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Review

Current Strategies to Modify the Functional Properties of Proteins Extracted from Pumpkin Seeds: A Comprehensive Review

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Abstract: The functional properties of pumpkin seed proteins remain unutilized in numerous food and industrial applications. Several current approaches aim to improve the functional properties of pumpkin seed proteins, allowing their innovative potential to develop and modify significantly. Several strategies can be implemented to alter the functional properties of proteins isolated from pumpkin seeds. The first is enzymatic hydrolysis, regardless of whether, proteases may free peptide binding and profoundly impact the protein structure and functionality. Thermal treatment can include heating and cooling to replace protein conformation and increase solubility, emulsification, and gelation properties. Chemical modification techniques, including acylation and glycation, can also be used to improve stability, viscosity, and foaming ability. Functional properties and, where possible, ingredients with many applications may include exceptional possibilities for proteins modified in food preparations, such as dairy replacements, plant-based meat analogues, and free gluten that have an outstanding aspect, satisfactory quality, and nutritional profiles. As multiple different proteins act as precursors of active peptides, they can also be used to generate bio-specific foods. This review briefly provides information about various types of protein extraction techniques and functional properties that are modified by different types of processing technologies.

Keywords: pumpkin seeds; protein modification; extraction; alternative protein

Citation: Pandey, V.K.; Singh, K.; Suthar, T.; Srivastava, S.; Rustagi, S.; Ungai, D.; Kovács, B.; Shaikh, A.M. Current Strategies to Modify the Functional Properties of Proteins Extracted from Pumpkin Seeds: A Comprehensive Review. *Horticulturae* **2024**, *10*, 1194. https://doi.org/10.3390/horticulturae10111194

Academic Editor: Rosario Paolo Mauro

Received: 19 September 2024
Revised: 4 November 2024
Accepted: 7 November 2024
Published: 13 November 2024

1. Introduction

Seeds are basic to nutrition and represent a major source of both macronutrients and micronutrients needed for human and animal health. Proteins stand out as some of their main macronutrients and seeds such as pumpkin, chia, and flax contain a complete array of amino acids needed for muscle, immune function, and cellular repair [1]. Seeds provide healthy fats as well, specifically, unsaturated fatty acids (such as omega-3 and omega-6 found in flax and chia seeds). Fatty acids have been linked with fewer instances of cardiovascular diseases, better brain performance, and reduced inflammation [2]. Seeds with healthy vitamins, minerals, and dietary fiber add even more micronutrients. Research shows that pumpkin seeds are rich in magnesium and zinc (two essential immune-supporting minerals to support bone and metabolic health). Sunflower seeds also provide considerable vitamin

E, an antioxidant that may protect cells against oxidative stress [3]. Seeds are also high in fiber, which is good for digestion and has also been related to a lower risk of long-term ailments such type 2 diabetes and heart disease.

Pumpkin seeds, also known as pepitas in North America, are derived from the pumpkin (Cucurbitaceae family) and are gaining attention due to their high nutraceutical and therapeutic value [4]. These seeds are rich in protein, vitamin E, provitamins, carotenoids, saponins, fiber, and essential minerals such as calcium, magnesium, iron, sodium, and copper. Phytochemicals in pumpkin seeds, such as polyphenols, phytoestrogens, and fatty acids provide health benefits, including cardiovascular support and the treatment of sex hormone imbalances in menopausal women [5]. Additionally, pumpkin seed oil has demonstrated antibacterial and antioxidant properties, while phytoestrogens like secoisolariciresinol and lariciresinol exhibit protective effects against hyperlipidemia and osteoporosis [6]. These phytoestrogens also play a role in reducing hormone-dependent tumors. Pumpkin seeds contain high levels of vitamin E, specifically α- and γ-tocopherol, which have anti-aging, antioxidant, and free-radical-scavenging properties [7]. The emerging role of plants as an alternative source for producing therapeutic proteins through post-translational maturation has garnered attention, given their potential to replace microbial and animal cell manufacturing systems [8].

