of their cleavage specificity. Taken together, *in silico* hydrolysis is an effective method for providing theoretical hydrolyzates in an efficient and low-cost manner; however, the results must be confirmed by experiments.

3. Bioactivity prediction of peptides

As illustrated by Ruiz Ruiz et al. [42], structural properties, such as chain length and physicochemical characteristics including hydrophobicity, molecular charge, and side-chain bulkiness of the amino acid residues, have large effects on the specific bioactivities of food-derived peptides against various molecular disease targets. Based on those theories, researchers have developed a series of methods for predicting the activities of peptides, such as the online software PeptideRanker, QSAR modeling, and molecular docking, in which the structures of peptides are essentially significant.

3.1. Sequence comparison

The function of peptides is closely related to the amino acid sequence [43], and peptides with similar amino acid sequences may exhibit similar bioactivity. If different peptides have the same key amino acid residues in the active site, they likely have the same functions. Based on this theory, sequence comparison of the targeted peptides manually or automatically using special tools for the BAPs is the general experimental design.

Sequence comparison also can be used to predict the specific biological activities of peptides. For example, Jollès et al. [44] isolated an undecapeptide that inhibit platelet aggregation through the detecting of analogous features between fibrinogen and κ-casein. A novel peptide derived from β-casein showed remarkable sequence similarity with fragment 54–65 of hirudin, and thus has been predicted as a potent thrombin inhibitor [9]. It has been reported that in many cases, the biological activities of peptides are attributed to the presence of certain key amino acids with specific properties. For instance, the amino acids valine (V) and proline (P) are very important in most antihypertensive peptides [45]. Furthermore, most short, hydrophobic, and cationic peptides exhibit antimicrobial properties [46]. Moreover, histidine (H), cysteine (C), proline (P), methionine (M), and aromatic amino acids have been reported to contribute to the antioxidant activity of food peptides. In addition, known DPP-IV inhibitory peptides were reported to consist of hydrophobic amino acid residues (Trp, Leu, Ile or Phe) at the N-terminus and/or a Pro/Ala at position 2, and/or Pro at the C-terminus [47].

3.2. Prediction of peptide structures

The biological activity of any peptide depends on the amino acid position in the sequence. Typically, physico-chemical methods can be used to elucidate peptide structures including circular dichroism (CD), fourier transform infrared spectroscopy (FITR), electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), and X-ray crystallography [48]. Among them, X-ray crystallography and nuclear magnetic resonance spectroscopy are the most powerful tools for determining the three-dimensional (3D) structure of peptides [49]. However, these two techniques have some limitations because they are time-consuming and/or typically costly [49]. At the moment, computational methods have been developed to evaluate 3D structures based on sequences, which have been classified into three main categories, including homology (comparative modeling), threading, and *ab initio* [50].

Recently, several online servers have been developed to predict peptide structures, such as Pep-Fold [51], PEPstrMOD [52], Protinfo [53], Hmmstr/Rosetta [49,54], and I-Tasser [55]. For example, 3D structures of antiviral peptides (AVPs) were modeled using the PEP-FOLD program and the 3D model for each AVP was chosen according to the PEP-FOLD server, considering the lowest energy model indicating peptide stability [56]. Different software programs, such as Discovery studio, Sybyl, and ArgusLab can predict the poses of peptides and further refine them by energy minimization and/or molecular dynamics simulations. For instances a defensin B peptide 3D structure was predicted using Discovery studio software, which may be useful for structure-based drug design studies in the future [57]. After the peptide structure is obtained, further analysis required. Moreover, Discovery Studio 2017 software can be used to predict the structure of molecules based on the Force Field theory, which is a concept of a set of parameter and equations for use in molecular mechanics simulations [58].

