Bioactive Peptides from Unconventional Food Sources: A Novel Radical Approach in Food Value Addition

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Abstract

Introduction: Bioactive peptides are small peptides of 2-20 amino acids encrypted in the proteins of protein-rich unconventional food sources. Bioactive peptides show therapeutic functions such as antioxidative, immunomodulatory, opioid, anti-hypertensive, antimicrobial, etc.

Objective: Unconventional protein-rich food sources may be employed to fabricate bioactive peptides using enzyme technology.

Methodology: Protein-rich unconventional food sources such as *Bellamya bengalensis Lamellidense marginalis* have been used to fabricate bioactive peptides using different proteases *in vitro*. The ultrafiltration technique isolated the peptides. Functional properties of protein isolates from the meat of *Bellamya bengalensis* were characterized as foaming property, solubility index of protein, and emulsifying property. The anti-hypertensive property of the bioactive peptides was determined and compared with lisinopril, a standard drug. *In vitro* experiments and *in silico* molecular docking studies were carried out to find the most powerful inhibitory peptide against ACE, the major enzyme of the Renin-Angiotensin System. Using isothermal titration calorimetry, the results were further authenticated, and the actual mechanism of action of their anti-hypertensive property was found.

Results: The hydrolysates were ultrafiltered, the smallest fraction was evaluated in MALDI-TOF MS, and five peptides were sequenced via *de novo* sequencing. Lisinopril is a synthetic analog of the Angiotensin Converting Enzyme. The BP's binding affinity to ACE's active site was compared with lisinopril. The thermodynamics of the inhibition was checked by ITC and further validated by *in silico* molecular docking study. The ACE-inhibitory potential of the BP positively correlates with the presence of hydrophobic amino acids.

Conclusion: BP from different food sources have been excised and studied for their functional properties. Some have anti-hypertensive properties and may be of particular interest in the context of hypertension. BP from dietary proteins exposes various biological activities. These BP with anti-hypertensive effects may serve as a value-added food and pharmaceutical product.

Keywords: Bioactive peptide, Unconventional food source, Bellamya bengalensis, anti-hypertensive

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Introduction

Proteins occupy an important part of our daily diet with diverse functions for maintaining the normal physiology and homeostasis of the body. Several diseases like diarrhea, GI ulcer, and cancer reduce dietary protein absorption and bioavailability of the protein. The present research in enzyme biotechnology has opened up vistas of protein food product development. Proteases from animal, plant and microbial sources were isolated. Their application for the modification of proteins obtained from different food protein sources led to the development of new protein-rich food products. Protease hydrolysis of native protein leads to the formation of small peptides called bioactive peptides. These are the fragments derived from protein that has high bioavailability and bioaccessibility and influence health by acting as a good source of protein in case of a diseased person. They provide numerous potential physiological functions in the body.[1]

In recent years, extensive research in the field of dietary proteins led to the development of bioactive peptides with various biological activities, including antioxidative, anti-hypertensive, hypocholesterolemic, and immunomodulatory properties, in addition to their nutritional value. The demand for incorporating such bioactive peptides in a healthy diet regime is growing very fast. The prophylactic activities protect against many lifestyle diseases like hypertension, myocardial infarction, hypocholesterolemia, etc.[2,3] Recent studies have shown that biologically active peptides from dietary proteins exert many other functionalities beyond energy production. Bioactive peptide sequences are encrypted inside the amino acid sequence of the parent protein, which are released by digestive enzymes during gastrointestinal transit or by fermentation or ripening during food processing.[4,5] Several BP has been studied, mainly of animal origin, particularly milk and egg-derived peptides. BPs derived from livestock products like bovine milk, egg, cheese, and dairy appear to be very important products with various functionalities.[6,7]

Novel nutraceuticals and functional food researches directed towards the bioengineering of BPs, including their

