

## BIOACTIVE PEPTIDES AND HEALTH EFFECTS

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### ABSTRACT

*Bioactive peptides are organic substances formed by amino acids joined by covalent bonds known as amide or peptide bonds. Although some bioactive peptides exist free in its natural source, the vast majority of known bioactive peptides are encrypted in the structure of the parent proteins and are released mainly by enzymatic processes. Some bioactive peptides have been prepared by chemical synthesis. Bioactive peptides attributed to different health effects, including antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities,*

*opioid activities, enhancement of mineral absorption and/or bioavailability, cytomodulatory and immunomodulatory effects, antiobesity, and anti-genotoxic activity. The growing interest in bioactive peptides has incentivized the scientific community and the food industry to exploring the development of new food additives and functional products based on these peptides. The present review highlights the recent findings on the identification, bioassays, and use of bioactive peptides, as well as their potential use as food additives and in the development of functional products.*

### INTRODUCTION

Bioactive Peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may influence health [1]. Currently, more than 1500 different bioactive peptides have been reported in a database named 'Biopep' [2]. The value of proteins as an essential source of amino acids is well documented, but recently it has been recognized that dietary proteins exert many other functionalities in vivo by means of biologically active peptides. Inactive within the sequence of the parent protein, such peptides can be released by digestive enzymes during gastrointestinal transit or by fermentation or ripening during food processing [3]. Bioactive peptides and proteins play important roles in the metabolic functions of living

organisms and, consequently, in human health. They display hormone or drug-like activities and can be classified based on their mode of action as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative. Bioactive peptides have been considered the new generation of biologically active regulators that can prevent, for example, oxidation and microbial degradation in foods. They can be used for the treatment of various medical conditions, thus increasing the quality of life [4]. Recently, functional foods and nutraceuticals have received much attention, particularly for the impact that they can have on human health and their use in the prevention of certain diseases [5]. Consequently, considerable interest has been devoted to

the production and properties of bioactive peptides the past few years [6]. Even though bioactive peptides have been identified and isolated from several natural sources, and their activities

investigated in many disciplines, the present review is mainly concerned with bioactive peptides in the context of different food matrices.

## SOURCES

Bioactive peptides are predominantly encrypted inside bioactive proteins [7]. By far, bovine milk [8], cheese [9], and dairy products [10] are the greatest sources of bioactive proteins and peptides derived from foods. However, they can also be obtained from other animal sources such as bovine blood [6], gelatin [11], meat, eggs, various fish species such as tuna, sardine, herring and salmon. Some vegetal sources of bioactive peptides and proteins are wheat [12], maize, soy [2],

rice [13], mushrooms, pumpkin, sorghum [14], and amaranth [15]. In vivo, encrypted peptides can be liberated during gastrointestinal (GI) digestion by enzymes such as trypsin or by microbial enzymes. Currently, bioactive peptides and nutraceutical proteins are being developed to improve human health by preventing or alleviating medical conditions such as coronary heart disease, stroke, hypertension, cancer, obesity, diabetes, and osteoporosis [16].

## PHARMACOLOGICAL PROPERTIES AND HEALTH BENEFITS

Currently, the relationship between chemical structure and activity of a peptide can not be predicted. The activity of a peptide depends on its structure, i.e. the amino acid composition, the type of *N* and *C*-terminal amino acid, the length of the peptide chain, charge character of the amino acids forming the peptide, the hydrophobic/ hydrophilic characteristics of the amino acid chain, among others. For instance, peptides with higher ACE inhibitory activity usually have aromatic or basic *N*-terminal amino acids, higher quantity of hydrophobic and positively charged amino acids in *C*-terminal [17].

### Antihypertensive activity

Among the various bioactive peptides, antihypertensive peptides probably seem to be the most studied peptides from exogenous sources such as food. These food-derived antihypertensive peptides have not only been well researched but have also been put to practical use as functional and designer foods. Since hypertension has become a serious health problem,

especially in developed countries, and has been considered a risk factor for developing cardiovascular diseases, there has been a growing interest in antihypertensive peptides for their effectiveness in lowering blood pressure. These peptides have been found effective in preventing or treating hypertension mainly by inhibiting the angiotensin-converting enzyme (ACE), which plays a key role in the regulation of blood pressure and electrolyte homeostasis. IPP and VPP peptides are generally described and analysed as inhibitors of the angiotensin I-converting enzyme [18].

### Cholesterol-lowering effect

Conditions like hyperlipidemia, especially hypercholesterolemia, is one of the most important risk factors contributing to the development of cardiovascular diseases. In search of the treatment and prevention of hypercholesterolemia, numerous synthetic drugs and natural extracts with cholesterol-lowering effect have been explored for their potential. Many proteins

and their peptides are known to exert a cholesterol-lowering effect (e.g., soy protein, soy 7S globulin, soy protein hydrolysate, enterostatin, soy glycinin fragment, milk  $\beta$ -lactoglobulin hydrolysate, pork protein hydrolysate), among which soybean is the most well recognized source of hypocholesterolemic proteins and peptides. Milk is another important source of bioactive peptides with cholesterol-lowering effect [19].

### Antioxidant activity

Peptides derived from milk proteins have shown antioxidant properties that prevent peroxidation of essential fatty acids. For instance, the addition of a Leu or Pro residue to the *N*-terminus of a His-His, dipeptide will enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants [e.g. butylated hydroxytoluene (BHT)]. On the other hand, it has been demonstrated, that digestion of casein produces phosphorylated peptides that exhibit both hydrophilic and lipophilic antioxidant activity due to both metal ion sequestering and quenching of ROS [20]. The fish processing industry produces more than 60% byproducts as waste. To use these wastes, and to add value to several underutilized fish species, protein hydrolysates from fish proteins are being prepared using enzymes. These fish protein hydrolysates contain small bioactive peptides with antioxidant activity [21]. Soy peptides have shown increased antioxidant activities compared to intact proteins [22]. Soy protein hydrolysates prepared from native and heated soy protein isolates using different enzymes had varying degrees of hydrolysis (1.7–20.6%) and antioxidant activities (28–65%) [22]. Antioxidant proteins and peptides have also been identified in egg, potato, and gelatin [23].

