Anti-diabetic Bioactive Peptides

Subjects: Food Science & Technology

Contributor: Fernando Rivero-Pino, Francisco Javier Espejo Carpio, E. M. Guadix

Bioactive peptides released from the enzymatic hydrolysis of food proteins are currently a trending topic in the scientific community. Their potential as antidiabetic agents, by regulating the glycemic index, and thus to be employed in food formulation, is one of the most important functions of these peptides. The future applicability that these molecules have due to their biological potential as functional ingredients makes them an important field of research, which could help the world population avoid suffering from several diseases, such as diabetes.

Keywords: bioactive peptide ; diabetes ; dipeptidyl peptidase IV ; glucosidase ; protein hydrolysate ; functional food

1. Introduction

Antidiabetic food-derived peptides obtained by enzymatic hydrolysis of food proteins are an important topic in the research, due to their health benefits in human. These peptides are obtained by the action of enzymes called proteases. Different food-grade proteases might have different specificities [1], and from the same substrate, the pool of peptides produced would be different, and would show different properties. A protein hydrolysate is the mixture of peptides that originally formed the protein, after its hydrolysis. The complex structure of proteins in the native state hides the functionality of the peptides, preventing them from exerting their bioactivity by association with some other molecules. This research topic is important in the scientific community and it has also industrial relevance due to the economical impact it has concerning the pre-treatment of diseases.

2. Bioactive Peptides

The similarity of food-derived peptides to the structure of human regulatory peptides makes them suitable for interacting with some enzymes and receptors involved in human metabolism. In this way, the most important improvement of proteins after hydrolysis, concerning functional food, is the bioactivity development. Lately, the proportion of the world population suffering an illness has increased, and prevention and pre-treatment are considered good options for most of them. At the economical level, the cost savings, compared to those associated with the treatment of the disease, are high [2].

Bioactive peptides are considered to be regulator molecules operating at different levels in the organism. As was previously mentioned, protein hydrolysis during digestion releases peptides that exert bioactivity in humans, but the intake of peptides with improved bioactivity, compared to those obtained naturally, is seen as a good option for humans[3]. This is due to the specificity of food-grade proteases employed in the industry, which are able to release peptides that digestive proteases cannot. Bioactive peptides can exert physiological effects at a cardiovascular, digestive, endocrine, immune and/or nervous level [4].

Obtaining bioactive peptides from food proteins is preferably carried out by enzymatic hydrolysis rather than chemically, or via microbial fermentation. Enzymatic hydrolysis requires mild reaction conditions, and is specific and controllable. The reaction itself is simple, needing the substrate (protein) and the enzyme(s) (protease(s)). The reaction conditions (pH and temperature) are determined by the protease, and many factors, such as enzyme/substrate ratio or substrate concentration, must be taken into consideration too.

3. Identification of Bioactive Peptides

3.1. Fractionation

Having obtained a bioactive protein hydrolysate, different technologies allow the separation of peptides based on different physicochemical properties (molecular weight, polarity or charge). The main technologies employed for fractionation are chromatography or membranes. The concentration and purification of bioactive fractions from hydrolysates is an important step in industrial implementation. Moreover, identification of the actual bioactive sequences in these fractions would

enable us to verify their actual bioactivity, and their bioavailability, stability and functionality in the context of nutrition. These protein hydrolysates contain a pool of peptides obtained by the cleavage of different enzymes, and their respective abilities to inhibit DPP-IV or digestive enzymes will determine how bioactive they are. There are numerous studies reporting the production of DPP-IV inhibitory peptides [5][6]. Numerous authors have also identified α-glucosidase inhibitory peptides [7][8] and α-amylase inhibitory peptides [9].

Chromatography is a laboratory technique for separating compounds. There are numerous types of chromatography, distinguished by their characteristics. In terms of peptide purification, size exclusion chromatography (SEC) and reverse-phase chromatography (RPC) are the most widely used. These two separate peptides depending on their size and their hydrophobic characteristics, respectively. Usually, the combination of both techniques is adequate to obtain fractions that can be injected into a mass spectrometer (MS) so as to identify the peptides contained therein.