3.3. Molecular docking approaches

Molecular docking methodologies play an important role in the planning and design of new drugs; these approaches aim to predict and estimate the binding modes and affinities of a small molecule within the binding sites of target receptors [59]. Presently, molecular docking is widely used to screen for food-derived BAPs and illustrate their biological mechanisms. Particularly, the ACEI peptides were derived from milk [22], Silkworm pupa [60], and rice bran [24]; dipeptidyl peptidase IV (DPP-IV) inhibitory peptides were from amaranth seed proteins [61]; and antithrombotic peptides were screened from milk [9]. Molecular docking normally consists of four main procedures, namely protein structure selection and preparation, ligand preparation, docking, and analysis of the results, as shown in Fig. 3. Choosing a suitable receptor molecule is the first and important step in this process; therefore, here we listed the PDB codes of the molecules reported in the literature for molecular docking, as shown in Table 2. However, notably, although molecular docking has been widely employed in bioactive substance design, discovery, and analysis studies as a standard computational tool, some theoretical and computational challenges must be overcome to increase the accuracy of prediction. Furthermore, the results of molecular docking required to be validated by experiments.

4. Relationship of structure and bioactivity

4.1. QSAR and peptides

QSAR refers to the association of the structural characteristics of molecules to their biological or chemical properties [12], which has been extensively applied in food chemistry, including the aspects of BAPs, sensory peptides and so on, but can easily be expanded to other areas of food research. Generally, the structure and activity of BAPs mainly focus on antimicrobial, ACE-inhibitory, antioxidant, and renin and dipeptidyl peptidase IV (DPP-IV) inhibitory peptides. QSAR modeling mainly consists of four steps: (i) building a BAPs library, to collating the sequences of target peptides to be used for building the QSAR model(s); (ii) describing peptides using scalar descriptors of constituent amino acids; (iii) building QSAR model(s); and (iv) confirmatory studies with synthetic peptides [12].

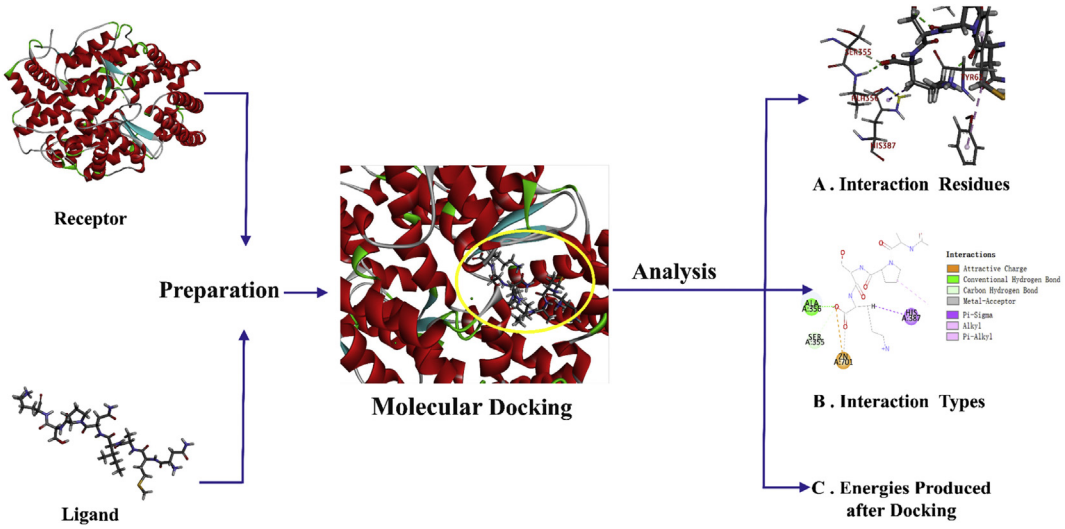

Fig. 3. General procedures for molecular docking.

Table 2
Classical PDB codes of molecules used in the research of various activity mechanisms by molecular docking.

Activity	Receptors	PDB code of receptors	Software	Reference
ACE inhibitory	ACE	1O8A	Auto Dock; Discovery Studio; Molegro Virtual Docker	[24,85,86]
		1O86	Discovery Studio; AutoDock; SYBYL	[13,37,87]
		1UZF	Molegro Virtual Docker	[86]
Renin inhibitory	Renin	2V0Z	Discovery Studio	[68]
Dipeptidyl peptidase IV (DPP-IV) inhibitory	DPP-IV	1R9M	ClusPro 2.0	[61]
		1ORW	AutoDock	[88]
		1WCY	AutoDock	[88]
Xanthine oxidase (XO) inhibitory	Xanthine oxidase (XO)	3BDJ	AutoDock	[88]
Antithrombotic	Thrombin	2BVR	Discovery Studio	[9]
		2ZC9	AutoDock	[89]
		1KTS	SYBYL	[90]
Factor Xa inhibitory	Factor Xa	1NFY	Molecular Operating Environment (MOE)	[91]
		2W26	Discovery Studio	[92]
Acetylcholinesterase inhibitory	Acetylcholinesterase (AChE)	1B41	Discovery Studio	[93]
		1EVE	Discovery Studio	[94]
Sweetness	Sweet taste receptor, T1R2/T1R3	Constructing homology models	Schrödinger	[95]
Antimicrobial	ecKAS III	1HNJ	AutoDock	[96]
COX-2 inhibitory	COX-2	1PXX	Schrodinger	[97]
		6COX	AutoDock	[98]
		1CX2	GOLD	[99]
Human secretory phospholipase A2 (PLA2) inhibitory	PLA2	1KQU	FlexPepDock server	[100]