### Antimicrobial activity

Antimicrobial peptides (AMPs) are an abundant and diverse group of molecules that are produced by many tissues and cell types in a variety of invertebrate, plant, and animal species. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers [24]. These peptides are involved in the inhibition of cell growth and in the killing of several microorganisms, such as bacteria and fungi. Antimicrobial peptides are usually below a MW of 10 kDa and encoded within the sequences of native protein precursors, may also be generated *in vitro* by enzymatic hydrolysis [25]. AMPs constitute a promising alternative as therapeutic agents against various pathogenic microbes [26]. More than 60 peptide drugs have reached the market for the benefit of patients and approximately 140 peptide therapeutics are currently being evaluated in clinical trials [27]. Casein Derived Antibacterial Peptides show inhibitory activity against *Streptococcus mutans*, *Streptococcus sanguis*, *Porphyromonas gingivalis*, *Streptococcus sobrinus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. For instance, after digestion with chymosin enzyme, casein- $\alpha$ 1 provides two peptides known as caseicin A and caseicin B that inhibit several pathogens (*Staphylococcus*, *Sarcina*, *Bacillus subtilis*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes*). On the other hand, Lf a Whey Protein Derived Antibacterial Peptide shows bacteriostatic effects *in vivo* and *in vitro* against *Bacillus stearothermophilus*, *B. subtilis*, *Clostridium spp.*, *Haemophilus influenza*, *Streptococcus mutans*, *Vibrio cholerae*, *E. coli*, and *Legionella pneumophila* [28].

### Cytomodulatory and anticancer activity

Proteins, peptides, and amino acids have been implicated in preventing the development of different types of

cancer. Dairy milk proteins and their peptide derivatives play a role in cancer prevention. CPP has also demonstrated anticarcinogenic activity [29]. The anticancer activities of these proteins may, at least partially, be attributed to encrypted bioactive peptides. Numerous peptides in different sizes from various sources have been indicated to render anticancer effect in *in vivo* studies [30]. By acting as specific signals that may trigger viability of cancer cells, there is increased evidence that milk-derived peptides may possess cytomodulatory activities. Bioactive peptides with cytomodulatory activities have been found during bacterial hydrolysis of casein by commercial yogurt starter cultures that affected colon cell Caco-2 kinetics *in vitro* [31]. Bioactive peptides with antiproliferative activity towards leukemia cells have been found during digestion of

bovine skimmed milk with cell-free extract of the yeast *Saccharomyces cerevisiae* [32].

### **Anti-inflammatory activity**

Proteins and peptides from egg, milk, soy, and plant sources have shown anti-inflammatory properties. Ovotransferrin is an egg White protein well known for its antibacterial activity [33]. It is often present in large amounts in chickens, during inflammation and infection processes. This protein also has immunomodulating effects on chicken macrophages and heterophil-granulocytes and can inhibit proliferation of mouse spleen lymphocytes. Immunomodulatory peptides derived from hydrolysates of rice and soybean proteins act to stimulate ROS, which triggers non-specific immune defence systems [34].

## **APPLICATIONS AND PRODUCTION IN FOOD INDUSTRY**

### **Nutraceutical**

Nutraceuticals are substances of natural origin that can be extracted from various sources (e.g. fruits, plants, lignocellulosic biomass, and algae) and that have important health benefits when incorporated into food or pharmaceutical formulations. In recent years, functional foods and nutraceuticals have attracted much attention, particularly for their impact on human health and prevention of certain diseases [35].

### **Functional ingredients**

Peptides derived from the milk of cow, goat, sheep, buffalo, and camel

exert multifunctional properties on human health. Additionally, medicinal plants are a rich source of natural antioxidants that are increasingly used in food manufacturing, because they provide valuable nutritional and therapeutic properties and retard oxidative degradation of lipids thus improving, the quality and nutritional values of foods regarded as functional [36]. For instance, the effects of *Allium sativum* on total phenolic content, proteolysis by o-phthaldialdehyde assay, antioxidant activity by radical inhibition and capacity to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase activities *in vitro* were higher in camel milk yogurt (MY) than cow MY [37].

## **CONCLUSIONS**

For development of various health-promoting functional and designer foods, bioactive peptides attracted increasing interest as prominent candidates. Numerous products based on the health-

promoting properties of the bioactive peptides have already struck the market and many products are under development, exploiting the potential of food-derived bioactive peptides. This

trend is likely to continue alongside with increasing knowledge about the functionalities of the peptides. Research continues to uncover novel bioactive peptides and to reveal their possible functions and health benefits.

Enzymatic hydrolysis occurring during digestion or fermentation can liberate an enormous amount of bioactive peptides whose activities span from antimicrobial, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative. As a result of this broad spectrum of activities, bioactive peptides have the potential to be used as food additives and ingredients of pharmaceuticals for the treatment or prevention of some medical conditions

and life style diseases, such as obesity, diabetes type II and hypertension.

Despite the significant progress in the isolation and purification of bioactive peptides from several natural sources, as well as the assessment of their bioactivities, there still are several obstacles to overcome, particularly from the technological viewpoint to produce them at large scale without losing activity. The increasing interest of the scientific community in the identification, purification, chemical synthesis and uses of bioactive peptides and the food industry to use bioactive peptides in commercial products will contribute to improving human health.

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