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Plant Seed Proteins: Chemistry, Technology and Applications

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Abstract

This review deals with the significance of plant seed proteins. Plant seed proteins are known to provide various beneficial activities like antimicrobial, antihypertensive, antiviral and antioxidant. They are essential source of amino acids; act as a source of nutrition booster. Present review elaborates on extraction of proteins and hydrolysis with their advantages and disadvantages, their nutritional property, health benefits and challenges associated with the peptides.

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Introduction

Plants and their products have been used for their potential nutraceutical properties. Plant proteins are considered as an economical and environmentally sustainable source of protein as compared to that of animal proteins. The needs of the people in developing country like India could be met through the plant proteins to cure protein malnutrition. The 80% of energy and 70% of protein is supplied from plant source[1]. According to the studies reported by Pimentel & Pimentel, (2003), animal and fish proteins are unsustainable sources compare to that of soy, legume, canola and cereal[2]. Legumes and seeds are a major portion of plant and familiar for substantially as a source of nutrition[3].

A large volume of wastes and by products are produced in fruit industries during its processing such as making of juices, jellies and candies. This waste includes large no of seeds, peels which are generally used as animal feed. The recent trend is focused on the utilization of such wastes in making value added functional food products[4].

Consumption of seeds in human diet as protein source and other bioactive constituents has been known from last two decades. There have been numerous studies and reports indicating the nutritional, functional health benefits provided by seeds in human diet[3,4,7], such as carbohydrate content[8], antioxidant activity[9], fatty acid content[10] and protein content[11].

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The consumer diet is nowadays more focused on functional and health benefits with balance nutrient profile instead of targeting energy providing diet[12] which lead to increased intake of natural and health foods[13]. Research towards exploring inexpensive plant-based protein supplements, as well as developing new food products[14], has resulted in the investigation of the potential of underutilized dicotyledonous seeds (with substantial traditional knowledge) for humans, as well as for livestock consumption[15,16].

The present review deals to introduce the plant seeds as source of nutrition booster, extraction of proteins and hydrolysis, nutritional property, health benefits and challenges associated with the peptides.

Plant Based Sources of Proteins

Seeds could be used an alternative booster of protein to overcome the protein deficiencies and to replace animal proteins which are high in cost. The present section deals with the protein from different seeds Table 1.

Table 1: Different type of seeds with its nutritional significance

Seeds	Scientific name	Description	Reference
Date	*Phoenix dactylifera L*	rich source of protein	17
Belinjau seed	*Gnetum gnemon L.*	rich in protein, crude fiber, carbohydrates, total dietary fiber and encompassed with essential amino acids, fatty acids and minerals	18
Kiwi seed	*Actinidia chinensis Planch.*	rich source of lipids, polyphenols, and crude fibers	19
Cumin seed	*Cuminum cyminum*	antimicrobial, anti-carcinogenic and antioxidant, antidiabetic	20,21
Watermelon seeds and Wood apple seeds	*Citrullus lanatus* and *L acidissima*	large amounts of content proteins and ample amount of minerals	16
Grape seeds	*Vitis vinifera L.*	rich in lipids, carbohydrates and proteins essential amino acids	23,24
Tamarind seed	*Tamarindus indica L*	Source of essential amino acids, gums and carbohydrates	25

Extraction and Hydrolysis Methods of Proteins

Protein extraction in plants is technically interesting due to high abundance of some proteins in plant extracts which interfere with the resolution of proteins of similar molecular weight as well as protein quantization[26]. Hence, this section deals with the different methods that are used during extraction of seed proteins and the factors affecting on efficacy of extraction based on different reported literature (represented in Fig 1).

