Review

An overview of chia seed (*Salvia hispanica* L.) bioactive peptides' derivation and utilization as an emerging nutraceutical food

Roshina Rabail[1], Moazzam Rafiq Khan[1], Hafiza Mahreen Mehwish[2], Muhammad Shahid Riaz Rajoka[2,3], Jose Manuel Lorenzo[4,5,*], Marek Kieliszek[6,*], Abdur Rauf Khalid[7], Muhammad Asim Shabbir[1,*], Rana Muhammad Aadil[1,*]

[1]*National Institute of Food Science and Technology, University of Agriculture, 38000 Faisalabad, Pakistan,* [2]*School of Pharmaceutical Science, Health Science Center, Shenzhen University, 518060 Shenzhen, Guangdong, China,* [3]*Food and Feed Immunology Group, Laboratory of Animal Food Function, Graduate School of Agricultural Science, Tohoku University, 980-8572 Sendai, Japan,* [4]*Centro Tecnológico de la Carne de Galicia, Avda. Galicia Nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain,* [5]*Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain,* [6]*Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences—SGGW, Nowoursynowska 159 C, 02-776 Warsaw, Poland,* [7]*Department of Livestock and Poultry Production, Faculty of Veterinary Sciences, Bahauddin Zakariya University, 60800 Multan, Pakistan*

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1. Abstract

Chia (*S. hispanica* L.) is an annual herbaceous plant that has gained popularity for its seeds of high-quality vegetative proteins, richest contents of omega-3 polyunsaturated fatty acids (Ω-3 PUFA), soluble dietary fiber, and great gelling ability, as well as its high contents of bioactive peptides of antioxidative and nutraceutical potential for many other clinical biomarkers. Such health protective bioactive peptides should be utilized for supplementation in the food and nutrition industries. This review was therefore designed to align the researches done on chia bioactive peptide's derivation, processing, consumption and to identify their antioxidative and nutraceutical po-

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tential for various disease biomarkers. The evidence gathered is fairly compelling for the health-promising nutraceutical and clinical potential of chia seed bioactive peptides as antioxidants, dipeptidyl peptidase-IV inhibitors (DPP4), angiotensin-converting enzyme (ACE) inhibitors, and anti-inflammatory drugs. Their assimilation into everyday diets has the potential to open new doors in health departments and food sectors.

2. Introduction

The hunt for natural protein sources with bioactive ingredients for the creation of functional foods with a high nutritional impact and concurrent health benefits is rising [1]. But there is still a gap among experiments done in laboratories and the innovations at industrial levels, which needs to be filled [2]. Such utilization of bioactive constituents into the routine diet for therapeutic purposes is known as alternative medicine [3], which has been proven effective for preventing, slowing the progression, and overall complete regression of many diseases. This surge in demand for functional foods has directed human efforts toward the fortification of their bioactive constituent to deliver potential health benefits and disease prevention [3–5].

Dietary protein serves as a source of exogenous peptides that can perform comparable regulatory activities as endogenous peptides (hormones) do in our body to control our endocrine and neurological systems. These exogenous peptides are referred to as "bioactive peptides" because of their physiological relevance and nutritional significance. These peptides typically range in length from 2 to 20 amino acids (AA) and can be found in any encrypted section of a protein sequence [4, 6]. Numerous epidemiological and clinical research back up the idea that higher consumption of functional foods from plant origin can help to reduce the risk of many chronic ailments [5, 7]. Moreover, these vegetative food sources continue to be an essential primary source of protein in developing countries, therefore increasing the agricultural output of such beneficial crops to provide balanced and appropriate nutritional intake for the population is critical [1].

Among those functional foods, chia (*Salvia hispanica* L.), a herbaceous annual plant that belongs to the Lamiales order, Lamiaceae family, Nepetoideae subfamily, and Salvia genus [8], has attracted worldwide interest owing to its nutraceutical advantages. Several studies have shown that the chia seed might be a useful tool in the fight against many diseases [9, 10]. The word "chia" has been adapted from the Spanish word "Chien" or "Chian" meaning "oily" [11]. While all of its leaves, flowers, and seeds can be used and its beautifully marbled grey to dark brown seeds has been reported for their proven health protection against multiple metabolic disorders. With the progression of research, chia seeds have emerged as plant-based nutraceuticals and have gained the attention of nutritionists due to their balanced nutritional composition of proteins, fibre, omega-3 polyunsaturated fatty acids (Ω-3 PUFA), vitamins, minerals, and antioxidants. The higher amounts of α-Linolenic Acid (ALA) in chia seeds have been reported as cardio and hepatoprotective [9, 12, 13]. Its dietary fiber benefits the digestive system, whereas its anti-diabetic and anti-cancer potential have also been reported. Based on all these nutritional benefits, it could be considered as an emerging "superfood" [13–15].

All in all, chia seed has now been recognized by dietitians as a product with a long list of possible health advantages about various ailments. Multiple recent studies have shifted their focus from its rich Ω-3 PUFA and high dietary fibers towards the type and nature of proteins and digested peptides to identify their bioactivity potential for various maladies. There was a need to combine and analyze such scientific researches done on identification, derivation and isolation of chia seed bioactive peptides, along with their bioactivity mapping for various disease biomarkers. Therefore, this review has been designed to summarize and align the available literature on nutritional profile of chia seed, its various edible forms for daily utilization, derivation and isolation of its bioactive peptides and highlighting their bioactivity against various disease biomarkers.

3. The nutritional composition of chia seed

The nutritional composition of chia seeds is elaborated in Fig. 1. Previous literature revealed healthy fat contents ranging from 31 to 35% [14–18], with distinctive ALA, that is vegetative Ω-3 PUFA, content ranging from 59 to 60% [17, 19] along with the cardioprotective ratio of Ω-6 to Ω-3 as 1:3 [17]. The primary fatty acids were ranked in order of abundance: ALA > linoleic acid > oleic acid > palmitic acid > stearic acid. Chia oil's Ω-3 to Ω-6 PUFA ratio ranged from 3.18 to 4.18, which is the highest documented range for vegetable oils [18]. Chia seeds have been reported to be among the richest sources of high-quality vegetative proteins and dietary fibers. The protein content was reported as 18.9–23% [14, 19, 20] with the presence of many peptides of bioactive potentials as antioxidants, antidiabetic, anti-inflammatory, and hypotensive behaviors [21, 22], ash as 4.5% [20] fibre as 35.5% [14] holding insoluble/soluble dietary fiber ratio of 4.3 [20]. Chia seeds have also been reported to be rich in vitamin E by possessing 8.02 μg/100 g with high amounts of iron, potassium, calcium, phosphorus, vitamin A and various forms of antioxidants like flavonoids and cinnamic acid [11, 14]. Moreover, chia seeds are a rich source of polyphenols and antioxidants including caffeic acid, rosmarinic acid, myricetin, and quercetin, etc. [23]. Antioxidant analysis revealed 27 phenolic acids, flavonoids, proanthocyanidin-related phenolics, and procyanidin dimers (A, B1, B2, and B3) [10].