In recent years, a large of studies have been conducted to estimate the activities of related compounds and predict structures of high-activity peptides using QSAR methodology. Food-derived ACEI peptides are with the function of inhibiting the angiotensin-I converting enzyme (ACE), which have been intensively studied and recognized as blood pressure lowering ingredient. Using ACEI peptides as examples, a QSAR model with good predictive capacity was constructed by Jing et al. for ACEI tripeptides derived from milk; four tripeptides were selected based on the constructed model and validated *in vitro* [62]. Similarly, Qi et al. selected four potent tripeptides, GEF, VEF, VRF, and VKF, based on the established QSAR models, and the results of *in vitro* evaluation showed good agreement with the predicted values [63]. Peptide bitterness is a major challenge in industrial application as bitter peptides are frequently produced during the enzymatic process to produce functional BAP hydrolyzates or during the aging process in fermented food products [64]. Moreover, most mammals including humans instinctively reject bitter substances to avoid ingesting bitter-tasting toxic substances [65].

QSAR approaches have been employed to explore the properties of peptides that contribute to bitterness. Bulky hydrophobic amino acids at the C-terminus and basic amino acids at the N-terminus are highly correlated to the bitterness of peptides [64]. Interestingly, a QSAR approach was used to analyze ACEI activity associated the bitterness of peptides, and significant correlations between increased ACE inhibition and bitter-taste were found in dipeptides [66]. The association was mainly attributed to the importance of hydrophobicity for both properties. Moreover, Wang et al. reported that medium-sized peptides are generally more bitter than larger/smaller peptides [67]. Limited structural variations in dipeptides

may make it difficult to have features that limit the effect of C-terminal hydrophobicity, which is necessary for ACE inhibition, on bitter taste.

Although QSAR modeling plays an important role in the development of functional protein foods by predicting active peptides by elucidating structure-activity relationships, QSAR methodology is limited in predicting rare types of BAPs, as it is difficult to establish the model and the related physical properties. Many studies have focused on peptides with ACEI and antibacterial activity, as well as some bitter peptides, which may be attributed to the fact that the amino acids of these peptides show some regularity.

4.2. Bioactivity mechanism clarity

Bioactivity mechanisms typically refer to the pathways and methods by which active substances exert their biological functions. Activity mechanisms of newly identified or previously reported peptides are remarkably important for further understanding the functional activities of BAPs. From the perspective of pharmaceutical molecules as well as BAPs, elaborating their interaction mechanisms appears crucially important for further understanding their functional activities. In previous decades, studies of the active mechanisms of functional substances mainly relied on various spectroscopic technologies, such as fluorescence spectra and CD spectra, isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and bio-layer interferometry (BLI).