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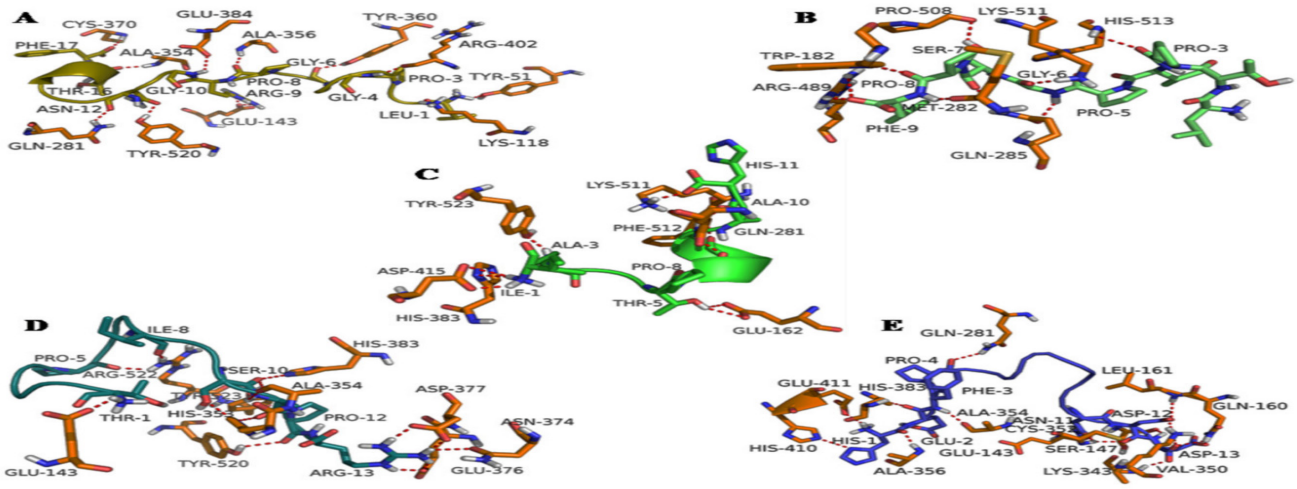

Figure 3: In silico studies: Docking poses of identified peptides (A. LNPGAGLPRGPNGADTF; B. LTPVPGSPF; C. IIAPTPVPAAH; D. TIGAPDGIPSAPR; E. HEFPGVVVGANDD) with angiotensin-converting enzyme (PDB file 1O86.pdb) at the core of the enzyme adapted from Dey TK, Chatterjee R, Mandal RS, Roychoudhury A, Paul D, Roy S, Pateiro M, Das AK, Lorenzo JM, Dhar P. ACE Inhibitory Peptides from *Bellamya bengalensis* Protein Hydrolysates: In Vitro and In Silico Molecular Assessment. *Processes*. 2021; 9(8):1316.