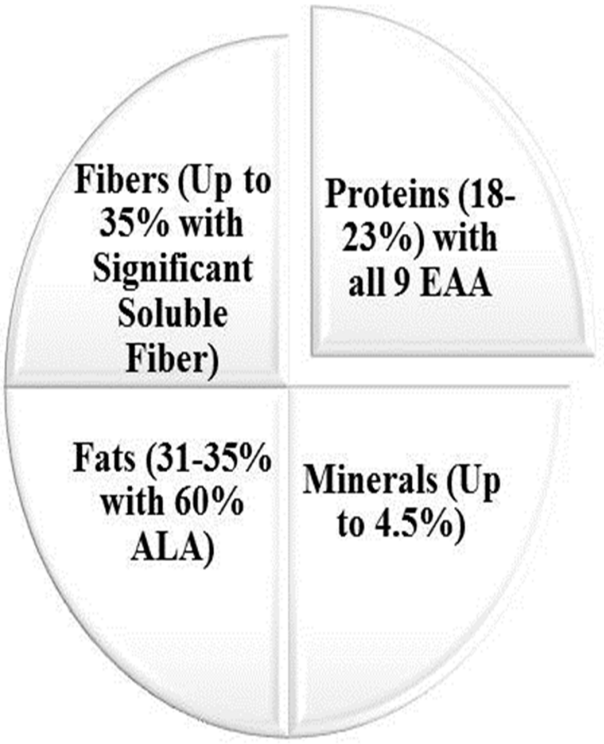

Fig. 1. Nutritional composition of chia seed.

An examination of the AA makeup in chia seed revealed a total of 10 AA, including all nine essential AA (leucine, isoleucine, lysine, phenylalanine, methionine, tryptophan, threonine, histidine, and valine), and this quality makes it a complete protein source for human nutrition [24], where arginine, phenylalanine, leucine, valine, and lysine are exogeneous, while glutamic, alanine, aspartic acids, serine, and glycine are endogenous AA [11]. As a result, these seeds have been hailed as the super new golden seed, and also known as the seed for the twenty-first century, and have been chosen for present use [25].

4. Dietary utilization of chia seed

Chia seed has been remained a seed of hidden potential for strength and energy in history and was consumed by Aztec soldiers and pre-Columbian populations in the 16th century during their battles and expeditions [8]. Highly nutritious gluten free bread was prepared by incorporating 5%–14% whole chia flour in the recipe [26]. Chia seed mucilage spreads by breaking the primary layer of cells and swells, resulting in the appearance of gel that has been widely used in drinks, salads, and ice creams. It can be consumed as whole seed topped on desserts, socked to make gel in beverages, in its flour form alone, or by its supplementation in other foods like yogurts, fruits, salads, and bakery, etc. [8]. It is available not just as seeds, but also as oil, which also has health-protective and anti-inflammatory properties due to rich Ω-3 PUFA [27]. Fig. 2 depicts the many modalities of chia use, where its leaves are generally consumed as a herb but its seeds are used in a variety of edible forms such as raw or roasted whole seeds, ground and supplemented, creating oil, protein, peptide, or gel-based dietary products.

The addition of chia in bakery products not only provides nutraceutical and nutritional benefits but also increases its textural properties of holding water contents and increasing sensorial appraisal due to its gelling ability and rich protein and fiber contents as compared to ordinary wheat flour [26, 28].

5. Bioactive peptides in chia seed and their derivation

All naturally existing proteins can be processed to yield peptides, but it is better to use the leftover policy at the industrial level because 10–50% of protein content is wasted in leftover by-products of agro-industry that can serve as bioactive peptides. We can utilize the solid residual by-products of the chia seed oil extrusion process that is known as chia expeller to produce value-added products in the food and medicinal industry. Therefore, utilization of this chia expeller has been gaining popularity to produce maximum bioactive peptides by using different techniques, as highlighted below. These peptides can serve many functional benefits in the food industry due to their antioxidative and nutraceutical potentials [1, 22, 29]. Therefore, bioactive peptides and their nutraceutical benefits have driven human interest towards the plant food proteins and their utilization as predictive medicine, hence modulating current food trends towards fortification of functional bioactive peptides [4, 6].

Bioactive peptides remain inactive when bound inside the parent protein sequence of plants, animals, or marine foods. They need to be activated by hydrolysis/digestion using enzymes, fermentation, chemical or gastrointestinal digestion The resultant peptides formed can act as regulatory factors of hormone-like activity as hypotensive, anti-cancer, hypocholesterolemic, and immunomodulatory agents that represent the potential nutraceutical properties of health-enhancing for food and drug [8]. Besides, the human body can produce these bioactive peptides from dietary proteins during enzymatic hydrolysis in the gastrointestinal tract, but in laboratory proteolysis, specific proteases are used to obtain bioactive peptides of a specific activity. These industrial food-grade proteinases can be derived either from microorganisms (proteinase K, collagenase, pronase, subtilisin A, Flavourzyme® and Alcalase®) or plant (Papain, ficin, bromelain) or animal sources (trypsin or pepsin, chymotrypsin) [6]. The most adapted methods used for protein digestion to yield peptides of interest are microbial fermentation and enzymatic hydrolysis that could be microwave or ultrasound-assisted followed by any suitable separation technique, i.e., ion

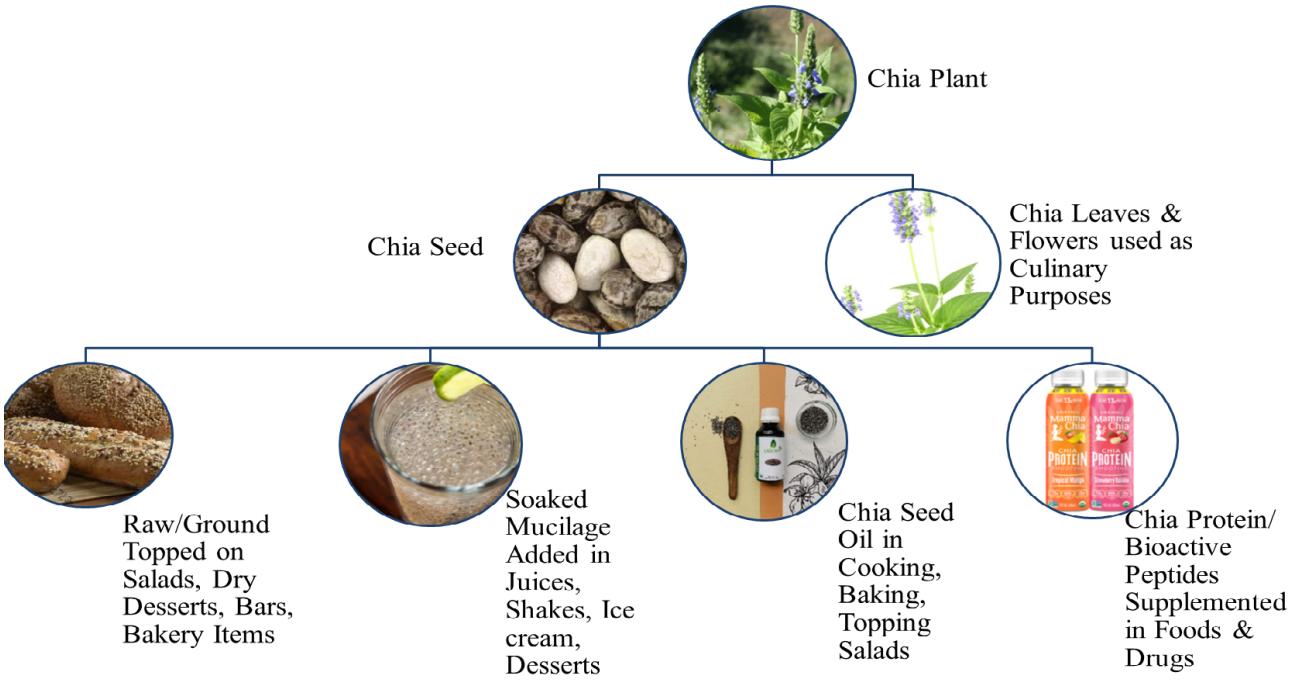

Fig. 2. Chia (*Salvia hispanica* L.) utilization in various dietary forms.

