PLANT SEEDS AS THE UNDERUTILISED WEALTH AND NUTRITIONAL SOURCE OF BOOSTER PROMOTER

Sachin K Sonawane[1,2], Anjana Anandhan[2], Tanika Nair[2], Sonal Patil[1,2], Arya S. S.[1*]
[1]*Food Engineering and Technology Department, Institute of Chemical Technology, NM Parekh Marg, Matunga Mumbai, India*
[2]*Food Science and Technology, School of Biotechnology and Bioinformatics, D. Y. Patil University Navi Mumbai, India*
*E-mail: shalu.ghodke@gmail.com/sac007s@gmail.com

Abstract
Seeds are neglected over years but they can be proven as potential sources of many important nutrients and functionalities due to presene important proteins in them. The efficacy of these protein functionalities depends on the extraction parameters. Such extracted proteins have bioactive capacities in order to preserve foods, functional bevarages etc. This review focuses various aspects of plant seeds including their extraction procedures, hydrolysis methods, bioactivity and their application in the field of food industry. Plant seeds are characterized by presence of proteins, fats, polyphenols, and possess a great antioxidant activity. These seeds have been shown to be thermo resistant, oil and water absorptive, radical scavengers and prone to undergo encapsulation. Various studies carried out for extraction (acid, alkali, enzymatic) of proteins from plant seeds have been accompanied by use of response surface methodology. However, alkali extraction is the most efficient and cheapest method of extraction followed by precipitation and commonly employed in industry. These protein hydrolysates have been shown to possess antioxidant activity as well as metal ion chelation activity and reducing activity. These proteins have a broad spectrum of applications including in various industries including food, cosmetics industry and also in nanoemulsions. Plant seed proteins have been successively employed in food industry as additive, preservative as well as in nutritional bevarages.

Keywords: *seeds, plant proteins, protein extraction, protein optimization*

Received: 27.07.2018 Reviewed: 15.10.2018 Accepted: 29.10.2018

1. INTRODUCTION

Seeds can be served as a potential source for functional food ingredients, as they are rich source of monosaturated and polysaturated fats as well as essential amino acids and minerals. Many other nutrients including dietary fiber, phytochemicals and vitamins are also present in them (Sonawane and Arya, 2018). These fruit seeds can find a wide scope of application in food industry because of their nutritional and economical value. Seeds from different fruits can act as a good source of sugar fiber and phenolic antioxidants (Sonawane and Arya, 2018). Also, the extracts obtained from such seeds have some medicinal value including prevention of high blood pressure since seeds are good source of antioxidants along with flavonoids, phenolic procyanidins (Sonawane and Arya, 2018). Processing of plant seeds leads to some alterations in their functional as well as nutritional quality.

Asogwa and Onweluzo (2010) studied the effect of processing on nutrient composition of seeds and reported significant difference between the nutrient composition of processed seeds and raw seeds. Boiled seeds had higher amount of protein than roasted seeds. On the other hand processing of seeds by heating is lead to reduction of vitamin content due their high sensitivity to oxidation (Davy et al., 2010). However as far as convenience, quality and safety are concerned, seed processing methods such as heat treatment inactivates anti-nutritional factors in raw seeds there by increasing palatability and nutritional value (Apata and Olegbobe, 1994).

This review focuses various aspects of plant seeds including their extraction procedures, hydrolysis methods, bioactivity and their application in the field of food industry.

2. Plant seeds

Fruit seeds have always been neglected over a period of time. But these seeds are a potential

source of various nutrients those can be included in human diet. These can be used in the day to day diet depending upon their nutritional composition and other functional characteristics.

It is a well-known fact that watermelon (*Citrullus vulgaris*) seeds are great source of protein and fat which utilized in various food preparations. However, bioaccessibility of these nutrients is a key concern today. Jyothi and Kaul (2011) explored this issue for water melon seeds. They analyzed chemical composition; functionality and mineral bioaccessibility from defatted flour and protein isolate which formulated from watermelon seed meal (WSM). The proteins have good protein digestibility and seeds were an adequate source of iron and zinc.

El-safy, Salem, & El-ghany, (2012) examined the nutrient, functional properties, protein digestibility, and antinutritional factors of various seed flours. These seed flours were papaya, apple, watermelon, guava, orange, prickly pear, apricot and paprika seed flours. All flours had higher amounts of protein. These flours had substantial amount of minerals which would be introducing food supplements. They also proposed the use of these seeds flours in order to over-come the deficiency of certain amino acids.