Membrane technology allows the separation of a sample into retentate and permeate. In this case, the pore size of the membrane would make the peptides separate into different fractions, depending on their molecular weight. Different molecular weight cut-off (MWCO) membranes would separate the peptides depending on their size, enabling one to identify the most bioactive fractions[10], which are usually the smallest ones, and to discard larger peptides, which are generally non-bioactive. Lacroix and Li-Chan [11] ultrafiltered dairy protein hydrolysates using an Ultracel Amicon ultrafiltration unit model 8400, with membrane MWCOs of 10 kDa, 3 kDa and 1 kDa, and reported a higher DPP-IV inhibitory activity for < 3 kDa fractions. Considering a large-scale production of hydrolysates, purification by membranes would be an adequate means of obtaining different-sized fractions.

3.2. Peptide Sequence Identification

The identification of peptides is generally carried out by mass spectrometry (MS) analysis of the most bioactive fractions after chromatographic purification or membrane separation. MS is an analytical technique that measures masses of atoms and molecules after their conversion to charged ions, with or without fragmentation, by an ionization process. This process allows one to identify unknown compounds, and to elucidate their structure and chemical properties. Characterization is done by their mass to charge ratios (m/z) and relative abundances. In this case, controlled fragmentation allows the determination of amino acid sequences in order to identify peptides. A protein hydrolysate is a mixture of peptides, some of them bioactive and some others not. The importance of the identification resides in the fact that the full characterization of the peptides involved in regulating the disease enables the manufacturer to claim the health-promoting property of the fortified product.

Food-derived peptides from food proteins play a crucial role in the regulation of glucose homeostasis, due to their implication at different levels (e.g., glucagon-like peptide 1 regulation) and due to their capacity to inhibit digestion-related enzymes.

α-amylase inhibitory peptides are not as broadly studied as the α-glucosidase and DPP-IV inhibitors described. Some authors have suggested that peptides with branched chains (such as Lys, Phe, Tyr and Trp) and cationic residues are preferably bound to α-amylase [12]. Ngoh and Gan[13] reported the importance of Gly or Phe at the N-terminal and Phe or Leu at the C-terminal. However, the α-amylase inhibitory peptides' features should be further researched, in order to establish similar statements as those concerning the DPP-IV inhibitory or α-glucosidase inhibitory peptides.

Concerning the α-glucosidase inhibitory peptides, Ibrahim et al.[14] summarized the structural properties of α-glucosidase inhibitory peptides. What is remarkable is the importance of amino acids containing a hydroxyl or basic side chain at the N-terminal (which could be expected from trypsin hydrolysis), and of proline within the chain and alanine or methionine at the C-terminal. Nonetheless, factors such as the length of the peptide, its hydrophobicity and its isoelectric point are not extremely important. Ser-Thr-Tyr-Val (STYV) has been reported as the most potent glucosidase inhibitory peptide.

Diverse features have been described for DPP-IV inhibitory bioactive peptides, such as the hydrophobic N-terminal[15] ideally tryptophan [16], and proline or alanine as the penultimate N-terminal residue, or a low molecular mass [17]. Among the 222 peptides analyzed by Liu et al. [14], over 88.4% had a molecular weight lower than 1000 Da, and more than half had one lower than 500 Da. Ile-Pro-Ile (IPI) has been reported as the most potent DPP-IV inhibitory peptide (IC_{50} = 5 μM)[5] .

The identification of bioactive peptides is a key point in this field of research. However, there are still limitations to this procedure due to the high number of molecules (free amino acids, small-/medium-size peptides, polypeptides, oligomers, undigested proteins, etc.) contained in a protein hydrolysate. Considering the presence of high molecular weight

molecules, it is sometimes hard to identify low molecular weight peptides (<4 amino acids length), which are usually those responsible for the bioactivity [18]. In this regard, bioinformatics analyses play an important role in the identification of bioactive molecules.

3.3. Bioinformatics Analysis

Bioinformatics analyses should be taken into consideration given their potential use in identifying, characterizing and producing bioactive peptides [19]. The most remarkable analyses described below are in silico analysis, molecular docking and the Quantitative Structure–Activity Relationship.

The first approach to identifying bioactive peptides is the employing of informatics tools that use knowledge about proteins and proteases. Thus, having the sequences of the protein and knowing the selectivity of the enzyme, one can expect to obtain the resulting peptides after the cleavage. This method has advantages concerning its feasibility, but it also has disadvantages regarding the numerous protein structures that a substrate can have, and the fact that, depending on the reaction conditions, the proteases can act one way or another. One application for this analysis would be in identifying in which protein we could expect to obtain a peptide that it is known to have antidiabetic properties. Databases largely cited in the literature are BIOPEP, ExPASy-PeptideCutter or Enzyme Predictor, that are capable of performing virtual hydrolysis, that is, in silico digestion.