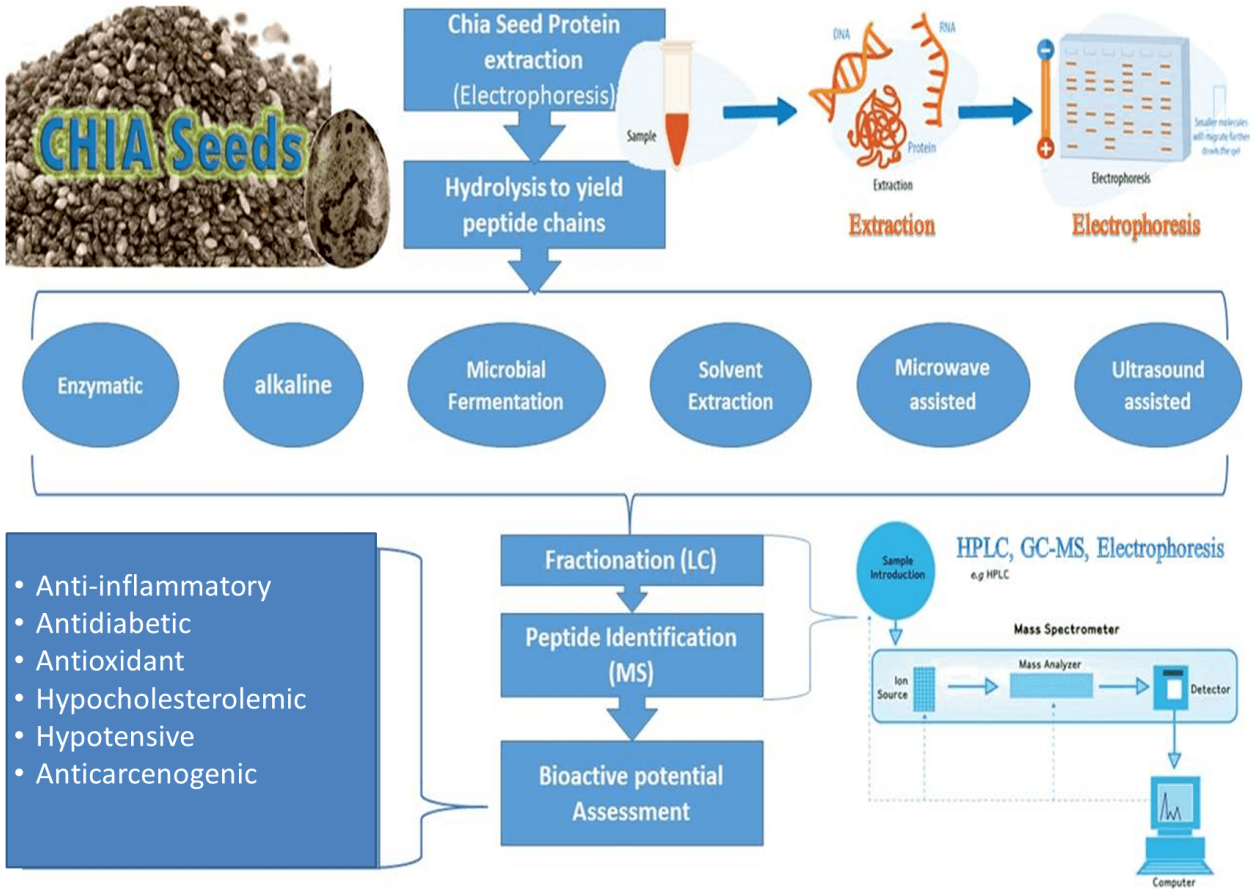

Fig. 3. Schematic diagram for chia seed bioactive peptides derivation.

Table 1. Chia seed bioactive peptides derivation and separation techniques.

Year	Method	Derivation	Separation	Results & Bioactivity	Reference
2020, 2013	Enzymatic hydrolysis + Enzymatic (Microbial Fermentaion)	Two different proteases (Papain and Alcalase®), extraction pH10-12; precipitation pH 3.5–4.0. Diethyl ethoxymethylenemalonate	-	Alcalase® and papain both increased the degree of hydrolysis, peptides content, protein solubility, antioxidant capability. But antioxidant activity was highest by Alcalase® at 60 mins of hydrolysis	[30, 31]
2020	Microwave assisted, Enzymatic (Microbial Fermentaion)	Sequential enzymatic (alcalase followed by flavourzyme)	size exclusion chromatography (SEC)	The <3 kDa peptides exhibited enzyme inhibitory activities towards elastase, tyrosinase, hyaluronidase, and collagenase	[21]
2019	Microwave assisted, Enzymatic (Microbial Fermentaion)	Ultrasonication to remove mucilage, alcalase + flavourzyme	SDS- PAGE	Peptides of molecular weight 25 kDa; highest antioxidant and ACE inhibition activity observed	[32]
2019	Enzymatic hydrolysis	Papain	SDS-PAGE and MALDI-TOF/MS	Peptides of low molecular weight <15 kDa; increased antioxidant activity	[1]
2019	Enzymatic hydrolysis	Pepsin and Pancreatin	ultrafiltration membranes with molecular weight cut-off	Peptide fractions (<1, 1–3, 3–5, 5–10, and >10 kDa); All of the peptides had anti-inflammatory activity, however the peptide fraction between 1 and 3 kDa had the greatest anti-inflammatory impact	[33]
2019	Enzymatic hydrolysis	Sequential enzyme digestion with pepsin + pancreatin	SDS-PAGE	Digested albumin, globulin, and glutelin showed increased antioxidant potentials	[34]
2019	Enzymatic (Microbial Fermentaion)	Alcalase + flavourzyme	UHP 76 (Advantec MFS) ultrafiltration unit	Peptide fractions (>10, 3–10, and <3 kDa); the peptides generated by flavourzyme were superior antioxidants, while <10 kDa showed best action against lipid oxidation	[29]
2018	Enzymatic (Microbial Fermentaion)	Alcalase + flavourzyme	ultrafiltration through a membrane with 3 kDa nominal molecular weight	Peptides with lower molecular weight than 3 kDa reduced 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase)	[35]
2018	Isoelectric pH gradient	Isoelectric precipitation method at different pHs (pH 2.0–6.0)	SDS-PAGE	Protein isolates ate pH 3.0 showed highest anti-inflammatory activity, while at pH 6.0 showed antioxidative	[36]

exchange chromatography (IEC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), size exclusion chromatography (SEC), affinity chromatography (AC) and polyacrylamide gel electrophoresis (PAGE) as explained in Fig. 3.

The methodology followed to derive bioactive peptide should be chosen very carefully as, the final molecular weights are highly affected by the method adopted i.e., enzymatic, alkaline, microwave-assisted, ultrasound-assisted, nature of extraction solvent, purification protocol, and drying techniques, etc. Moreover, the consequent molecular weights, amino acid sequences (AAS), their interrelationship, and their hydrophobic or hydrophilic nature of derived peptides are responsible for their functional potential and nutraceutical advantages [22]. Table 1 summerizes the latest techniques adapted for peptide derivation from chia protein.