Currently, newly-developed computational approaches can aid in elaborating the interaction mechanisms of BAPs with receptors from the binding sites and binding types between the receptor and ligands. Therefore, combined *in silico* and traditional methods have been applied in active mechanism studies to study the bioactive mechanisms of BAPs deeply. For instance, He et al. evaluated the potential molecular mechanisms responsible for the ACE and renin-inhibitory activities of three rapeseed protein-derived peptides (TF, LY, and RALP) by fluorescence spectroscopy, CD spectroscopy, and molecular docking techniques in combination with enzyme inhibition kinetics; their enzyme inhibition kinetics showed competitive, non-competitive and mixed-type peptide-dependent inhibition of peptides with renin and ACE inhibitory activities [68]. Intrinsic fluorescence intensity data revealed the binding effects of peptides against ACE and renin molecules. CD data showed that the inhibitory mechanism involved extensive peptide-dependent reductions in the α-helix and β-sheet fractions of ACE and renin protein conformations. Molecular docking studies confirmed potential ACE or renin inhibitory activity mechanisms at the molecular level. Similarly, the inhibitory effects of potato patatin-derived peptides WG and PRY on ACE and renin activities were investigated trough kinetics, intrinsic fluorescence, and molecular docking analysis by Fu et al. [69]. The results indicated that PRY is a more potent ACE and renin-inhibitory peptide than WG. The corresponding enzyme inhibition kinetics results showed that WG and PRY inhibited ACE activity through mixed-type and competitive modes, respectively, while renin showed mixed-type. PRY exhibited stronger affinity towards ACE and renin molecules, compared to WG, as determined by analysis of intrinsic fluorescence intensity. Molecular docking data confirmed that the higher inhibitory potency of PRY may be attributed to the formation of more hydrogen bonds with the active site or non-active sites of enzyme that distorted the configuration necessary for catalysis. Ni et al. combined enzyme kinetics experiments, isothermal titration calorimetry, and molecular docking simulation to investigate the active mechanisms of ACEI hexapeptide TPTQQS [70]. The results showed that the hexapeptide inhibits ACE in a non-competitive manner and bound to ACE via interactions of the N-terminal Thr1, Thr3, and Gln4 residues with the residues on the lid structure of ACE, and the C-terminal Ser 6 attracted the zinc ion, which is vital for ACE catalysis. Displacement of the zinc ion from the active site resulted in inhibition of ACE activity. The structural model based on docking simulation was supported by experiments in which the peptide was modified. Thus, *in silico* methods overcome the drawbacks of spectroscopic and thermodynamic methods and illustrate the key amino acids corresponding to the interactions.

4.3. Simulation mutation of bioactive peptides

Considerable studies involving peptide mutation were carried out to either construct peptides with pH-dependent activities [71] or enhance the activities of peptides, such as antibacterial and ACE inhibition activities. Till date, integrated computational methods, involving QSAR, molecular docking, and molecular dynamics simulations, have been used to modify natural bioactive peptides to improve their activities. For instance, considering that the ACE inhibitory peptides with Trp at the C-terminus show high ACE inhibitory activities, four novel tripeptides VKW, YAW, KYW, and TAW, were designed by modification with Trp at the C-terminus based on the original peptides VK, YA, KY, and TAY; consequently, their activities were increased by 27−1450-folds compared to those of their corresponding original peptides. Moreover, molecular docking and molecular dynamics simulation showed that modification with Trp can enhance the stability of ACE/derived peptide complexes by increasing binding affinity and the number of interaction sites with important amino acid residues, indicating that modification with Trp at the C-terminus is an effective method for designing novel ACE inhibitory peptides [72].

Molecular dynamics simulation, binding free energy analysis, kinetic and inhibition studies, and systematic mutation energy map were successfully combined to design novel human secretory PLA2 inhibitory mutants based on a known inhibitor. Eight peptides were successfully identified to show potent inhibition potency. Further structure examination revealed that the designed peptides can form intensive nonpolar networks of van der Waals contacts and hydrophobic interactions at their complex interfaces with PLA2, conferring considerable stability and affinity for the formed complex systems [73]. Eight antibacterial peptides with unnatural amino acids (uABPs) were successfully designed and explored by integrated *in silico-in vitro* methods. The results of *in vitro* antibacterial activity tests showed that four of the eight uABPs were potent with a minimum inhibitory concentration of $<50\ \mu g\ mL^{-1}$, and F[Nle]W[Hag]RWWV[Orn]L exhibited the highest activity in all tested candidates. Molecular dynamics simulations revealed that the designed uABPs are amphipathic helix in solution, but they unfold when they become spontaneously embedded in an artificial lipid bilayer that mimics the microbial membrane [74].