Pure protein can extracted using alkaline extraction which is a commonly practiced to obtain a high yield[27]. Different buffers like acetate-urea, SDS-buffer, NH-40 buffer phosphate and Tris- HCL buffers can also be used to enhance the extractability of protein with addition of chemical ingredients (like citric acid, cysteine hydrochloride, polyethylene glycol, and mercaptoethanol. The common activity of protease can be obstructed by high pH or high alkali extraction which causes ionization of phenolic compounds and prevents formation of hydrogen bonding with protein[28,29]. To prevent protein oxidation by using reducing agent like β-mercaptoethanol; protein extraction can be enhancing using KCl, EDTA, and SDS with buffers[30,31].They are TPP (three phase partitioning) well known biosepration method is also available to extract the protein from the sources.

Protein Hydrolysis Method

Different hydrolysis method for hydrolysis of protein is given in Table 2.

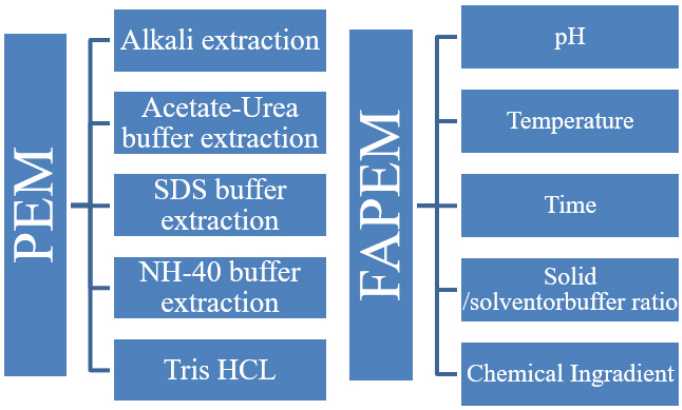

Fig. 1: A simplified flow chart showing methods of protein extraction (PEM) and factors affecting on protein extraction

Table 2:

Acid hydrolysis	Alkali hydrolysis	Enzymatic hydrolysis
oldest methods of hydrolysis	simple and straight forward process	Most acceptable method to developed bioactive peptides
Application: used as flavor enhancers	Protein hydrolysates prepared by this method commercially use in food industry	Bioactive peptides, supplements
Hydrolysis accomplished with sulfuric and hydrochloric acid	Alkali used or alkaline pH maintain	Different digestive and gastrointestinal enzymes, employed in hydrolysis [26,27,28]
Mechanism: Formation of free amino acid or smaller peptides due to disruptions of proteins	Solubility of protein enhance by heating with addition of alkaline agents like calcium, sodium or potassium hydroxide	Enzymes are very target specific cleave the peptide bond at particular amino acids
Factors for hydrolysis: • Concentration • Type of acid • Temperature ranged between 120–140°C • 32–45 psi pressure • 2–8 h time of hydrolysis • 50–65% concentration of protein.	Desired degree of hydrolysis at desired time by maintaining temperature within typical range that is 27–55°C can be achieved.	Factors: enzyme substrate ratio, time, temperature
Disadvantage: •uncontrolled process •many amino acids get destroyed during the process due to high concentration of acid, for example: tryptophan, methionine, and cysteine	Disadvantage • amino acids like serine and threonine are destroyed	Advantage • controlled process • does not require high end physiological conditions • enzyme generally works at mild situations

Properties of Peptide

The peptides which are hydrolyze by using acid, alkali of enzymatic method which is inactive part of the protein composed with bi, tri of poly peptides chain possess various properties. The human body endangered to stress and exposure to toxic materials interrupting normal functions of body leading to various health conditions. Physiological homeostasis or health-promoting agents have potential to control these abnormalities. Consumes are looking for more healthy, nutritional oppositionists derived from natural sources falls under the category of functional foods and nutraceuticals have arisen as alternative to chemotherapy to protect from diseases[34].

Protein is well known constituent and key for building blocks of human body. Raw food proteins force their action in their original form or on the hydrolysis both in vivo and in vitro. After hydrolyzed proteins get into small fractions and amino acids to get absorb into body by digestive track enzymes. These dietary peptides possess beneficial pharmacological properties[35] which are recognized as bioactive peptides. The activities shown by peptides depicted in Fig 2.