The growing conditions of pumpkins, especially of species *Cucurbita pepo* and *Cucurbita maxima*, are very limited and are influenced by several agronomic factors that influence growth, yield, and nutritional quality. One of the most important factors is climate as pumpkins are warm-season crops, which do best when the temperature is between 20 and 30 °C; the plants perform best in bright sunlight and grow better in relatively humid climates [9,10] (Saud et al., 2019; Hosen et al., 2021). Pumpkins are a very yield impacted crop based on soil environment. The most suitable soil type for them is a loam or sandy loam soil that is both well-drained and rich in organic material and has a mildly acidic to neutral pH (6.0–7.5). The introduction of organic matter is advantageous, as it increases the soil's fertility and holding capacity of water, aiding both root growth and plant health [11]. High soil wetness can cause root diseases and so fields must be prepared to drain every necessary product in space. Fertilization, especially using nitrogen and potassium, contributes to healthy vegetative and reproductive development of the vine, although care should be taken to apply the fertilizer based on a nutrient balance to promote excess vine growth and poor fruit yield [12].

An investigation revealing the chemical properties of pumpkin seeds identified moisture, carbohydrate, fiber, total ash, and oil content as key components [13]. Despite their nutritional profile, improper water solubility, complexity, reactions to pH, salinity, and temperature limit their functionality. Indigestible polysaccharides like hemicellulose and lignin embedded within pumpkin proteins further reduce bioavailability [14]. Pumpkin seeds are recognized as a functional food due to their pharmacological and biofunctional properties [15]. Recent studies have explored their use in food formulations, such as biscuits, where pumpkin seeds were shown to enhance protein content when added to casein-based diets [16]. However, gluten-free protein formulations face challenges due to texture issues, largely due to the absence of gluten proteins. Studies demonstrate that plant protein enrichment, particularly with pumpkin seeds, can improve the texture and protein content of gluten-free bread [17]. This has led to the production of baked goods with improved textural properties and water-holding capacities [18].

Pumpkin seed proteins have potential antioxidant properties, scavenging free radicals and reducing oxidative stress. They can donate protons, inhibit lipid peroxidation, and chelate metal ions, counteracting oxidative damage linked to chronic diseases like cancer and diabetes. Pumpkin seed proteins also have anti-inflammatory properties, modulating immune responses and preventing chronic inflammation [19]. In cellular models, pumpkin seed protein hydrolysates inhibit pro-inflammatory mediators, potentially lowering inflammation-induced diseases like arthritis and metabolic syndrome. These proteins also protect the heart area by reducing blood pressure and enhancing the blood lipid profile.

They have ACE-inhibitory and antihypertensive effects, reducing the risk factor for cardiovascular disease. Additionally, pumpkin seed proteins help preserve cardiac health by reducing bad cholesterol levels and raising good cholesterol levels, establishing a healthier lipid balance [20].

Villamil et al. [21] studied the incorporation of pumpkin seed powder into pasta, meatballs, bakery products, and dairy beverages, and found that the manufacture of these novel snacks has had a positive effect on their physicochemical properties. The powder was produced using pumpkin skin, with the pulp and all the seeds that go to waste. The shell has a good level of bioactive compounds like tocopherols, carotenoids, and antioxidant activity, in addition to total phenolic content. It increased the fiber content, antioxidant activity, and total phenolics when incorporated into these products. The seeds mainly have lipids. It was observed that, when included in products, an increase of protein and PUFAs occurred, along with a decrease in moisture, SFAs, viscosity, and pH. This is a good case of upcycling to improve the nutritional profile in food formulation and environmental sustainability, as waste reduction by-products from pumpkin have potential uses. A study of the textural and sensory properties of these food products are still currently underway.

Furthermore, supplementing bread with plant proteins like pumpkin seed proteins reduces glycemic index, making it a healthier option. The manufacturing process of pumpkin seed oil results in several by-products, including seed cake, which has potential as a nutraceutical source but remains underutilized [22]. Approximately 11,500 tons of pumpkin seeds produce oil, with the remaining pumpkin seed cake often used for livestock feed or composting [23]. Studies on pumpkin seed protein isolate (PSPI) show that various treatments, such as ultrasonic and alkaline processing, enhance gel formation, hydrophobicity, solubility, and protein quality, surpassing the performance of traditional soy protein isolates [24]. Well drained sandy to loamy soils with good fertility and organic matter are ideal for growing pumpkin. Field peas do best in soil with a pH from 6.8 to 7.0. Good yields are dependent on proper soil preparation, such as ploughing and organic compost or manure incorporation. Pumpkins can be easily spread in a long, linear row, or allowed to sprawl around their intended area. Since pumpkins are very sensitive to cold, it is a requirement that seeds be sown once the danger of frost has passed. Seeds are planted in the ground and plants must be well-watered throughout different periods of vegetation. These are usually grown in hills or raised mounds and spaced 4–6 feet apart, with the technique dependent on the variety of pumpkin. Fruit requires pollination, and nature does it best with bees.