5.1 Enzymatic hydrolysis

When protein is hydrolyzed enzymatically at a specific pH and temperature, it is known as enzymatic hydrolysis. Any crude or purified proteolytic enzyme can be used to hydrolyze proteins into hydrolysates holding short peptide sequences. The simultaneous or sequential addition of enzymes depends upon the temperature and optimal pH of the enzymes. After enzymatic hydrolysis, the supernatant can be separated from the mixture by centrifugation, cross-flow membrane filtration, freeze-drying, desalting, and column chromatography like gel filtration, which quickly desalt the peptides of low molecular weight [4]. During the enzymatic hydrolysis of bioactive peptides, many factors like enzyme specificity, enzyme to substrate ratio, hydrolysis time, and protein pretreatment before hydrolysis can affect the resulting peptide structural physicochemical properties. Enzymatic hydrolysis may be performed using specific or non-specific single or multiple proteases for the production of angiotensin-converting-enzyme (ACE) inhibitors and antioxidant peptides [37–39]. Papain-based hydrolysis was used to yield peptides of low molecular weight of <15 KDa and SDS-PAGE and MALDI-TOF/MS were used for the determination of the molecular weight of resulted peptides to get their potent health ben-

efits of radical scavenging in experiments [1]. Chia defatted flour, which has been utilized to produce low-cost animal feed, now has recently moved into the manufacture of chia protein hydrolysates (CPH) to produce peptides with antioxidant qualities and other health advantages. Flavourzyme and Alcalase based sequential hydrolysis of chia flour was done and resultant hydrolysates were ultrafiltered to yield three fractions F1 (>10 kDa), F2 (3–10 kDa), and F3 (3 kDa). The antioxidant capabilities of peptides corresponding to F3 fractions were the greatest, but those of F2 was equally good. Flavourzyme generated hydrolysates were excellent antioxidants, followed by Alcalase-prepared hydrolysates [29]. In another study, protein was isolated by alkaline extraction and acid precipitation method from defatted chia flour and was enzymatically hydrolyzed. Protein suspension was treated with pepsin at pH 2 for 45 min followed by pancreatin treatment at pH 7.5 for 45 min [33]. On total and fractioned proteins, gastrointestinal enzymatic hydrolysis was performed in a sequential enzyme digestion with pepsin at pH 2.0 for 2 h at 37 °C and pancreatin at pH 7.5 for 2 h at 37 °C. Digestion was halted by immersing the samples in a 75 °C water bath for 20 min. The digested total protein and protein fractions were fractioned using a 100–500 Da molecular weight cut-off membrane (Spectra/Por®, Biotech CE Membrane) [34]. Previous studies on different nature of proteins also resulted in similar high biological activity, e.g., pepsin alone or its combination with pancreatin, trypsin, and/or chymotrypsin for hydrolysis of plant proteins was reported to yield high ACE inhibitory potential peptides [37, 40]. Among the selection of suitable pH range for peptide derivations, pH 3.0 and 4.0 were found best for obtaining maximum chia protein concentrate using water or 1 M NaCl as the solvent respectively, whereas 1 M NaCl resulted in greater yield when compared to water [36]. The enzymatic method is overall preferred over others like microbial fermentation due to its short reaction time, ease of predictability, scalability, more success in releasing the peptides [41].

5.2 Microbial fermentation

Microbial fermentation is done by involving culture media of bacteria or yeast on protein substrates for protein hydrolysis. Microbial fermentation is preferred over enzymatic hydrolysis when the lowered cost of the protocol is concerned as microorganisms are a cheap supply for producing proteases [42]. The growing microorganism secretes its proteolytic enzymes, which act on protein to release peptides. Most often, the preferred bacterium is cultivated in a broth at a suitable temperature. Then suspension of microbial cells in sterile water is done that can be used as a starter for inoculation into sterilized protein substrate. The extent of analysis depends on the used strain, protein type, and fermentation time. The utilization of lactic acid bacteria such as lactobacilli is a successful technique for the development and commercialization of novel bioactive peptides. Proteolytic activity of lactobacilli varies according to strain and species, i.e., each species has a varied proteinase composition, resulting in a wide range of proteolytic activities [43]. This demonstrates *Lactobacillus* strains' strong potential for producing new hydrolysates and bioactive peptides of great interest. For example, utilization of *Lactobacillus brevis* for stronger ACE inhibition ability has been reported when compared with other species of *Lactobacillus* origin. Hence protein hydrolysates functionality may differ between cultures due to different proteolytic systems [4]. Similarly, microbial enzyme Alcalase® was used to enzymatically hydrolyze the chia protein-rich fractions for 60 min, followed by Flavourzyme® for up to 150 min. Hydrolysates generated were tested for ACE-inhibitory, antioxidant, and antibacterial properties. The hydrolysate generated at 150 and 90 min had the strongest ACE-inhibitory and antioxidant activities respectively. Antioxidative and antihypertensive peptides are better liberated using fermentation [41, 44]. Mucilage on chia seed hinders proper hydrolysis of chia seed proteins, therefore ultrasonic treatment was employed for its separation. Following that, defatted chia seed meal was microwave-assisted hydrolyzed utilizing sequential enzymatic (Alcalase® followed by Flavourzyme®) hydrolysis. The hydrolysate was then separated using ultrafiltration with a 3 kDa cutoff membrane. Chia seed hydrolysates exhibited superior antioxidative activity *in vitro*. DPP-IV inhibition was also greater [21, 32]. Microwave-assisted hydrolysis was used to yield chia seed-derived bioactive peptides after removal of chia seed mucilage using a combination of ultrasonic treatment and vacuum-assisted filtration. Hence, bioactive peptides derived by microwave and enzymatic hydrolysis from chia seed can be employed in therapeutic foods as antimicrobial agents [21].

5.3 Fractionation and bioactivity mapping of chia seed peptides

Following the synthesis of the peptide, they are allowed to undergo a separation technique that includes centrifugation and washing to remove reagent residues as well as side reaction products. Following that, the peptides are cleaved and filtered. The most often used procedures for peptide purification include HPLC, IEC, SEC, AC, and capillary electrophoresis [42]. Peptides when separated by denaturing sodium dodecyl sulfate SDS-PAGE after being released enzymatically, revealed globulin as the most abundant protein profile, followed by albumin [45]. Similarly, upon fractionation chia protein revealed globulins as a major fraction (52%), where globulin fraction comprises predominantly 11S and 7S proteins, according to sedimentation coefficient studies. All of the reduced fractions had molecular weights of 15–50 kDa [41]. In another previous study, electrophoresis revealed four bands (104–628 kDa) of proteins including albumins, globulins, prolamins, and glutelins with denaturation temperatures of

