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Review

Vegetable foods: A cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities

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ABSTRACT

Despite less explored than foods from animal origin, plant derived foods also contain biologically active proteins and peptides. Bioactive peptides can be present as an independent entity in the food or, more frequently, can be in a latent state as part of the sequence of a protein. Release from that protein requires protein hydrolysis by enzymatic digestion, fermentation or autolysis. Different methodologies have been used to test proteins and peptides bioactivities. Fractionation, separation, and identification techniques have also been employed for the isolation and identification of bioactive proteins or peptides. In this work, proteins and peptides from plant derived foods exerting antihypertensive, antioxidant, hypocholesterolemic, antithrombotic, and immunostimulating capacities or ability to reduce food intake have been reviewed.

Contents

1. Introduction

Many plant foods possess biological properties that make them to be considered as potential functional or health-promoting foods. Some of these properties are attributed to biologically active peptides and proteins. A protein or peptide is a functional ingredient if it has been successfully demonstrated its beneficial effect on one or more functions of the body beyond its nutritional effects, so that their effect is significant for health, in general, or enables to reduce the risk to suffer a disease [1]. Bioactive peptides and proteins can present diverse activities (antioxidant, antihypertensive, hypocholesterolemic, immunostimulating, etc.). Among peptides, it is possible to differentiate between bioactive peptides that are found naturally in foods as independent entities and those peptides obtained by the hydrolysis of a precursor protein, being in a latent state as part of the sequence of that protein. As a result of proteolytic processes occurring during *in vivo* digestion of proteins or as a result of its

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processing (fermentation, autolysis, *in vitro* digestion, etc.), these peptides can be released showing certain activities. Obviously, direct ingestion of functional peptides requires they were not susceptible to digestive proteases and peptidases, thus, retaining their biological function.

As a consequence of the interest for plant foods containing bioactive proteins and peptides is the development of transgenic foods expressing gens that accumulate high levels of them. This is the case of the hypocholesterolemic peptide lactostatin, derived from bovine milk β-lactoglobulin, which has been expressed in the glutelin fraction of transgenic rice [2,3]. Another examples are the modification of the α′-subunit of soybean β-conglycinin with three peptides (novokinin, Leu–Pro–Tyr–Pro–Arg, and rubiscolin) with hypotensive, hypocholesterol, and memory-enhancing activities, respectively, [4] and the introduction of a peptide from enterostatin, peptide produced in the intestine and having hypocholesterolemic activity, in soybean β-conglycinin α′-subunit [5]. On the other hand, chemical modification of peptides by amino acid substitution or by the introduction of bioactive fragments in an amino acid sequence has also been a common practice to increase food bioactivity [6].

In the search for new proteins and peptides with biological activities, bioinformatics constitutes an important tool. Indeed, bioinformatics enables the prediction of protein structure–function relationships, the identification of protein domains, and the computer simulation of proteolytic processes. All this information can be extracted from the vast number of biologically active peptides that have already been isolated. Structural motifs in active peptides serve as a source of information to be used in the search for new bioactive molecules. All this information is ordered in databases such as PepBank (http://pepbank.mgh.harvard.edu), EROP-Noscow (http://erop.inbi.ras.ru), BioPD (http://biopd.bjmu.edu), PeptideDB (http://www.peptides.be), APD (http://aps.unmc.edu/AP/main.php), BIOPEP (http://www.uwm.edu.pl/biochemia/index_en.php), etc. BIOPEP database contains information of proteins from 54 plants hydrolyzed with 21 endopeptidases. These data revealed that wheat gliadins were the most susceptible plant proteins for bioactive peptide release [7,8].

This work has been focused on bioactive proteins and peptides from plant derived foods and has revised antihypertensive and antioxidant activities in addition to other less frequent activities such as cholesterol reduction capability, immunomodulation, opioid activity, etc.