Lima et al (2014) accomplish with a study that deals with the characterization of various different seeds (cherry, jackfruit, orange, casaba melon, peach and Surinam cherry) for their centesimal as well as functional properties. The seeds have been processed into flours and various methods have been employed for their characterization such as infrared spectroscopy, XRD, thermo gravimetric analysis and low field NMR. They found protein content in those seeds to be ranged between 10% and 32%. The seeds had an ample amount of lipids also ranged from 3-39%. They also had a high value for ash content (3.9%). The starches extracted from these seeds were having A-type of crystals. Seeds were thermo resistant as indicated by thermo gravimetric analyses.

Bhat and Yahya (2014) evaluated belinjau (*Gnetum gnemon L.*) seed flour for nutrients. They also studied antioxidant activity and functional assets. The seeds are rich source of various nutrients like essential amino acids, fatty acids, minerals. Protein, crude fiber, carbohydrates and total dietary fibers were 19.0, 8.66, 64.1, 14.5% respectively. Antioxidant compounds such as total phenols, tannins and flavonoids were present in these seeds in generous amounts. Antioxidant activity was measured as inhibition of DPPH and FRAP in ethanol extracts. It shows water (5.51g/g) and oil absorption (1.98 g/g,) capacities, emulsion capacity (15.3%) and stability (6.90%) and foaming capacity (5.78%). Presence of various functional groups was revealed by FTIR spectral analysis. Bhat and Yahya noted the use of these flours as a raw material in the development of low cost nutritious functional foods owing to their nutraceutical value.

In another study conducted by Elinge et al. (2012), pumpkin seeds were evaluated for nutritional analysis and the values for ash, lipid, fiber, protein and carbohydrates were found to be 5.5, 38.0, 1, 27.48 and 28.03% respectively. These seeds were composed of elements like potassium (273mg/100g) and many others.

Xu et al. (2016) described phenolic profile of apple seeds. It was seen that phloridzin is the chief phenolic compound in the seeds that accounts for various functions in the seeds within range of 240.45–864.42 mg/100 g d.w.. Górnaś, Rudzińska, & Segliņa (2014) studied lipophilic compounds such as palmitic acid, oleic acid and linoleic acid.

Martinez et al (2010) in their article about walnut discussed about implication of the walnut kernel. These seeds are rich in oil so it can be served as a nutrient-dense food. The oil can be consumed without refining and extracted by using screw pressing techniques. The oil extracted from walnut shows higher percentage of unsaturated fatty acids in which linoleic in higher range compared to oleic and linolenic acids. Various minor components are also present in walnut oil including

tocopherols, phospholipids, sphingolipids, sterols, hydrocarbons and volatile compounds. Martinez et al (2010) observed fewer amounts of phenolic compounds in extracted oil as compared to seed and found rich in proteins. The oil extraction residue was found to be rich in proteins. Hence these seeds have scope in the formulation.

Cattaneo et al (2016) studied characterization of the Prosopis alba seed (South American tree species) which generated as waste during production of pod flour. 62% of protein was observed in pod flour. They also observed fat free polyphenol that is 1150 ± 20 mg GAE/100 g flour. Pod flour also composed with β-CE/100 g of flour. Various flavonoids composition of polyphenolic extracts found in pod flour. The extract possesses ABTS scavenging activity and hydrogen peroxide scavenging activity. The authors suggested its use as a new alternative while formulating functional foods or food supplements (Cattaneo et al., 2016).

There are few more seeds tabulated in Table 1 with their nutritional significance.

Table 1. The nutritional composition of different seeds

Seeds	Nutritional significance	Reference
Carica papaya Linn. seeds	Composed of hydroxycinnamic acid kaempferol-3-glucoside,phenolic derivative such as p-hydroxybenzoic acid, and also possess antioxidant activity	Kadiri, Akanbi, Olawoye, 2016
Avocado, Jackfruit, Longan, Mango and Tamarind seeds	antioxidant activity and total phenolic content	Soong, 2004
Ziziphus jujube seed	antioxidant activity and stability of encapsulated peptide from *Ziziphus jujube* seed	Kanbargi, Sonawane, Arya, 2017
Lemon, Orange and Grapefruit seeds	Illustrate dietary fibers extracted from defatted press meals seeds	Karaman, Yılmaz, Tuncel, 2017

Day (2013) has stated about increasing utilization of plant proteins in order to develop protein-rich foods without animal proteins in the human die would be done by increasing utilization of plant protein. It will help diminish the strain of the environment posed by the exhaustive animal husbandries. Plant proteins can be recognized as essential amino acid supplement. They also perform an integral role in fundamental development of foods.

3. Extraction and optimization of protein from different sources of plant

This section deals with the extraction of protein from different sources of plant and different method of extraction.