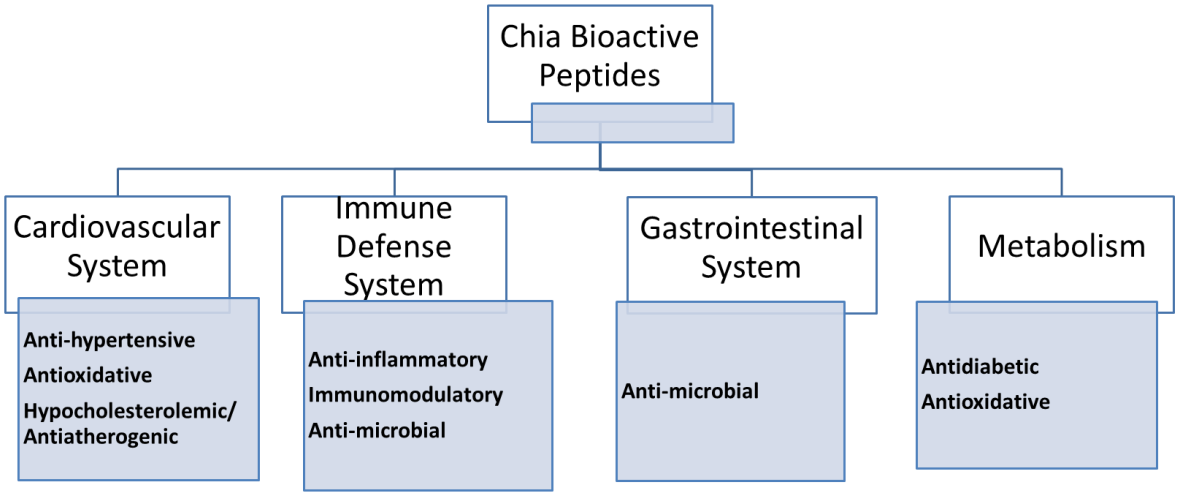

Fig. 4. Nutraceutical potentials of chia seed bioactive peptides.

103, 105, 85.6, and 91 °C, respectively. The seed flour protein had an excellent balance of essential AA, notably methionine + cysteine [24]. Moreover, centrifugation at 2500 rpm was implied to remove the insoluble contents, and then fractionation was done using ultrafiltration membranes to separate chia seed peptide fractions (>10, 5–10, 3–5, 1–3, <1 kDa) [33]. After yielding and fractionating bioactive peptides, their nutraceutical active status can be examined using different bioinformatics tools and databases, including PepBank, BIOPEP, and Antimicrobial Peptide Database [6]. Whereas AAS similarity searches can explain detailed examination and cross-evaluation of activity against various biomarkers by using PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool) or BLAST programs. Appendix Table 2 (Ref. [8]) reveals similar bioactivity mapping from AAS similarity, after tabulating chia proteins as previously derived [8] and identifying their bioactive peptide positioning for multiple disease biomarkers. The specificity of bioactivity of a peptide derived from any food for different diseases depends mainly on their structure and physicochemical properties, including hydrophobicity, molecular size, and the bulkiness of side-chain AA residues (AAR) [41].

Twelve proteins are found responsible for the metabolic functions like metabolism and cell division of seed, whereas the remaining eight are related to lipid production and storage [8]. The ability of 3 kDa chia seed peptides to suppress aging-related enzymes such as collagenase, hyaluronidase, tyrosinase, and elastase was tested. SEC was used to extract additional fractions, which were then evaluated for enzyme inhibitory activity. The 3 kDa peptides inhibited the enzymes elastase, tyrosinase, hyaluronidase, and collagenase. The second SEC fraction showed stronger enzyme inhibitory activity and included these 7 peptides (APHWYTN, DQNPRSF, GDAHWAY, GDAHWTY, GDAHWVY, GFEWITF, & KKLKRVYV) having 19–29 enzyme–peptide pair interactions towards these enzymes. These findings suggest that chia seed peptides may help to promote skin health by providing protection against aging-related enzymes and reducing the breakdown of the protein matrix on the skin [21].

6. Bioactivity of chia seed peptides

Chia seed consumption has been gained due to its nutritional composition being rich in Ω-3 PUFA, dietary fibre, and good quality proteins. The high concentration of protein and essential AA in chia seeds has also been proved a promising bioactive peptide source [8]. As elaborated in Fig. 4, chia seed protein has been well documented for its antioxidant, antihypertensive, and anti-inflammatory properties [21].

The bioactive potential of chia-derived peptides was reported active for ACE inhibitors, DPP-IV inhibitors, and antioxidant capacity. Hence reinforcing the health benefits of chia for its hypotensive, hypocholesterolemic, hypoglycemic and antioxidant potential [8]. Chia seed is a rich source of antioxidants like chlorogenic acid, myricetin, caffeic acid, quercetin, and kaempferol, which is believed to possess protective effects on cardiac, hepatic, aging, and carcinogenic characteristics. Its rich contents of dietary fibre are beneficial for controlling diabetes and assisting the digestive system, whereas its healthy Ω-3 PUFA, gluten-free protein, phenolic compounds, vitamin, and minerals also play a role in therapeutic effects of controlling diabetes, hypertension, dyslipidemia, inflammation, oxidative stress, blood clotting, laxative, anxiety, depression, immunity and vision improver [46].

6.1 Antioxidative potential

Chia seed peptides were found highly antioxidant using sequential and traditional hydrolysis with microwave treatment [32]. Chia seed consumption in either way has also been reported active for its radical scavenging property

and deactivating 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radicals [44, 47] and also have the potential for scavenging synthetic 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals [48]. *In vitro*, such peptides demonstrated a significant radical scavenging action against DPPH and ABTS [1]. Albumin, globulin, and glutelin from chia seed also showed nitric oxide, hydrogen peroxide, superoxide, and DPPH scavenging capability and inhibited 5-lipoxygenase (5-LOX), cyclooxygenase-1 and 2 (COX-1-2), and inducible nitric oxide synthase (iNOS) enzymes [34]. Globulin and albumin from chia seed protein were reported best for their high antiradical activity against ABTS and DPPH. The highest capacity to chelate the ferrous ion was demonstrated by peptides from prolamin and globulin fractions. The findings confirmed the antioxidative properties of chia seed, which should be included in the human diet regularly [45]. Chia derived peptides were found protective in the prevention and treatment of neurodegenerative disease. Three peptide fractions (1, 1–3, and 3–5 kDa) were produced from chia protein enzymatic hydrolysis and were analyzed for mediating human microglial clone 3 (HMC3) cells was assessed. The 1–3 kDa fractions from chia showed the most neuroprotective efficacy in HMC3 cells, which might be attributable to anti-inflammatory and antioxidant mechanisms of action, as indicated by the decrease of proinflammatory mediators (TNF-α, IL-6, NO, and H_2O_2) and ROS. The neuroprotective impact of F 1–3 kDa is a relevant study target for future research [49].

6.2 Antihypertensive potential

CPH produced in either way was found active for ACE inhibition activity [32]. Albumin and globulin from chia seed protein were reported with higher ACE activity [45]. Chia seed bioactive peptides consumption showed inhibition activity of ACE directly proportional to the duration of peptides hydrolysis process [44]. The inhibitory effect of ACE by chia seed protein fractions was reported to be strongest for albumin and globulin [25].