2. Antihypertensive peptides

Antihypertensivity is the main bioactivity found in plant food-derived peptides. Despite there are different biological pathways regulating blood pressure in living organisms, hypotensive peptides act mainly on the rennin-angiotensin system. The renin-angiotensin system starts by the conversion of angiotensinogen to the pre-hypertensive hormone angiotensin I (Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu) by the action of renin which is secreted by kidneys. Angiotensin I is further converted to angiotensin II (Asp–Arg–Val–Tyr–Ile–His–Pro–Phe), the active form of the hormone, by the action of angiotensin converting enzyme (ACE). Angiotensin II raises blood pressure by acting directly to blood vessels, symphatic nerves, and adrenal glands [9]. Moreover, ACE can also inactivate the antihypertensive vasodilator bradykinin. Potent synthetic ACE inhibitors are commercialized for the regulation of blood pressure. Nevertheless, these synthetic compounds yield side effects such as coughing, taste disturbances, and skin rushes. Food derived ACE inhibitor peptides could be an alternative to synthetic drugs since they do not present those after-effects.

There are different vegetable foods showing antihypertensive properties. Table 1 groups the research works focused to the determination of antihypertensive proteins and peptides from plant foods. They have been classified according to the way peptides and proteins have been obtained: peptides and proteins not encrypted in any parent molecule and peptides obtained by enzymatic hydrolysis, fermentation, gastrointestinal digestion, and autolysis. Accumulated information from all these research works has resulted in a clear relationship between peptide structure and ACE inhibitory potency. Both the type of amino acids and peptide sequences determine peptide activity. Most ACE inhibitors peptides present short sequences ranging from 2 to 12 amino acids. Best antihypertensive peptides contain hydrophobic amino acids such as Pro, especially, at C-terminal position or positively charged amino acids such as Lys or Arg at C-terminal position [76]. The spectrophotometric method described by Cushman and Cheung [77] is the most widely employed for measuring ACE *in vitro* inhibition. This method is based on the hydrolysis of hippuryl–histidyl–leucine (HHL) by ACE to yield hippuric acid (HA) and histidyl–leucine. The HA is extracted into ethyl acetate and quantified by measuring its absorbance at 228 nm. The inhibitory potency is expressed as the IC_{50} value which is defined as the concentration needed to inhibit 50% of the enzyme activity. Nevertheless, this method involves several tedious steps and ethyl acetate extraction of HA can also co-extract unhydrolyzed HHL which also absorbs at 228 nm, overestimating ACE activity. Alternative methods not comprising any extraction step have been proposed. Wu et al. [26,78] designed a rapid and sensitive method that used reversed-phase chromatography (RPC) for the separation of HHL and HA. Some years later, Li et al. [79] developed an extraction-free method based on the specific reaction of HA with benzene sulfonyl chloride in the presence of quinoline. More recently, other authors have proposed a rapid and sensitive method that simultaneously quantified HA and HHL by ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) [80]. Nevertheless, this method has never been applied in the case of peptides but to synthetic drugs.

Peptides not obtained from a parent molecule and naturally occurring in foods have been observed in garlic, wakame seaweed, buckwheat, broccoli, and edible mushrooms. Extraction methods, in all cases, were very simple and consisted of protein extraction with water or an organic solvent such as MeOH. In most cases, a subsequent fractionation step was performed to finally isolate bioactive peptides. Most active peptides were obtained in wakame, garlic, and buckwheat. Indeed, seven different dipeptides were firstly isolated from garlic (*Allium sativum L*) by Suetsuna [10] despite some years before they could identified up to 16 peptides in a garlic extract [81]. The same authors identified ten peptides with antihypertensive properties from wakame (*Undaria pinnatifida*) [11]. Moreover, two tripeptides with sequences Gly–Pro–Pro and Tyr–Pro–Lys have also demonstrated hypotensive activity in buckwheat, a recognized functional food in China, Japan, and Poland [12], and in broccoli [13], respectively. Nevertheless, most naturally occurring peptides have been observed in mushrooms. Indeed, mushroom *Tricholoma giganteum* demonstrated native ACE inhibitory activity provided by a tripeptide that kept active after *in vitro* incubation with proteases [14]. Another mushroom that has also been studied for its content in peptides with antihypertensive activity is *Pleurotus cornucopiae*. Two peptides with IC_{50} values of 0.46 and 1.14 mg/mL were isolated and submitted to simulated gastrointestinal digestion. Fig. 1 shows the MS/MS spectra corresponding to these peptides and used for their sequence identification. Results suggested that purified peptides became stronger ACE inhibitors after gastrointestinal digestion [15]. Kokean et al. [16] evaluated the effect of cooking on antihypertensive properties of Hatakeshimeji