Plant-based proteins are an important part of the food industry. They retain good nutritional and functional properties which are served as food ingredients. Mainly used plant proteins involve use of protein isolates from grain legumes. The downside of the route is poor economic efficiency and low yield of the specific protein isolation. Although various studies have been accomplish for the same, there is still an absence of systemic studies for isolation of protein with designated properties using diverse raw materials. Sussmann et al. (2013) investigated legumes as raw material in which he studied importance of procedure limitations on the overall protein yields.

Rao et al. (2011) developed wood apple protein concentrate (WSPC) from wood apple seed meal (WSM). They found protein contents in WSM and WSPC to be 33.79 and 77 g/100g respectively. WSPC was recognized as source of essential amino acids. Mostly recognized essential amino acids were leucine, phenyl alanine, valine, iso-leucine and threonine in WSPC. Potential use of wood apple protein concentrates for industrial application have been shown owing to their higher water absorption capacity (WAC), lower oil holding capacity (OHC), stable foam and presence of essential minerals.

Juice manufacturing industry always has seeds as a byproduct of their processing. Water melon seeds (*Citrullus lanatus Cv Mateera*) are good example of the same. These seeds contain good quality of extractable proteins.

Wani et al (2006) developed model like central composite design variables for extraction of seed protein were as follows:

i. 40 to 60ºC of extraction temperature
ii. 0.3 to 1.5 % of NaOH concentration (w/v)
iii. 5 to 25 min extraction time
iv. 30:1 to 70:1 as solvent/meal ratio

From above set up they found protein yield ranges between75.49 to 86.08%. The model obey was second-order with coefficient of determination of 0.846. The alkali concentration show the effect on protein yield. These outcomes support in scheming method of ideal protein withdrawal from source of watermelon seeds (Abas Wani, Sogi, Grover, & Saxena, 2006).

Wani, Kaur, Ahmed, & Sogi (2008) employed RSM- CCD tool to recover protein from meal of watermelon seed. They design four independent variables as follows:

i) 40 to 60ºC of extraction temperature
ii) 0.03 to 0.14 (g/L) NaOH concentration
iii) 5 to 25 min extraction time
iv) 30:1 to 70:1 solvent/meal ratio

By considering protein yield as response and it ranges between 72.03 and 81.52 g/100 g seed meal and model obey was second-order equation. This equation was providing by four independent and response variables. The interaction between processing factors and response were understood by using contour plots graphs. Coefficient of determination (0.80) and standard error (0.906) exposed predicted values obtained for protein yield. Assenting studies discovered that the protein yield was 80.71 g/100 g seed meal under optimum conditions such as 0.12 g/L of alkali concentration, 15 min. of extraction time at the ratio 70:1 (v/w) solvent/meal by maintaining extraction temperature 50.1ºC (Wani, Kaur, Ahmed, & Sogi, 2008).

In another study conducted by Ronny et al (2010), protein fractions were extracted sequentially from defatted ripe bitter melon seed (*Momordica charantia*) using:

1. Water soluble – albumin - 49.3%
2. 1 M sodium chloride solution- globulin - 29.3%
3. alkali/pH 11.0 - glutelin - 3.1%)
4. alcohol soluble- prolamin - 18.3%

The molecular weight of all the fractions was typically about 45 and 55 kDa. The T_d of these fractions was higher such as albumin (111.9), globulin (117.3), and glutelin (133.6) also have unique protein profiles. The authors recommend their use as novel food ingredients in order to impart novel characteristics to the food.

Amza, Amadou, Zhu, & Zhou (2011) isolated proteins from gingerbread plum seed. The physicochemical and functional properties were carried out to understand the effect of extraction and isolation. Alkali solution and isoelectric precipitation techniques were employed in favor of protein extraction from defatted seed flour. Physicochemical and functional properties of freeze dried gingerbread plum seed protein isolate (FGPSPI) and vacuum dried gingerbread plum seed protein isolate (VGPSPI) were assessed. To overcome protein deficiency, this could be source for fortification of protein into food products which are deficient in protein.

Bekhit, Carne, & Birch (2014) driven physicochemical and functional properties of hemp, flax and canola protein isolates and also scrutinize influence of defatting process, acid and alkali on extraction. The protein extracts produced with acid and alkali extraction process showed maximum emulsifying activity as well as emulsion stability. The highest water absorption was shown by alkali extraction treatment. Flax protein isolate and acid hemp protein isolate extracted from alkali shows larger size of droplet and shows highest CS (creaming stability). An improvement in amino acid profiles were observed due to the extraction accomplished in acid and alkali. This will be helpful to meet human nutrition requirements.