6.3 Anti-inflammatory potential

Several chia protein peptides interacted with inflammation and atherosclerosis indicators. For several peptides, this interaction proved more effective than the pharmacological controls [34]. Digested total proteins and isolated protein fractions from chia yielded albumin, globulin, prolamin, and glutelin peptides interacted with COX-2, p65- nuclear factor kappa B, LOX-1, and toll-like receptor 4. Anti-inflammatory potential was marked positive for H_2O_2 release, NO production, pro-and anti-inflammatory cytokines (IL-10, IL-1β, IL-6, and TNF-α,) production. All the chia derived peptides exerted an anti-inflammatory potential whereas peptide fraction between 1–3 kDa was marked with the highest anti-inflammatory effect as they lowered the levels of NO (65.1%), ROS (19.7%), prostaglandins (34.6%), TNF- (24.1%), MCP-1 (18.9%), IL-6 (39.6%), and IL-10. Hence, chia peptides suppressed the expression and release of inflammation-related indicators [8]. Chia proteins concentrate at pH 3.0 (100–1000 μg/mL) demonstrated an anti-inflammatory effect with values ranging from 56.32% in a dose-dependent manner [36]. Chia consumption was found immunostimulant when chia seed 150 g/kg diet was given. It improved the antibodies concentration (IgE) in rats [50, 51]. Five % of chia seeds consumption in high fat and carbohydrate diet of rats reduced hepatic and cardiac inflammation [12]. *In vitro* activation of peritoneal macrophages was studied by CPH [33].

6.4 Hypolipidaemic and hypoglycemic potential

Peptides from chia protein with molecular mass less than 3 kDa inhibited the velocity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) by up to 80.7 percent. It was suppressed by pravastatin by 81.5%. The bioactive peptides discovered in this study are structurally different from known statins, they constitute a unique class of HMG-CoA reductase inhibitors that can directly interact with this enzyme to block the mevalonate pathway and prevent hypercholesterolemia [35].

6.5 Antimicrobial potential

Chia seed bioactive peptides were derived with microwave-assisted hydrolysis using sequential enzymes and fractionated into $<$3 kDa (3–10) fractions. Their antimicrobial potential towards *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* was examined. Overall results were conclusive for remarkably increased membrane permeability of *E. coli* and *L. monocytogenes* resulting in wrinkling and pronounced deformations in bacterial cell membrane integrity along with the decrease in the growth rate of the bacteria [29].

7. Conclusions

This review reveals that most of the peptides identified from chia seeds up till now have been proved to exert their active potential against various disease markers like antioxidative, hypoglycemic, immune-modulatory, anti-inflammatory, hypocholesterolemic, and antihypertensive potential. When these bioactive peptides are consumed daily either by direct consumption or indirect in the form of supplemented products, nutraceutical benefits can be attained for various disease biomarkers as highlighted above. Although there is considerable work done on the production, processing, and utilization of bioactive peptides from various proteins there is still a need to go for comprehensive nutraceutical studies on selected proteins and their peptides to identify their bioactivity for various individual disease biomarkers. In the case of chia proteins and their bioactive peptides, there is a wide gap to be filled in research and

literature to draw the attention of nutrition professionals to discover the mechanisms behind their role and their modes of implementation in daily life.

8. Future recommendations

This paper summarizes the nutraceutical benefits of chia seed derived peptides, their bioactivity potentials along with the most adapted techniques used for their derivation. Still, there is a lot more gap to be filled by clinical investigations and critical evaluation of the chia seed derived peptides.

9. Author contributions

RR, MRK, HMM, MSRR, JML, MK, ARK, MAS, RMA—manuscript preparation, MK, JML, RMA—Supervision.

10. Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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12. Conflict of interest

The authors declare no conflict of interest.

13. References

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Appendix: See Table 2.

Keywords: Chia hydrolysates; Antioxidative; Anti-inflammatory; Antihypertensive; Chia bioactive peptides

Send correspondence to:

Jose Manuel Lorenzo, Centro Tecnológico de la Carne de Galicia, Avda. Galicia Nº 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain, Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain E-mail: jmlorenzo@ceteca.net

Muhammad Asim Shabbir, National Institute of Food Science and Technology, University of Agriculture, 38000 Faisalabad, Pakistan, E-mail: asim-shabbir@live.com

Marek Kieliszek, Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences—SGGW, Nowoursynowska 159 C, 02-776 Warsaw, Poland, E-mail: marek_kieliszek@sggw.edu.pl

Rana Muhammad Aadil, National Institute of Food Science and Technology, University of Agriculture, 38000 Faisalabad, Pakistan, E-mail: dilrana89@gmail.com

Table 2. Bioactivity mapping in amino acid sequences of chia peptides.