5. Prediction of physicochemical properties of bioactive peptides

5.1. Prediction of accumulation

Aggregation is a universal and widely examined topic for proteins and peptides and limit their production and biotechnological and pharmaceutical applications [75,76]. Moreover, more than 20 human disorders have been reported to be associated with the aggregation of proteins and peptides *in vivo*, including Alzheimer's, Parkinson's disease, and type II diabetes (see review article [77]). Taking aggregation into consideration, many drug-aimed active polypeptides are frequently abandoned at an early stage of development. Therefore, measuring the aggregation is of great importance for functional proteins and peptides. The biophysical and biochemical methods used to investigate aggregation are

summarized by Dobson [77]. Additionally, online software has been developed to predict protein and/or peptide aggregation, such as AGGRESCAN (available at http://bioinf.uab.es/aggrescan/), and PASTA 2.0 server (available at http://protein.bio.unipd.it/pasta2/). Human calcitonin is a 32-residue polypeptide hormone synthesized and secreted by C cells of the thyroid, which can be used to treat osteoporosis, Paget's disease, hypercalcemia, and musculoskeletal pain [78]. However, its aggregation is a serious problem during production, storage, and administration. To overcome the aggregation problem, Fowler et al. rationally designed of aggregation-resistant bioactive peptides, human calcitonin-like variants, which significantly reduced aggregation propensity without a loss of physiological activity, by using a semiempirical approach and a more quantitative approach involving an *in silico* selection procedure [75]. Therefore, an ideal method for altering aggregation properties of BAPs is to predict small mutations, preferably single-point, that result in a large change in aggregation rate with the aid of bioinformatics; this method can also be used for high-throughput screening.

5.2. Prediction of solubility

Solubility, which mainly refers to the aqueous solubility for peptides, is also an important factor and must be considered when evaluating the peptides; this property influences the absorption, distribution and elimination of peptides in the body [79]. Moreover, it is reported that the poor solubility of compounds may mask toxicity and other adverse effects [79]. Therefore, solubility issues are fundamental for discovering functional substance as well as BAPs. Traditional methods for measuring for solubility are not compatible with the high-throughput analysis, making it difficult to screen potential drugs among numerous potential bioactive compounds during the process of drug discovery. An increasing number of *in silico* methods have been developed to predict the solubility of substances using 1D, 2D, and 3D parameters [79–81]. To overcome low solubility of peptides, several methods have been adopted, such as the fusion of target polypeptides to a solubilizing protein fusion partner, glycosylation with hydrophilic carbohydrates, addition of short solubility enhancement peptide tags, and site-specific modification [82]. Betaine has been used to increase the solubility of proteins and peptides by site-specific modification [82].

It is noteworthy that the solubility of peptides is nearly related to physico-chemical properties, particularly amino acid composition. Some hydrophilic residues (aspartic acid, glutamic acid, and serine) contribute significantly more favorably to peptide solubility than other hydrophilic residues (asparagine, glutamine, threonine, lysine, and arginine) [83,84].

6. Conclusions and future outlooks

Bioinformatics technologies have widely applied to study BAPs derived from food proteins by providing conformation information, predicting potential activities, illustrating molecular interaction mechanisms, and improving peptide properties. *In silico* integrated BAPs studies methodologies have partially broken the limitations of traditional research methods. However, some limitations remain, such as the lack of knowledge of proteins and protein sequences in the virtual enzymatic hydrolysis, biomarkers (key proteins) of specific bioactivities, detailed structure information and 3D structure of the receptor in molecular docking; additionally, as many as possible of the known active compounds with IC_{50} in QSAR, and the BAP bioavailability in the body are very essential. Moreover, the predictive power of bioinformatics, accuracy of proteomics, and gap between the simulations and metabolism *in vivo* should been taken into consideration for further development of bioinformatics.

Bioinformatics is also a promising technique in the fields of not only peptides but also other chemicals, especially for bioactive chemicals related to fundamental and practical studies. Therefore, the development of bioinformatics in these fields depends on several aspects as follows: (i) purification and characterization of key proteins as a donor or receptor in a biological course; (ii) future development of the proteomics, particularly foodomics; (iii) molecule screening, amino acid sequence identification and structure characterization of biomarkers for a specific purpose; (iv) bioactivity mechanism clarification of bioactive peptides or proteins as a functional component; (v) intensive development of bioinformatics tools to precisely evaluate or predict the interactions of donors and receptors, especially on the molecular level, *i.e.*, active sites, interactive amino acids, interactive bonds; (vi) other new findings in biochemistry, chemistry, physical-chemistry, computational chemistry, and X-ray crystallography.

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