Jongjareonrak et al. (2015) employed central composite design in order to extract proteins from waste material which obtained from Sangyod Phatthalung (*Oryza sativa L.*) rice bran after removal of oil.

It contains 12.8% protein. Protein extraction process was optimized using central composite

design taking into consideration three factors viz:

I. 0.05 to 0.2M sodium hydroxide concentration
II. 30-60°C extraction temperature
III. 60-240 min. extraction time

Influence of these parameters on extraction yield as well as the protein was determined. Response Surface Methodology gave best formulation to achieved yield were 0.13M concentration of sodium hydroxide at 49°C for 170 min. 68.3% of protein solubility was seen at pH 10. It shows good functional properties at pH 10 could be considered for food ingredient or protein source.

Ditaxis heterantha, a plant which belongs to *Euphorbiaceae* family, is known to produce yellow pigmented seeds which have various food coloring applications. These seeds have 20% of protein as reported by Espino-Sevilla et al. (2013). These proteins are extracted and further fractionated on the basis of their solubility. Various researchers have fractionated these proteins into three fractions viz:

i. glutelins
ii. albumins
iii. globulins

Each of these fractions possesses good amino acid profiles. Reported denaturation temperature for globulins and glutelins is between 100 and 106^{o}C, while in case of albumins this temperature has been reported to be 76^{o}C. Espino-Sevilla et al (2013) stated about the beneficial functional properties of these proteins which can be further explored to be used in food formulations.

Studies have been carried out in order to extract proteins enzymatically from rapeseed (Brassica rapa). Pectinase treatment enriches proteins recovery from these seeds. There was no need to adjust pH through cold pressing which recovered proteins in water-lean conditions.

Rommi et al. (2015) studied effect of pH conditions and physiochemical properties of the protein concentrate. pH 6 found to be the optimum for proteins extraction. Recovery of protein improved due to hydrolysis of carbohydrates which possessed better protein solubility at pH 6. The water extracts shows higher dispersion stability due to smaller particle size as compared with isoelectric precipitates from alkaline extraction (Rommi et al., 2015).

Mazlan et al. (2014) studied protein extraction from fermented and non-fermented Perah Seed (Elateriospermum tapos). They employed response surface methodological (RSM) approach for the same. A Box-Behnken design (BBD) was used having three independent variables which are NaOH concentration (6, 8 and 10%), extraction time (10, 20 and 30 minutes) and solvent/meal ratio (50:1, 100:1 and 150:1, v/w). The optimization extraction was obtained at 5.5% of solvent extraction, 40:1 ratio of solvent/meal and at 32 minutes of reaction time. It was found that surface concentration and solvent to meal ratio affected on protein output from fermented seeds. In case of non-fermented seeds only solvent extraction was found operative on protein result. Maximum protein yields observed in case of both fermented and non-fermented Perah seeds were 18.0 g/100g and 5.0 g/100g seed meal respectively (Mazlan, Muhamad, Hassan, 2014).

Tea leave pulps have also been used for the purpose of extraction of proteins. Shen et al. (2008) employed method like alkali and enzymes for extraction of proteins from the same. Four enzymes were employed for the purpose:

i. neutrase
ii. alcalase
iii. protamex
iv. flavourzame

56.4% of yield was observed in alkaline method as compared to enzymatic extraction (less than 20% rate of extraction). The authors proposed and performed use of combination of two enzymes to enhance protein yields. Alcalase and protamex at 1:3 weight ratios increased the yield of proteins to 47.8%. They also investigated the influence of various extraction conditions on protein yields, mentioned below:

i. volume - weight ratio (V/W),

ii. time for extraction (t),
iii. pH,
iv. temperature (T),
v. agent concentration (c)

Orthogonal analysis techniques were employed and produce combinations of extraction conditions. Further purification was carried out for the extracted tea protein for the investigation of molecular weight distribution and amino acid describing. They observed the presence of 7 types of proteins and a soy protein similar amino acid composition in these tea proteins.

The general method of plant protein extraction is usually followed by precipitation. But it is always observed that the yields are not satisfactory and the proteins thus obtained are non-specific. Sussman et al. (2013) investigated various processing parameters and different raw materials in order to get higher protein yields. The proteins thus obtained had better properties in terms of textural properties which resembled fat like texture.