Sr. #	Protein fragment	AAR	Amino Acid Sequence (AAS) Representing Bioactive Potentials As: Antithromboti = Red Font; Hypotensive = Blue Font; Immunomodulating = Green Font; Antioxidant = Bold Letters; ACE inhibitor = Double Strike Letters; DPP-IV Inhibitor = Underlined Letters
1	Ribulose bisphosphate Carboxylase large Chain	473	MSPQTETKASVGFKAGVKEYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTV WTDGLTSLDRYKGRCYHIEPVPGEKDQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLR IPVAYVKTFQGPPHGIQAERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRGGLDFTKDDENVNS QPFMRWRDRFLFCAEAIYKSQAETGEIKGHYLNATAGTCEEMMKRAIFARELGVPIVMHDYLTGGFTAN TSLAHYCRDNGLLLHIHRAMHAVIDRQKNHGMHFRVLAKALRLSGGDHIHSGTVVGKLEGERDITLGFV DLLRDDFVEKERSRGIYFTQDWVSLPGVIPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAP GAVANRVAVEACVLARNEGRDLAAAEGNAIIRLACKWSPELAAACEVWKEIKFEFPAMD
2	Tubulin beta chain	450	MREILHIQGGQCGNQIGSKFWEVICDEHGVDPTGRYKGDGSESDTQLERINVYFNEASGGRYVPRAVLM DLEPGTMDSIRSGPYGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELIDSVLDVVRKEAENCDCLQGFQV CHSLGGGTGSGMGTLLISKIREEYPDRMMLTFSVFPSPKVSDTVVEPYNATLSVHQLVENADECMVLDN EALYDICFRTLKLSTPSFGDLNHLISATMSGVTCCLRFPGQLNSDLRKLAVNLIPFPRLHFFMVGFAPLTSR GQHYISLTVPELTQQMWDSKNMMCAADPRHGRYLTASAMFRGKMSTKEVDEQMLNVQNKNNVKSSV CDIPPTGLKMSSTFVGNSTSIQEMFRRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLVAEY QQYQD A
3	Elongation factor 1-ALPHA	449	MGKEKIHISIVVIGHVDSGKSTTTGHLIYKLGGIDKRVIERFEKEAAEMNKRSFKYAWVLDKLKAERERGI TIDIALWKFETTKYYCTVIDAPGHRDFIKNMITGTSQADCAVLIIDSTTGGFEAGISKDGQTREHALLAFTL GVKQMICCCNKMDATTPKYSKARYDEIKEVSSYLKKVGYNPEKIPFVPISGFEGDNMIERSTNLDWYKG PTLLEALDAVQEPKRPSDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVTFGPTGLTTEVKSVEMH HHEALQEALPGDNVGFNVKNVAVKDLKRGFVASNSKDDPAKEAANIITSQVIIMNHPGQIGNGYAPVLDCH TSHIAVKFSELMTKIDRRSGKELEKEPKFLKNGDAGMVKMIPTKPMVVETFSQYPPLGRFAVRDMRQTV AVGVIKSVEKKDPSGAKVTKAAAKKGAK
4	Fatty acid desaturase 7 isoform1	440	MASWVLSGCGLKPLPRIYPMPRTVSSPNPSKLRISTADFSSDSSSLCSVGRGRNWGLNVSAPLRFQEVGEE ENEERESEVVNGFGGGDGFDPGAPPPFKLADIRAAIPKHCWVKNPWKSMSYVVRDVAVVFGLAAAAAY LNNWAVWPLYWFAQGTMFWALFVLGHDCGHGSFSNDPKLNSVAGHLLHSSILVPYHGWRISHRTHHQ NHGHVENDESWHPLSEKIYKQLDFVTKKLRFTLPFPMLAYPIYLWYSRSPGKKGSHFHPDSDLFVPNERK DVITSTVCWTAMVAILAGLSFVMGPLQLLKLYGIPYFGFVAWLDLVTYUHHHGHEDKLPWYRGKEWS YLRGGLTTLDRDYGWINNIHHDIGTHVIHHHLFPQIPHYHLIEATEAAKPVLGKYYKEPQKSGPLPLYLLG VLAKSMKKDHYVSDTGDIVYYQTDPKLN
5	Eukaryotic translation initiation factor3 subunit E	438	MASKYDLTPRIAPNLDRHLVFPLLEFLQERGLYPEEDILKAKIELLNHTNMVDYAMDIHKSLYHSDDVPQ DMIDRRAEVVGRLKALEDGAAPLIGFLQNPNAVQELRADKQYNLQMLKDRYQIGPEQIDALYDYAKFQF ECGNYSGAADYLYQYRALCTNSDKSLSALWGKLAAEVLMQNWDIALEELNRLKEIIDSKNFSSPLNQVQ SRIWLMHWSLFIFFNHDNGRTQIIDLFNQDKYLNAIQTNAPHLLRYLATAFIVNKRRRPQFKEFIKVIQQEQ YSHEDPITEFLACIYVNYDFDGAQLKKMKECEEVILNDPFLGKRIEEGNFTTVPLRDEFLEPSYTNVYEQLID HTKALSTRTYKIVHQLLENAPGQTARCRIHQRIDMGVLADKLNLNYEEAERWIVNLIRTSKLEAKIDSKL GTIIME NARLFIFETY
6	Clathrin adaptor complex	438	MPLAASAIYFLNLRGDVLINRLYRDDVGGNMVDAFRVHIMQTKELGTCPVRQIGGCSFFYMRISNVYVIVI VVSSNANVACAFKFVVEAVTLFKSYFGGSFDEDAIRNNFVLIYELLDEIMDFGYPQNLSPELKLYITQEG VRSPFSSKTADKPVPNATLQVTGAVGWRREGLVYKKNEVFLDIVESVNLLMSSKGSVLRCDVTGKILMK CFLSGMPDLKLGLNDKIGLEKESQLKSRPAKSGKTIELDDVTFHQCVNLTRFNSEKTVSFVPPDGEFELM KYRITEGVNLPFRVLPTIKELGRTRMEVNVKVKSVFGAKMFALGVVIKIPVPKQTAKTSFQVTSGKAKYS PSIDCLVWKIRKFPGQTEPTLSAEVELISTITEKKSWTRPPIQMEFQVPMFTASGLRVRFLKVWEKSGYNT VEWVRYITK AGSYEVRC
7	Fatty acid desaturase 8	429	MASFVISGCGLKPLPRIYPKPRSVQNSFSTSNLRISRPNQFSSSSIGINQKRNWGLGVSAPLRIQPLEEFNEE FDPAAPPFKLSDIKAAIPKHCWVKDPWRSVGYVVRDVVAVLGMAAAAAAYFNSWIVWPLYWFAQSTM FWALFVLGHDCGHGSFSNNPKLNSVFGHFLHSSILVPYHGWRISHRTHHQNHGHVENDESWHPMPEKIY NSLDSMAKKLRFTLPFPMLAYPIYLWTRSPGKKGSHYHPDSDLFVPAERKDVITSTVCWTAMAALLVGL SFVMGPIQLLKLYGIPYLGFVAWLDTVTYUHHHGHEDKLPWYRGKEWSYLRGGLTTLDRDYGLINNIH HDIGTHVIHHHLFPQIPHYNLIEATEAAKGVLGKYYREPKKSGPLPLHLLGDLVRSLKKDHYVSDTGDVV YYQTDPQLNGGQKS
8	Fatty acid desaturase 3 isoform1	393	MAVSSGARLSESGAEGGEPYAGQCEHLEGIGKRAADKFDPAAPPFKIADIRAAIPPHCWVKDPLRSLSY VAWDLIAVAALLAAAAYFDSWIFWPIYWAAQGTMFWALFVLGHDCGHGSFSDSTTLNNVVGHILHSSIL VPYHGWRISHRTHHQNHGHVEKDESWVPLPENLYKQLDFSTKFLRYKIPFPMFAYPLYLWYRSPGKTGS HFNPDSSLFKPNERDLVITSTVCWAAMVAFLLYASTIVGPTMLFKLYGVPYLIFVVWLDTVTYUHHHGY DKKLPWYRSKEWSYLRGGLTTVDQDYGIFNKIHHDIGTHVIHHLFPQIPHYHLVEATREAKRVLGNYYR EPRKSGPVPFHLIPTLLKSLSRDHYVSDNGDIVYYQTDSQLFSS KEI
9	Fatty acid desaturase 3 isoform2	386	MAVSSGADAEHHGHAQYEHLGKRAADKFDPAAPPFKIADIRAAIPPHCWVKDPLRSLSYVAWDVFVV AALLAAAAFFDSWIFWPIYWAAQGTMFWALFVLGHDCGHGSFSDNTTLNNVVGHVLHSSILVPYHGWR ISHRTHHQNHGHVENDESWVPLTENLYKQLDFSTKFLRYKIPFPMFAYPLYLWYRSPGKSGSHFNPYSSL FKPNERDLVITSTICWAAMVACLLYASTIVGPTMLFKLYGVPYLIFVVWLDTVTYUHHHGYDKKLPWY RSKEWSYLRGGLTTVDQDYGIFNKIHHDIGTHVVHHLFPQIPHYHLVEATREAKRVLGNYYREPRKSGA VPFHLVPTLLKSLSRDHYVSDNGDIVYYQTDGELFSSKEI

Table 2. Continued.