Pumpkins are warm-season plants and prefer a temperature range from 18 °C to 30 °C (65–85 °F) for growth. They still run the risk of damage as they are very susceptible to frost, with anything below 10 °C (50 °F) risking harm. Pumpkins need warm days and a long season (usually 75 to 120 frost-free days depending on the variety) to grow. They are well suited for areas with moderate to regular rainfall, although too much moisture can cause root rot as well as various fungal diseases. Pumpkins require an even supply of moisture, especially when fruiting, but it is also vital to have well drained soil so that the plants do not become waterlogged. Irrigation (when needed) maintains soil moisture at required levels, which is often the case in dry areas. The USA, India, China, Mexico, and Canada are leaders in efficient pumpkin production due to their favourable climatic conditions. Pumpkins are grown across much of North America and China, with the largest production area found in Illinois (USA), which supplies around 85% of all pumpkins used throughout the country each season [25]. Recently, researchers found that pumpkin contains the most abundant phenolic acid (p-hydroxybenzoic acid) [26]. Pumpkin seed oil, which contains medium-chain fatty acids (12–18 carbons), has been shown to have antimicrobial and cytotoxic properties [27]. Adequate consumption of pumpkin seeds provides healthy fats, zinc, and magnesium, offering significant nutritional benefits [28]. The physical properties of pumpkin seeds, including length (16.91 mm), width (8.67 mm), thickness (3 mm), and mass (0.203 g), have been documented [29]. Modifications such as pH shifting and heat treatment are commonly used to improve the quality and functionality

of pumpkin seed proteins [30]. Optimal temperatures, around 160 °C, are necessary to produce proteins with better nutritional value, as confirmed by electrophoresis and particle size analysis [31]. Therefore, pumpkin seeds hold vast potential as a source of bioactive compounds with functional properties that can be harnessed for food and nutraceutical applications [32]. However, the full utilization of these seeds, especially their by-products, remains a challenge in the food industry. Comparative information about the type of protein found in various fruits and their application has been mentioned in Table 1.

Table 1. Functional properties of proteins found in various fruit seed and their applications.

Source/Seed Type	Protein Type	Protein Properties	Applications in Food Sector	References
Pumpkin Seeds	Albumin	High solubility	Emulsifiers, foaming agents, binders	[33]
Sunflower Seeds	Globulin	Good emulsifying capacity	Salad dressings, baked goods, protein bars	[34]
Chia Seeds	Albumin, Globulin	High in essential amino acids	Nutritional supplements, baking ingredients	[35]
Hemp Seeds	Globulin, Albumin	Balanced amino acid profile	Protein powders, vegan products, Snacks	[36]
Sesame Seeds	Globulin, Albumin	Rich in antioxidants	Tahini, halva, baked goods, salad toppings	[37]
Flax Seeds	Globulin, Albumin	Source of omega-3 fatty acids	Omega-3 supplements, baking ingredients	[38]

2. Protein Extraction Methods

2.1. Solvent Extraction

There are different extraction methods based on solvents, such as water, alkali, acids, and organic acids. A combination of chemical methods with other methods has been used to improve protein recovery [39]. Standardization of different chemical methods was performed for the isolation of proteins based on maximum recovery with minimum loss. The nature of the sample protein and protein isolation efficiency is interdependent [40]. The solvent extraction of the protein was performed in three steps: sample defatting, extraction, and protein precipitation. These steps are important for increasing protein yield and contaminant reduction. First, for defatting, solvents were used to remove compounds that interfere with protein extraction. Different salts, ionic detergents, non-ionic detergents, aqueous extractions, alcohols, organic solvents, and modern techniques have been used for protein extraction. Finally, precipitation of isolated protein is done using chemicals or solvent after which the precipitate is recovered via centrifugation [41]. The yields from solvent extraction can vary, but typically range from 20% to 30% of the seed mass as protein isolate.