Salt-assisted extraction and precipitation was responsible for these. A d-optimal design was used for the purpose. The responses were analyzed while taking into consideration independent variables and dependent variables. The protein extracted from full-fat lupins shows highest yields i.e. 38% at pH was maintained at 5.0, 0.6 M concentration of NaCl and 1:7 as solid-to-solvent ratio of compared to other grain legumes. The authors also observed unique properties in terms of sensory attributes such as creamy, smooth and fat like characteristics.

Kiwi fruit (*Actinidia chinensis Planch*.) seed proteins have been studied by Deng et al. (2014). Kiwi fruit seeds are solid wastes which mostly are underutilized in the industry. They have used response surface methodology approach in order to study kiwi fruit seeds protein extraction. The yield was mostly influenced by pH and solid/liquid ratio. By applying processing condition that were temperature of 33.9^{0}C, throughout extraction pH 9.14 maintained for 49.2 min. with 1:8.4 (w/v) solid/ liquid ratio, 613.7 g/kg of protein with good digestibility was obtained (Deng et al., 2014).

Siow and Gan (2014) studied cumin seed protein isolate (CSPI) by employing BBD to optimize extraction parameters for protein. They also studied structural characteristics of these proteins. Along with that composition of amino acid, components of protein, structure of protein were also studied. The proteins thus extracted were found to have three potential components such as albumin (2S), globulin (7S), globulin (11S) and lectin. These CSPI composed with amino acid mixture such as Tyr, Glu, Asp, Arg, Leu and Phe. CSPI also have good antioxidant activity. They also observed different structure of proteins like intramolecular β -sheet, random coil, α-helix, β-turns and antiparallel β-sheet aggregates. Siow and Gan (2014) reported these isolates claim for ingredient of functional and strength-stimulating foods.

Lv et al. (2011) investigated grape seed by employing one factor as well as RSM. Solvent to meal ratio, temperature for extraction, pH and time for extraction play crucial role in yield protein. From the single factor test, the ideal length for extraction settings was attained. Based on the RSM analysis, 22.5/1 (v/w) of liquid to meal ratio, was temperature of 35^{0}C, throughout extraction pH 9.8 sustained for 29 min. (Lv, C; Jia, X; Li, M; Yang, J; Zhao, 2011).

Akasha, Campbell, Lonchamp & Euston (2016) extracted protein from date seed and claim the presence of storage proteins glycinin and β-conglycinin and also identified 91 over 300 proteins with confidence. They observed good emulsifying properties of date seed due to large protein oligomers.

4. Enzymatic hydrolysate optimization and bioactivity of protein hydrolysate from different plant and animal source

There are many reports available in scientific literature which show those protein hydrolysate possess antioxidant properties as well as many other properties which are mentioned below from literature.

A modified Osborne fractionation method was suggested by Dash & Ghosh to segregate

albumin, globulin, prolamin and glutelin sequentially from seeds of *Citrullus lanatus* (watermelon). These segregate factions were subjected to antimicrobial and antioxidant activities. Albumin and globulin were less effective against *A. baumannii* as compared to prolamin. The strongest antioxidant activity was exhibited by globulin (Dash & Ghosh, 2017).

Rafi, Halim, Amin, & Sarbon (2015) develop Lead Tree Seed Hydrosylate (LTSH) by using alacalase. For development of this hydrolysate they utilize RSM-CCD design in which set pH (7–9); temperature for hydrolysis (50^{o} C, 55^{o} C, 60^{o} C); time for hydrolysis (30 min, 60 min, 90 min); and enzyme/substrate for hydrolysis (1%, 2%, 3%) ratio as four independent variables. They experiential yield with antioxidant activity were studied. In this system, CCD provides 24 experimental points and 6 central points and provides optimized solution for hydrolysate that to maintain 9 pH, with 2% of an E/S ratio, 90 min of hydrolysis time with temperature 55°C. LTSH shows 92.79% ferrous ion chelating activity and robust reducing power was examined at A700 = 3.82 at the concentration of 20 mg/ml. It shows 76.21% of DPPH activity when IC50 is 1.99 mg/ml. LTSH also possesses hydroxyl RSA 66.72%; IC50 2.45 mg/ml. They supported LTSH as a natural antioxidant of functional food.

Zizyphus jujube falls under the category of medicinal plant and exhibited biological functions. Memarpoor-yazdi, Mahaki & Zare-zardini (2012) reported antioxidant peptides from *Zizyphus jujube.* They found that trypsin hydrolysate possess highest antioxidant activity and observed most potent antioxidant peptides, named fractions F3 and F6, identified as VGQHTR (MW: 678.36 ± 0.3 Da) and GWLK (MW: 482.27 ± 0.3 Da) respectively, using tandem mass spectrometry. They suggested the peptides derived from *Z. jujube* will helps for food preservation and medicinal purposes.