10	Fatty acid desaturase 2 isoform2	383	MGAGGRMSVPPAEEKAAKSDIVQRVPHTKPPFTLGDIKKAIPPHCFKRSIPRSFSYVVYDLVFASLFYYVATNYIHQLPHPLSYPAWILYGICQGCILTGVWVIAHECGHHAFSDYQWLDDTVGLILHSFLLVPFFSWKYSHRRHHSNTGSLERDEVFVPKVKTGVSWAAKYMNNPPGRLITLVVQLTLGWPLYLMFNVSGRPYDRFACHFDPNSPIYSDRERAQIFISDAGILAVTYGLYRLSVAKGLAWVLCVYGGPLLVVNGFLVLITFLQHTHPSLPHYDSSEWDWLRGALATVDRDYGILNTFHHNITDTHVAHHLFSTMPHYHAMEATKVIKPILGKYYQLDETPVFKAMFREVK ECIYVEPDEG EENKGVFWYN NKL
11	Fatty acid desaturase 2 isoform1	383	MGAGGRMSVPPADKKAKSDVIQRVPHAKPPFTLGEIKKAIPPHCFKRSIPRSFSYVVYDLIIASLFYYVATNYIHQLPQPLSYLAWTLYGICQGCILTGVWVLAHECGHHAFSDYQWLDDTVGLILHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKVKSGVSWTAKYMNNPPGRVITLIVQLTLGWPLYLMFNVSGRPYDRFACHFDPKSPIYSDRERAQIFISDAGILAVLYGLYRLMSVAKGLAWVLCVYGGPLLVVNGFLVLITFLQHTHPALPHYDSSEWDWLRGALATVDRDYGILNTFHHNITDTHVAHHLFSTMPHYHAMEATKAIKPILGKYYQLDETPVFKAMFREVKECIYVEPDEGEENKGVFWYN NKL
12	Actin	377	MADAEDIQPLVCDNGTGMVKAGFAGDDAPRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPIEHGIVSNWDDMEKIWHHTFYNELRVAPEEHPILLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDSLMKILTERGYMFTTTAERFIVRDIKEKLAYIALDYEQELETAKTSSAVEKNYELPDGQVITIGAERFRCPEVLFQPSMIGMEAAGIHETTYNSIMKCDVDIRKDLYGNIVLSGGSTMFPGIADRMSKEITALAPSSMKIKVVAPPERKYSVWIGGSILASLSTFQQMWIAKAEYDESGPS IVHRKCF
13	S-adenosyl methionine decarboxylase proenzyme	360	MDMPVSAIGFEGYEKRLFISFVEPGVFADPDGYGLRALTKAQLDEILDPAQCTIVASLKNDDVDSYVLSESSLFVYSYKILKTCGTTKLLSIPPILRLADGLGLTVSSVRYSRGSFIFPGAQPFPHRSFNEEVAVLDDHFSKLGLMSEAYVVMGDADEHEKWHVYSAYLEPSSDVEPVVYTLEMCMTNLDQKKASVFFKNQSSSATIMTDASGIRNILPESFICDFDFDPCGYSMNSIEGGAVSTIHVTPEDGFSYASFETGGYDFEKVDLTQLVERVLACFNPAKFSVAVRASIAGKELDSAFKLDIAKYGCAGRRCEVLGDGGSVIYCNFTSATGCGSPRSTLHLCWSESEDEFIEKK
14	Glyceraldehyde-3-phosphate dehydrogenase	337	MAKIKIGINGFGRIGRLVARVALQRDDVELVAVNDPFITVDYMTYMFKYDSVHGQWKHHELKVKDEKTLLFGEKPVTVFGERNPEEIPWASTGAEYIVESTGVFTDKDKAAAHLKGGAKKVVISAPSKDAPMFVVGVNEKSYTPDLDIVSNASCTTNCLAPLAKVINDRFGIVEGLMTTVHSITATQKTVDGPSAKDWRGGRAASFNIIPSSTGAAKAVGKVLPALNGKLTGMAFRVPTVDVSVVDLTVRLEKEATYDEIKAALKEESEGNLKGILGYTEDDVVSTDFVGDNRSSIFDAKAGIALSKNFVKLVSWYDNEW GYSTRVVDLI KHIHSTQ
15	Mono-acylglycerol acyltransferase	320	MSPENPSNFWGDTPEEIKKASQGVRNSKSYFDSPHGRLFTQSFLPLDPTRPVKASVFMTHGYGSDSSWMFQKFCISYAAWGYAVFAADMLGHGRSDGIRCYMGDLPKVAAASLAFFRSVRVSDEYKDLPAFLVGESMGGLATLLMYFQSEKDLWTGLIFSAPLFVIPESMMPSKVHLFAYGMLFGLADTWAAMPDNKMVGKAIKDPEKLKVIASNPMRYTGKPRVGTMRELLRQTEYAQNNFDKVTIPFFTAHGTSDGLAEWSGSQMLYDKASSEDKTLKLYEGMYHSLIQGEPDENANLVLADMRAWIDERVERYGKKN
16	Serine/threonine protein phosphatase	306	MPGHGDLDRQIFQLMECKPLSEAEVKILCDQARAILVEEWNVQPVKCPVTVCGDIHGQFYDLIELFRIGGNAPDTNYLFMGDYVDRGYYSVETVTLLVALKVRYRDRITILRGNHESRQITQVYGFYDECLRKYGNANVWKFFTDLFDYLPLTALIESQVFCLHGGLSPSLDTLDNIRALDRMQEVPHEGPMCDLLWSDPDDRCGWGISPRGAGYTFGQDIAAQFNHTNGLTLISRAHQLVMEGFNWCQEKNVVTVFSAPNYCYRCGNMAAILEIGEHMLEQNFLQFDPAP RQIEPDTTRK TPDYFL
17	FtsH protease	289	FDRNIVVPNPDVEGRRQILESHMSKVLKGEDVDLEIIARGTPGFSGAELANLVNVAAIKAAMDGAKAVSMADLEHAKDKIVMGSERKSAVISDESRRNTAYHEGGHALVAMFTDGALPVHKATIVPRGNALGMVSQLPDKDQTSVSRKQMLARLDVCMGGRVAEELIFGESEVTSGASSDLESATRMARSMVTRYGMSKQLGFVSHDYNDNGRSMSTETRLLIEQEVKDLLEKAYNNAKTILTTHSKELHALANELLDKETLTGAQVKALLENVKAQNTQ QQKQQQIVT
18	Peptidyl-prolyl cis-trans isomerase	229	MSGNHMISIVIAMVCIGVFRGSITAIATVPELGSARVVFQTNYGDIEFGFYHSVAPKTVEHIFKLVRLGGYNTNHFFRVDKGFVAQVADVGGGRTAPMNEVQRLFAEKTVVGEFSDVKHVRGILSMGRYSDPDSAQSSFSILLGDAPHLDGQYAIFGKVTKGDETLSKMEEVPTRKEGIFVMPTERITIESTYYYDTETESCEDDRLELKRRILASAVEIEKQRMKCFP
19	Oleosin	142	MADQHYGQFQSRPHHLQQHHPRSHQMVKAATAVTAGGSLLVLSGLTLAATVIALTIATPLLVIFSPVLVPAALAVFALAGGFLASGGFGVAALSVLSWIYKYMTGKHPVGADQLDTARTKLAGKARDMKDRVDHNVSVAQSS
20	Alpha-tubulin	74	MISNNTAVAEVFSRIDHKFDLMYSKRAFVHWYVGEGMEEGEFSEARED LAALEKDYEEVGAEGVDDED DE GEDY

AAN, Amino acid nomenclature; H, his; histidine; C, cys; cysteine; I, ile; isoleucine; S, ser; serine; M, met; methionine; V, val; valine; G, gly; glycine; A, ala; alanine; L, leu; leucine; T, thr; threonine; P, pro; proline; F, phe; phenylalanine; Y, tyr; tyrosine; R, arg; arginine; W, trp; tryptophan; N, asn; asparagine; D, asp; aspartic acid; B, asx; either of D or N; Q, gin; glutamine; E, glu; glutamic acid; Z, glx; either of E or Q; K, lys; lysine; X, undetermined amino acid. AAN & AAS adapted and reproduced from Grancieri *et al*., (2019) [8]. Table reveals similar bioactivity mapping from AAS similarity, after tabulating previously derived chia peptides, and identifying their bioactive potential for multiple disease biomarkers in following way (Antithrombotic potential indicated as Red Font; Hypotensive potential indicated as Blue Font; Immunomodulating potential indicated as Green Font; Antioxidant potential indicated as Bold Letters; ACE inhibitor potential indicated as Double Striked Letters; DPP-IV Inhibitor potential indicated as Underlined Letters).