2.1.1. Organic Solvent, Aqueous, Acid Solvent-Based Protein Extraction

Organic solvents result in pure protein products as they play an important role in extraction as well as precipitation. Because of the high solubility of proteins and isolated protein stability, the most used method is aqueous extraction [39], using cold or hot water (equal to or greater than 100 °C) at high pressure and then cooled at room temperature. This process is called Subcritical Water Extraction and has a temperature between 100 and 380 °C [42]. The sample was treated with subcritical water using a subcritical water extractor, as reported earlier, maintaining a sample–solvent ratio of 1:30. Then, the extraction vessel was pressurized at 20 bar using 99.99% nitrogen/carbon dioxide. The process of extraction required approximately one hour at 160 °C temperature. The vessel was then heated at a rate of 10 °C per min. Mixing was performed at a vibrating-platform frequency of 3 Hz. Then, the processing vessel was cooled quickly by using a water-bath at 202 °C following the next step i.e., extraction. During depressurization, nitrogen was expelled

through a valve. Whatman analytical filter paper, grade 1 was used to filter out the extract after placing it in a freezer at a temperature of approximately 4 °C [43].

2.1.2. Alkali Based Protein Extraction

Alkali (NaOH and KOH) are used to achieve high extraction and to maintain basic natural pH. Disulfide bond breakage in proteins improves protein yield and recovery, resulting from a basic pH [44]. Protein solubility increases with increasing solvent pH because of the ionization of acidic and neutral amino acids. This method yielded high protein yields. Temperature plays an important role in stabilizing the protein's structure, and its folding maintains covalent interactions within its structure [45]. Several studies have shown that factors such as sample-to-solvent ratio, alkali concentration, time interval, and temperature can be adjusted to obtain the highest protein yield at a lower cost. Protein extraction from pumpkin seeds was carried out with three independent variables, including extraction temperature, extraction time, and pH. The optimal procedure parameters for the protein extraction were 32.7 °C, 16.06 min, and a pH of 9.51. Proteins were then isolated under optimized conditions and used to develop new products (Figure 1) [46] (Rüger et al., 2017).

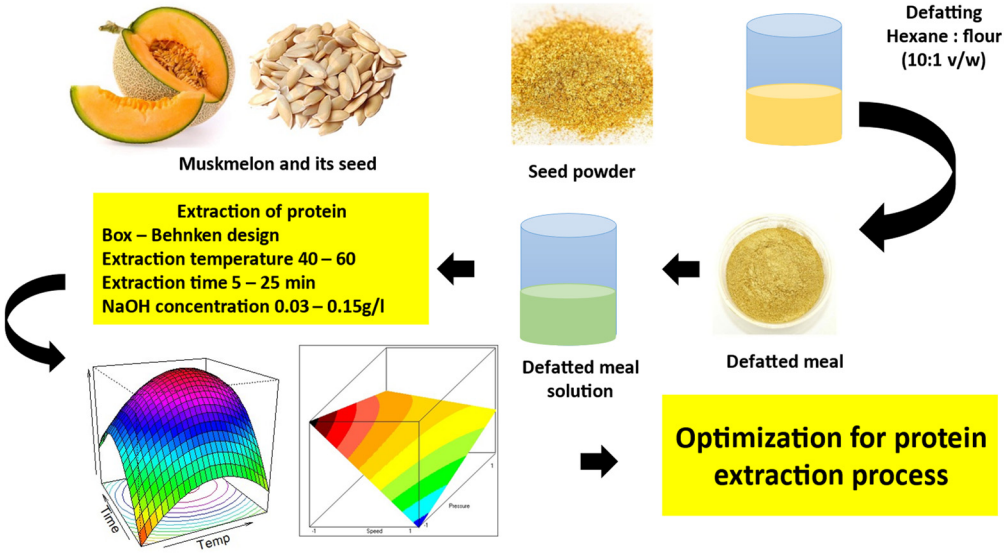

Figure 1. Schematic representation of alkali-based extraction of protein.