Kanbargi, Sonawane, Arya (2016) studied metal ion chelation, reducing power and antioxidant activity of protein hydrolysate which were extracted from Z. jujuba seeds. The protein concentration was found 5.72mg/g at the 50 mM of Tris-HCl buffer concentration by maintaining pH 7.5. The solubility of protein enhances at pH 2 and 3 that was 90 %. Various in vitro antioxidant assays were utilized to examine the antioxidant activity. Activity by ABTS was 8.09, 9.14 and 8.92 lM of TE/g respectively for alcalase, papain and protease hydrolysate. The DPPH scavenging activity 7.21, 17.54 and 8.36 were shown by alcalase, papain and protease hydrolysate respectively. The authors concluded that papain hydrolysate of *Ziziphus jujube* could be worthy foundation for antioxidant which shows progress in functional properties. Kanbargi, Sonawane, Arya, (2017) industrialized encapsulation peptides by using sodium alginate (2.5%), protein concentration (3%), calcium chloride (2%) used to recover bioavailability and organoleptic properties. Further, FTIR and SEM employed to understand cross-linking in calcium alginate beads. Antioxidant activity, metal ion chelation activity, and reducing power of encapsulated peptide were studied (Kanbargi, Sonawane, Arya, 2017).

Fan, Hu, Li, & Liu, (2014) used acid protease to hydrolyzed protein isolate which were extracted from *Cucurbita pepo L* (pumpkin) seeds to develop antioxidative peptides. They optimize hydrolysis by using RSM-BBD design. For this, they obtained second-order model for DPPH RSA. The experimental data shows that the model was fitted. They observed high coefficient value was 0.9918. This model provide optimize processing parameter for hydrolysis were 50°C of temperature 50°C, 6000 U/g enzyme amount was used and maintained at pH 2.5, 0.05 g/ml of substrate concentration for 5 h of hydrolyzing time. This hydrolysate shows 92.82% DPPH RSA.

Li, Jiang, Zhang, Mu, & Liu, collected the fraction from CPH. They used Sephadex G-25-gel filtration to collect CPH fractions from Fra.I to Fra.IV. All these fractions were subjected to reducing power, inhibition of linoleic acid autoxidation, DPPH/superoxide/hydroxyl radical-scavenging assay. Fra.IV shows 81.13% of antioxidant activity. This activity was very close to α-

tocopherol and lower to activity shown by 99.71% BHT in oxidation model of linoleic acid. 38.94% THAA was present in total hydrophobic amino acids and 125.62 kcal/mol amino acid residues in hydrophobicity were observed in hydrolysate which may one of the reasons for activity of hydrolysate. The MW distribution of Fra.IV ranged in between 200 to 3000 Da (Li, Jiang, Zhang, Mu, & Liu, 2008).

Eun, Morimae, Matsumura, Nakamura, & Sato, examined market samples of plant and milk protein which were hydrolyzed by using enzymes. Wheat gluten and soya protein hydrolysates were exploited in emulsion systems where possess strong DPPH activity. These hydrolysates also exhibit inhibition activity against model of oxidation of linoleic acid. Preparative isoelectric focusing was utilized to fractionated protein isolate. The fraction was collected on the based on amphoteric nature of peptides sample without adding ampholytes in isoelectric focusing. The acidic fractions of wheat gluten and soy protein hydrolysates possess stronger DPPH activity whereas basic fractions possess stronger ABTS activity. To understand fundamental of antioxidant activities of peptides in food would be done by acidic and basic peptide fractions (Eun, Morimae, Matsumura, Nakamura, & Sato, 2008).

Chen, Yang, Sun, Niu, & Liu, hydrolyzed walnut proteins by using pepsin for 3h. This shows highest DPPH, H_2O_2, chelate ferrous ion, exhibit RP and prevent oxidation of lipid. In hydrolyzed walnut proteins they identify Ala-Asp-Ala-Phe (423.23 Da) sequence. RP-HPLC-ESI-MS was used as analytical tool for sequenceing. Jahanbani et al., (2016) incubated Persian walnut (Chandler) seed proteins with pancreatic chymotrypsin and trypsin, and a microbial enzyme proteinase K. His study concludes that peptide fractions exhibit cell growth inhibition that was 63 % and 51% for breast cancer and colon cancer cells respectively. So this walnut protein hydrolysate exists as therapeutic peptides and effectively used as food additive (Chen, Yang, Sun, Niu, & Liu, 2012).