The molecular docking technique predicts the preferred conformation of a molecule, when bound to another in order to form a stable complex. It is usually employed to see how an identified peptide can bind with the enzyme. Different crystal structures of DPP-IV, α-amylase and α-glucosidase can be found in the RCSB Protein Data Bank. It is a good approach to execute a screening of the different compounds, so as to choose the best candidates [14] and to discover where the peptide would interact with the enzyme. Software widely employed for molecular docking and virtual screening includes AutoDock Vina and pepATTRACT.

Quantitative Structure Activity Relationship (QSAR) is an informatics tool that tries to predict the activity of a molecule based on its molecular features. This is based on the idea that structure and activity are related, and consequently, similar structures may well have similar activities. The combination of different bioinformatics techniques is a good initial approach to confirming the bioactivity of identified peptides.

Lacroix and Li-Chan [20] carried out an evaluation of the potential role of dietary proteins as precursors of DPP-IV inhibitors, via an in silico approach. Further, a structure–activity relationship was developed so as to theoretically predict the potential bioactivity of DPP-IV inhibitory peptides[21]. Ibrahim et al. [22] constructed a library of possible α-glucosidase inhibitory peptides based on the structural requirements of these kinds of biopeptides, which were subjected to in silico simulated gastrointestinal digestion and to molecular docking with glucosidase and amylase, in order to choose which peptides would be highly bioactive.

4. Stability and Functionality in Food Matrices

The food processing operations currently employed in the industry include thermal treatments (sterilization, pasteurization), non-thermal treatments (high-pressure homogenization or processing, ultrasound), storage (freezing and frozen), drying (dehydration, spray drying, freeze-drying) and separation (membrane processes). Some of these processes may well affect food protein functionality, due to physical and chemical changes. Proteins and peptides are prone to interact between one another, and with other molecules. The processing of food products containing proteins and peptides could, in consequence, reduce, maintain or enhance their bioactivity [23] . The amino acid residues would interact with molecules in different ways, also depending on the location of the peptides in the food matrix, ultimately affecting their native and denatured polymeric state. There are not too many studies on how food processing and/or storage modify peptide structure, and consequently their functionality and bioactive properties.

Recently, Harnedy-Rothwell et al.[24] fortified different food products (tomato-based soup and juice products) that were subjected to thermal treatments (sterilization and pasteurization) and stored at refrigerated temperature for 30 days. No modification of bioactivity was reported, indicating this treatment’s potential use on foods that could contain the bioactive protein hydrolysates.

5. In Vivo Evidences

Nowadays, considering the novelty of the research subject, literature concerning in vivo analysis with animals and humans is extremely highly needed, but unfortunately, also scarce. Evidently, this research point is the most important, and is the one that offers authentic evidence concerning the implementation of these bioactive peptides as nutraceutical

ingredients. The formulation of foods with legal claims to being a glycemic index-regulator due to the presence of these bioactive peptides would be the final step. For this purpose, plenty of evidence and verification in humans is required. The literature currently available on protein hydrolysates and bioactive peptides focusses mainly on in vitro analysis. In this regard, for the antidiabetic analysis, different analyses can be carried out, concerning the different metabolic routes involved in the disease.

The authentic evidence that bioactive peptides are adequate for employment in the food industry as nutraceuticals must overcome the clinical analysis carried out in humans. There are numerous studies reporting the efficacy of casein protein hydrolysates in humans, as a pretreatment for diabetes, which involve the observing of different parameters related to an adequate regulation of glucose blood level in type 2 diabetes patients [25][26][27].

In this review, we aimed to summarize the whole process that must be considered when talking about including these molecules as a bioactive ingredient. In this regard, at first, the production, purification and identification of bioactive peptides is summed up. The detailed metabolic pathways described included carbohydrate hydrolases (glucosidase and amylase) and dipeptidyl-peptidase IV inhibition, due to their importance in the food-derived peptides research field. Then, their characterization, concerning bioavailability in vitro and in situ, stability and functionality in food matrices, and ultimately, the in vivo evidence (from invertebrate animals to humans), was described.

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