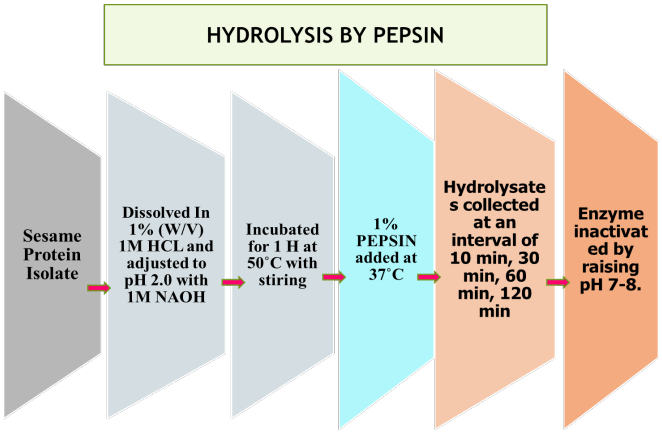

Figure 1: Hydrolysis of sesame protein by pepsin available in the market

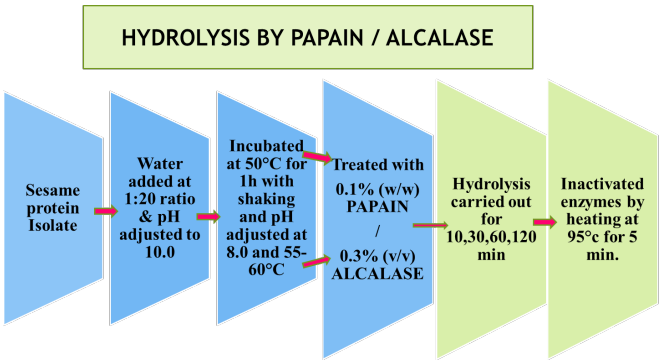

Figure 2: Hydrolysis of sesame protein by using papain and alcalase

application in respective physiological/health benefits.[8] In the present era, medical science concerned with discovering potential drugs of natural origins is increasingly gaining prominence owing to the increasing awareness about the side effects of synthetic drugs for treating many forms of chronic diseases. Consequently, radical research is being undertaken to find natural compounds with potential therapeutic benefits. With recent progress in nutritional sciences, Ayurveda and functional foods have received extensive attention. The worldwide nutraceutical and phytonutrient demand have increased over the past decade, and growth of the functional food industry is enormous with increased usage by a wide population for various therapeutic outcomes. Food-derived bioactive peptides are also of various types with different health-promoting functions. Bioactive Peptides (BP) are specific protein fragments that negatively impact body functions or improve health. Currently, more than 1500 different BP have been reported in a database named 'Biopep'.[9] Several dietary proteins may serve as a good source of bioactive peptides providing specific health benefits by promoting diverse activities including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, anti-hypertensive (some peptides inhibit angiotensin converting enzyme (ACE) that regulates the activation of Angiotensin II hormone, thereby regulating hypertension) activities beyond their regular nutritional values.[10] The BPs also get easily absorbed in the gastrointestinal tract and delivered to the target organs.[11] They can be released by different hydrolysis procedures like gastrointestinal digestion, fermentation, enzymatic proteolysis or by other food processing from multitude of plants and animal proteins especially from soybean seeds,[12] leguminous seeds, corn seeds, sesame seeds,[11] bovine milk,[11,1314] cheese,[15] and dairy products,[16] wheat,[15] barley, rice, rye, oat, millet, sorghum, and corn,[17] gelatin,[18] meat, eggs, various fish species such as tuna, sardine, herring, and salmon,[9] etc. Meat and fish-derived peptides have been shown to exhibit anti-hypertensive effects in vivo, along with antioxidant capabilities and other bioactivities such as antimicrobial and anti-proliferative activities in vitro.[9] Some examples of peptides with their known activities include[9] FSDKKIAK, EQLTK (Antimicrobial);

LKP, AKYSY, KRQKYD, KRPKHPIKH, SVPQPK, VVYPWYQ (Antihypertensive, Angiotensin Converting Enzyme); VECYGPNRPQF (Antioxidant) etc. Food proteins are hydrolyzed by enzymatic hydrolysis, food processing, microbial fermentation. Besides producing biopetides, protein hydrolysis improves functional properties like protein solubility, emulsification, foaming properties, etc.

Some studies reveal that enzymatically hydrolyzed fish protein is a rich source of biologically active peptides having certain health benefits (indicated above), most importantly the anti-hypertensive property. Arginine, valine, and leucine-rich purified peptides are known to be of low molecular weight peptides with anti-hypertensive, immunomodulatory, antibacterial, and antithrombotic activity.[19] These disclosures in the field of BP research have led to the development of new techniques to produce bioactive peptides with specific health benefits. Recent focus has been on producing Angiotensin Converting Enzyme (ACE) inhibitory peptides exhibiting anti-hypertensive activity from fish and its waste.

We have enzymatically produced bioactive peptides from sesame and mustard oil seed waste protein, from the flesh of *Bellamya bengalensis* and *Lamelidense marginalis*. Papain, pepsin and alcalase were utilized for the hydrolysis of protein. Bioactive proteins were isolated using membranes, and amino acid sequencing was done. *In vitro* anti-hypertensive activity of the peptides < 3 KD were studied. *In silico* binding studies were carried out to find the peptide with the highest anti-hypertensive effect.

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