Pelegrini et al. studied peptides from *Passiflora edulis*, which were purified and then characterized. The authors isolated Pe-AFP1 peptide which have MW 5.0 kDa and falls under the category of novel plant peptide. Red-Sepharose Cl-6B affinity column and reversed-phase chromatography on Vydac C18-TP were utilized to purify these peptides. They observed inhibiting effect of Pe-AFP1. They found of 32, 34, and 40 μg ml− 1of IC_{50} against filamentous fungi *Trichoderma harzianum*, *Fusarium oxysporum* and *Aspergillus fumigatus.* They intimate the contribution of Pe-AFP1 in future could assist in development of biotechnological products (Pelegrini et al., 2006).

Later on Pelegrini et al. (2008) isolate Pg-AMP1 peptides from *Psidium guajava.* In which peptides purify by using affinity column (Red-Sepharose Cl-6B) and characterization carried out by using reversed-phase HPLC (Vydac C18-TP). *Klebsiella sp*. and *Proteus sp.* are accountable for urinary and gastro-intestinal infection. Pg-AMP1 helps in the treatment against these principle pathogens. There amino acid sequencing falls under family of glycine-rich protein group. Pg-AMP1 introduces activity towards Gram-negative bacteria. Enterotoxin from *Escherichia coli* and other antibacterial proteins shows 3D structural homology (Pelegrini et al., 2008). Patrícia Barbosa Pelegrini & Franco, confirms that guava seed peptide have glycine-rich protein group. They also found cardiac-depressant activity, also helps to reduce blood sugar level.

Lin & Ng studied antifungal peptides from kale seeds belong to family *Brassicaceae* impeded mycelial evolution in an amount of fungal species with *Fusarium oxysporum*, *Helminthosporium maydis*, *Mycosphaerella arachidicola* and *Valsa mali*. This shows presence of N-terminal sequence. This is varied from those of antifungal proteins which have been described to date (Lin & Ng, 2008)

5. Application

5.1. Antifreeze proteins

Antifreeze proteins have potentially used in medicine, agriculture and food, particularly in ice cream. They are also known as ice-

structuring proteins because they suppress or adapt development of ice crystals. Structurally diverse group of proteins are also known as antifreeze proteins. Major source for this are fish, insects, plants, lichen and bacteria. Their ability to dramatically slow down recrystallization has led to proposals that they could be used in ice cream to preserve the fine circulation of ice crystals formed in the factory through the distribution chain, and thereby deliver a superior product to the consumer. A paper describes the origins and properties of ISPs, and focuses on their potential use in ice cream (Clarke, Buckley, & Lindner, 2004).

5.2. Additive

Park, Imazu, Matsumura, Nakamura, & Sato study the effect of wheat gluten hydrolysate (WGH) and its autofocusing fractions on cooked pork patties. Preparative isoelectric focusing which is also called as autofocusing techniques based on amphoteric nature was used to fractionate sample peptides from WGH. The CPP was preserved at 4 and 20°C for storage stability. The samples were store in dark. Lipids oxidation in the patties was abolished with assistance of WGH and autofocusing fractions. Acidic and basic autofocusing fractions suppressed oxidations which were greater extent as compared to WGH. This fraction was subjected to study the various antioxidant assays such as DPPH and H_2O_2 RSA, bleaching of β-carotene, ORAC, and Fe ($^{2+}$) chelating assays. The study proved that micro amount of peptides play crucial role in inhibiting oxidation of lipid in complex food system such as patties (Park, Imazu, Matsumura, Nakamura, & Sato, 2012).

5.3. Nutritional fortification of low pH food

The fortification of protein in low pH is possible by modifying functional properties with the help of enzymatic hydrolysis of proteins which is recent trend observed. Bitter-tasting peptides are main problem associated with hydrolysis that may be due to high degree of hydrolysis. Isoelectric soluble soy protein hydrolyzates can be harvest by using isolate or concentrate of soya as base material in many pilot scale plant. Adler-Nissen (1978) used food grade alcalase by setting hydrolysis parameter such as 2 % E/S ratio, at pH throughout 8.0 by maintaining temperature 50º C which produce degree of hydrolysis 10 for 2 h. Further it was inactivated at pH 4.2 by adding citric or malic acids. Initial trials with rat illustrate an acceptable quality of the hydrolyzates (Adler-Nissen, 1978).