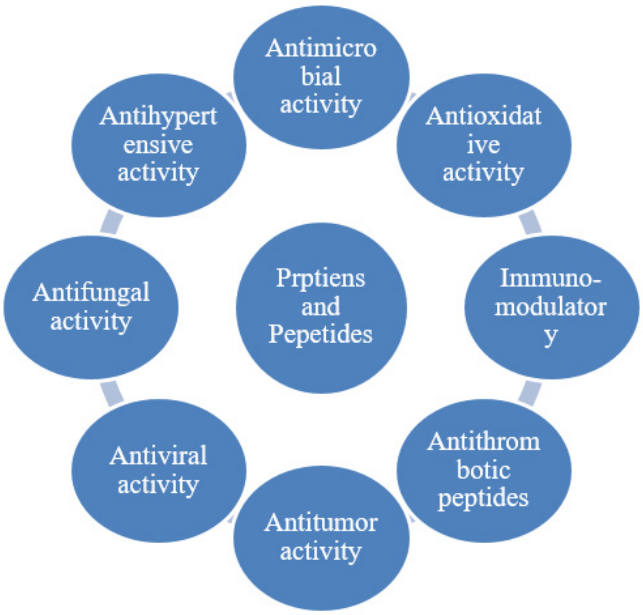

Fig. 1: Various activities shown by peptides

Antimicrobial Activity

Peptides which show activity against the micro organisms are known as antimicrobial peptides. One group of peptides acts on cytoplasmic membrane whereas another group performed neutral role the cytoplasmic membrane of the target microorganism[36,37]. Antimicrobial peptides are recognized by their influence on microorganism targeted to cytoplasmic membrane[36]. The magnetism between the peptide and the target cell created due to electrostatic binding which may be twisted due to cationic peptide and negatively charged outer cell membrane[37].

In this, electrostatic interaction removes Mg^{2+}, Ca^{2+} (divalent cations) from surface. The smooth entry of the peptide and subsequent peptide connection with the cytoplasmic membrane due to disruption of cell outer membrane and report auto-promoted uptake[36]. The cytoplasmic membrane creates an arrangement of the peptide to act on target cell and permeabilizes cytoplasmic membrane and/or translocate through it.

Factors such as electrostatic interactions, hydrophobicity and flexibility of peptide have influence on antimicrobial activity. *Foeniculum vulgare,*

Cucumis sativus, Ammi majus, Allium ascolinicum, Cichorium intybus and *Rumex vesicarius* plant seeds have shown an antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380)[38]. The excellent antimicrobial activity from *Cucurbita moschata* and *Lagenaria siceraria* seed proteins hydrolyaste against gram negative bacteria *A. baumannii* were reported by Dash & Ghosh, (2017)[34].

Song, Wei, Zhang, (2012) ascertained that the improvement in the hydrophobicity helps to initiate hydrophobic reaction between lipid acyl chains in bacterial membrane one of the crucial parameter to enhance the membrane permeabilization of bacteria. The amino acids like cysteine, histidine, proline, tyrosine, glycine, arginine, lysine and serine are accountable for antimicrobial property[41]. According Song, Wei, Zhang, (2012), if peptide contain major amino acids like lysine and arginine which are responsible for interaction with bacterial membrane.

Antifungal Activity

Plant antifungal proteins act as shield against fungal invasion and organized into different groups. This groups include thaumatin-like proteins, lectins, cyclophilin-like proteins, lipid transfer proteins, ribonucleases, storage 2S albumins, ribosome-inactivating proteins and many mores[42–44]. Different mechanism of action for this protein observed with degradation of fungal cell wall polymer, formation of pore, inhibition of DNA synthesis,[45]. An antifungal protein with 14.3 kDa extracted from the seeds of butterfly pea (*Clitoria ternatea*) showed lytic activity and fungicidal activity[46]. Bard *et al.,* (2014) segregated peptides from *Capsicum baccatum* seeds which exhibited inhibitory effects against α-amylase and antimicrobial activity[42].