2.2. Enzymatic Assisted Extraction (EAE)

The increased demand for high-quality protein foods has led to the development of non-conventional sources of proteins such as fungi, algae, food processing waste, and plants. Enzymatic-assisted extraction is applicable to emulsion-based foods. EAE is an acceptable approach for commercial recovery of high-quality plant proteins [47]. At room temperature, a combination of meal flour and distilled water at a ratio of 1:30 (*w/v*) was prepared using 1 N NaOH to achieve a basic pH of 10. After agitation for an hour at room temperature, centrifugation was done at 5000× *g* for 20 min. Using 1 N HCl, the isoelectric point (pH = 3.49) was maintained and then allowed to rest for 30 min at room temperature [48]. At ambient temperature the suspension underwent centrifugation at 5000× *g*. After the pellet was rinsed with 20 mL of distilled water, it was allowed to freeze dry. The parameters and response surface design were used to determine the factor and response relationship. This determination assists in the impact of hydrolysis conditions and for optimization. Independent factors, such as temperature, time, and enzyme-to-substrate ratio, were used [49]. According to Design-Expert calculations, pepsin enzyme (0.1 M hydrochloric acid, pH 1.5–2) was used at 1, 1.5, and 2% of concentrations, and hydrolysis time (independent variable) was set to 2, 3, and 5 h within a 200 rpm shake incubator. The temperatures used for hydrolysis were 30, 35, and 40 °C. To extract the

protein hydrolysate, the suspension was first heated at 85 °C for 15 min to deactivate the enzyme. Subsequently, the mixture was centrifuged at 4000× *g* for up to 30 min at 4 °C. After collection, supernatants were lyophilized [50]. As Kaewka et al. [51]'s method stated, the Degree of Hydrolysis (DH) was calculated by combining 10 mL of protein hydrolysate with 10 mL of 10% trichloroacetic acid (TCA) and centrifugation mixture. However, several other methods like the OPA (o-phthaldialdehyde) method, TNBS (2,4,6-Trinitrobenzene Sulfonic Acid) method, and pH-stat method are preferred in many studies due to their accuracy, reproducibility, and wide recognition in protein hydrolysis research. The Kjeldahl method was used to measure the nitrogen content (N) of the supernatant and total nitrogen. The equation used to determine the DH value is

$$\text{Degree of hydrolysis} = \text{N in TCA}/\text{N in whole hydrolyzed sample}$$

Bioactive protein hydrolysates are the result of the enzymatic hydrolysis of bioactive proteins and can be used as antioxidant and anti-hypertensive medicines for cardiovascular disease prevention. These components could be a better replacement for artificial food antioxidants [52]. The significant antioxidant activity of pumpkin seed protein hydrolysates can be attributed to the hydrophobic amino acid sequences that constitute a large portion of these hydrolysates. In addition, the results demonstrated that enzymatic hydrolysis could enhance functional qualities, such as emulsifying and foaming capacities, as well as oil and water absorption within food compositions, such as meat and bakery products, as well as some dietary supplements, thereby improving the chemical and physical characteristics and lengthening their shelf lives. This method is suitable for large-scale protein extraction and can be used for the development of various functional foods [53]. Enzymatic extraction yields food products of superior quality that are suitable for human consumption [54]. In addition, immobilized enzymes can be reused to effectively reduce the cost of protein extraction. The protein yield from enzymatic extraction ranges between 25% and 35%, depending on the enzyme type and processing conditions. Some studies have reported yields as high as 35% using proteases. This method is an economical alternative to the physical and chemical methods [55].

2.3. Ultrasound-Assisted Extraction

Ultrasonic waves create acoustic cavitation and hotspots at elevated temperatures and pressures, which facilitate the extraction of plant cell constituents [56]. Compared to other extraction methods, ultrasound assisted techniques have shown more benefits like increasing yield, extraction time reduction, saving energy, and decreases in carbon emissions of up to 40% [57]. Some major chemicals that are used in the extraction process are sodium hydroxide and hydrochloric acid. Defatted pumpkin seed samples were prepared, and the seeds were manually washed and cleaned for the removal or separation of impurities or contaminants. Cleaned seeds were then sent to tray dryers at 45 °C, for 48 h for drying. Seeds and kernels were separated manually. The kernels were ground into fine particles/powder [58]. Using an n-hexane–flour ratio of 30:1 (*v/w*), fats were removed from the prepared powder by stirring with a magnetic stirrer at 40 °C for 36 h, followed by centrifugation at 6000 rpm for 30 min to separate the solids. The separated solids were dried at 40 °C and powdered. They were stored at 4 °C until further use [59]. There are two independent variables whose effects on protein extraction were determined using the response surface methodology. The extraction process was optimized using face-centered central composite design. The two independent variables were ultrasound power and extraction time, and their effects on protein yield and protein recovery were evaluated (Figure 2) [60]. UAE has been shown to produce protein yields between 30% and 40%, with some studies reporting over 40% when combined with enzymatic treatment, as the ultrasound energy increases protein solubility and availability.