5.4. Cosmetic application

The proteins hydrolysate from *Vicia faba* seed shows antioxidant, antityrosinase and antibiofilm activities. It composed of seven peptides which isolated, identified and chemically synthesized. These peptides were further chemically synthesized to assess their antioxidant capacity, antityrosinase activity and antibiofilm ability against Pseudomonas *aeruginosa* PA14. Results showed that peptides P5, P6 and P7 identified as LSPGDVLVIPAGYPVAIK, VESEAGLTETWNPNHPELR and EEYDEEKEQGEEEIR respectively, displayed the highest DPPH radical scavenging activity (IC50 = 0.25–1.9 mM). Karkouch et al. (2017) suggest potentially used of bioactive peptides for cosmetic and pharmaceutical applications.

5.5. Nanoemulsion of protein

Patent N0.: US 6,716,450 B1 provides information in which bioactive proteins can be encapsulated by using nanocapsules. This made with branched or hyperbranched polymers and copolymers. This shows a core-shell arrangement. This core shell arrangement shaped a pocket volume suitable for complexing and holding enzymes and other bioactive molecules. They judge the stability at extreme temperatures and pH, soluble in aqueous or organic solvents. Its shelf life also extended by using lyophilized without loss of bioactivity (Patent N0.: US 6,716,450 B1).

Ji et al. (2015) prepared stable emulsion (O/W). Soy protein isolate and sodium caseinate were used to develop stable emulsion (O/W). This has outstanding storage stability and greater functional properties. This emulsion has polydispersity index and also possesses uniform droplets at nanoscale range. This was produced using high pressure homogenization. The authors employed DLS, NTA, TEM,

Rheometer and Turbiscan Lab analyzer to understand emulsion. Stability study was also investigated. pH, NaCl and SDS paramters were used to understand their effect on the droplet size, with SDS-PAGE application. Flexible protein SC and rigid globular protein SPI were adsorbed on the oil-water interface. Few molecules of protein liquefied in the aqueous phases which are free. The stability in the emulsion was observed due to repulsion of electro stat and possesses stabilization due to steric. The emulsion was excellent also proved by preservation ratio of vitamin A palmitate above 93%. This emulsion may contribute to improve nutritional quality by enhancing stability in storage. (Ji et al., 2015).

Emulsification and solvent evaporation techniques employed by Teo et al. to develop nanoemuslion. Whey protein isolates (WPI), lactoferrin and Tween 20 as emulsifiers were major composition of formulation. The concentration of protein varies from 0.25% to 1% (w/w) which shows decrease in particle size. Z-average diameter observed between 70 and 90 nm.Tthe concentration of 0.75% (w/w) protein with tween 20 found larger droplets in between 120–450 nm. They used following parameters to check the stability of nanoemuslion were as 30–90ºC temperature, pH was vary between 2 to 10 and 0–500 mM NaCl or 0–90 mM $CaCl_2$ of ionic strength. The nanoemulsion prepared with Tween 20 found unstable at 90 ºC for 15 min, whereas droplet aggregation at pH 4.5 and 5 were observed in WPI-stabilised nanoemulsions which also unsound at above 30 mM $CaCl_2$ concentration of salt. Author suggested emulsifiers could utilize to prepared stable emulsion (Teo et al., 2016).

5.6. Improved stabilization of nanoemulsions

To stabilize nanoemulsion in ice cream Yerramilli, Longmore, & Ghosh, (2017) uitilize pea protein isolate (PPI) with partial replacement of sodium caseinate (SC). They utilize1:1 mixture and individual SC and PPI with variation in concentrations of protein for formulation of nanoemulsions by using a high-pressure homogenizer. Presence of proteins at the oil droplet interface was confirmed by SDS-PAGE. The creaming observed in SC-stabilized nanoemulsions. Excess proteins may reduce flocculation may one of the reason of creaming. Aggregation and excessive droplet in PPI were futile to create stable flowable nanoemulsions. But mixture did not display any creaming or aggregation and remained stable for more than 6 months. The planned technique could be a unique system of developing plant proteins in the long-term stabilization of nanoemulsions in the food industry (Yerramilli, Longmore, & Ghosh, 2017).

6. CONCLUSION

However there has been many studies documenting a range of benefits of plant seeds, this study not only talks about the fascinating facts of fruit seeds and their bioaccessibility but also emphasizes onto the protein that can be extracted and further used in food sector as additive or for nutritional fortification and in non food sector such as cosmetics. The result of this study shows that the benefits will be stronger if the utilization is effective. Given the diversity, there is no doubt there will be more studies coming up bringing in light to other factors that need to be taken into consideration. Fruit seeds though neglected over ages can be proved as potential source of nutrients. Characteristics of fruit seed proteins make them a robust for various functionalities not only across food sector but in cosmetics industry as well. Many of such proteins also find therapeutic and biotechnological uses. Despite of all these benefits, these proteins are also attributed for their application in nanoemulsions and encapsulation technology.

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