Sm-AMP-X (33 residues) was isolated from chickweed (Stellaria media) seeds. This shows high activity against fungal phytopathogens and discovered as a novel antifungal peptide[48]. Actually this is consist of helix-loop-helix fold which stabilized by two disulfide bridges C1–C4 and C2–C3[48].

Antiviral activity

There are two ways to prevent viral infection. In first it can be blocked viral entry through interaction with the virus and another way is to obstruct virus entry through interaction with the host cell. The cationic peptides have potential to effectively inhibit viral infections. Peptides react with viral receptors by blocking the virus and prevent binding intra-cellularly[37]. Protein microbicides composed with antiviral lectins and antibodies which aid in blocking human immunodeficiency virus (HIV)[49]. He observed antiviral lectin griffithsin (GRFT) in the endosperm of transgenic rice plants (*Oryza sativa*) which exhibit persuasive neutralizing activity against HIV[50]. The GRFT isolated from *Griffithsia spp.* having MW of 12.7 kDa lectin which impedes HIV-1 by binding with mannose-rich glycans on the virus envelope glycoproteins[51]. The antiviral activity of GRFT against severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and other coronaviruses[52,53], *hepatitis C virus*[54,55], *Japanese encephalitis virus*[56] and *herpes simplex virus*[57] was validated.

Antihypertensive Activity

Blood pressure is risk factor in developing cardiovascular diseases which causes due to the Angiotensin II. These occur due to the deliverance of rennin from kidney which break downs the circulating angiotensinogen. Angiotensin I (decapeptide) is formed by the conversion of angiotensinogen which may cleave to the octapeptide (angiotensin II). This activity performed in the existence of angiotensin converting enzyme (ACE) which originates arterial constriction and elevation in blood pressure. Peptides produce by hydrolysis of lupin seed protein with pepsin able to inhibit the angiotensin-converting enzyme[58]. Peptides from canary seed prolamins showed an antihypertensive activity and reported as an accessible and cheap source to encourage better human health[54].

Antioxidant Activity

Protein hydrolysates composed of 2–20 amino acids or peptides longer than 20 amino acid residues showed bioactivity[60]. Recently, the scrutiny of novel functional food from vegetable proteins is a valuable source of protein to enrich food use[61].

Savadkoohi and Farahnaky (2012) reported that tomato seed proteins has nutraceutical properties[62]. Ziziphus jujbee, *C. Lantus* and *L. accidama* protein hydrolyaste shows antioxidant activity[58,59,60,61].

Applications of Peptides

Due to increased consumer awareness regarding use of natural preservatives; there is demand of healthy natural food additives derived from natural sources like plant seeds. Therefore, peptides derived from the plant seeds are in demand due to their antimicrobial property and having voluminous applications as in devices used in biomedical and food processing. The creation of antimicrobial packaging by fusing peptides into materials has an essential role in safety and quality of food. This employed to stretch food shelf life and to shrink growth of bacteria on the surface of product[63,64].

Many researchers enhanced the nutritional values of foods by employing protein hydrolysate from different plant sources like soybean, rice endosperm, and rice bran which may have their low costs and safety[65–68]. Akin & Ozcan, (2017), developed new healthy dairy fermented milk drinks (non-fat) using plant protein. This was formulated by using wheat gluten, soy protein isolate, rice protein, and pea protein isolate which improved the physico-chemical, nutritional and sensory properties.

From the industry point of view, the application of protein hydrolyzes are limited due to bitter taste which overcome using different approaches. Hydrolysis of bitter peptides with aminopeptidase, alkaline/ neutral protease and carboxypeptidase is one of the approaches with having disadvantage that hydrolyze the previously generated bioactive peptides. The masking additives like using monosodium glutamate, addition of cyclodextrins and encapsulation, could be preferably used to remove bitterness from the peptides.

Conclusion

From the above literature it can be concluded that plant seeds could be an alternative source of protein for replacement of animal protein that has associated with many health benefits. There is need to elaborate the research in plant seed protein to find health benefits and meet the demand of protein of growing population.

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