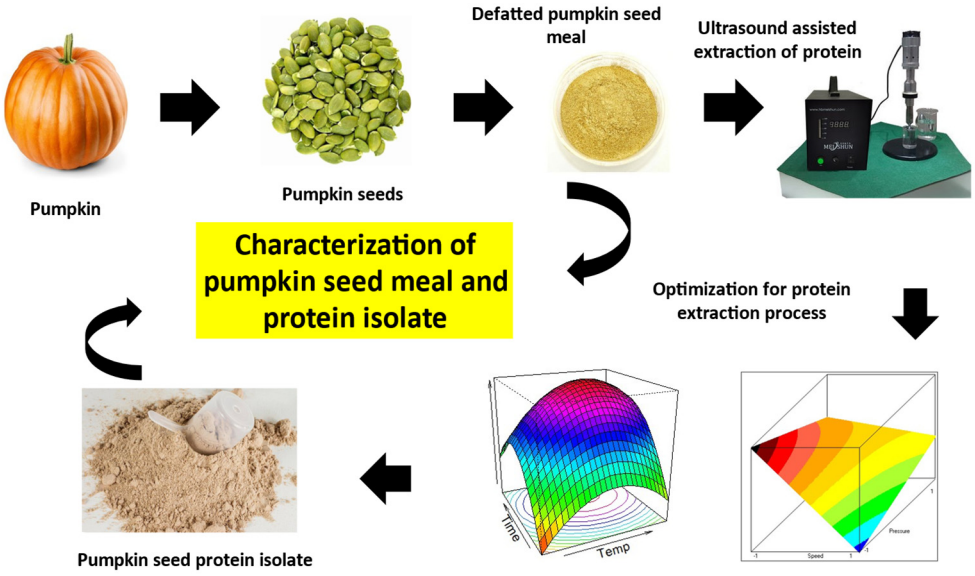

Figure 2. Ultrasound assisted extraction of protein from pumpkin seed meal.

Using an ultrasonic homogenizer, protein extraction was performed at a frequency of 20–25 kHz and a probe diameter of 6 mm. In a 100 mL beaker, defatted seed samples and 50 mL of sodium hydroxide (alkali solution) were taken. A solution probe was inserted in a beaker to a max. depth of 1 cm. Different independent variables (100, 200, and 300 W of ultrasound power and 10, 15, and 20 min of treatment time, respectively) were analysed using a homogenizer or by processing the solution. The temperature (32 °C), and pH (9.5) were fixed. The temperature was measured using a cool-water bath of the solution, and was controlled and continuously stirred for 20 min at 5000× *g* for 15 min at 4 °C. The sample was centrifuged [58]. After collection of supernatants, the precipitate was again dissolved in 0.1 N NaOH and stirred for 1 h. Again, centrifugation was performed at 5000× *g* for 10 min, and the supernatant was collected and combined with the previous supernatant [61]. Using 0.1 N HCl, the combined supernatant was acidified to a pH of 5 after centrifugation (7000× *g* for 25 min. at 4 °C). The precipitate was composed of pumpkin seed protein isolates (PSPI), and its protein content was calculated or determined by the Kjeldal method [58].

3. Characterization of Pumpkin Seed Protein

3.1. FTIR Spectroscopy

The molecular structural properties of pumpkin seed proteins were identified using Fourier Transform Infrared (FTIR) spectroscopy, including identification of functional groups (such as amide I and amide II) and α-helix types [62]. Fourier transform infrared (FTIR) analyses were performed on amide I band, corresponding to C=O stretch vibration in peptide bond, using a Vertex 70 series spectrophotometer with a Pike MIRache ATR accessory (PIKE Technologies, Madison, WI, USA). Room temperature infrared spectra were recorded in the spectral range from 600 to 3000 cm^{-1} (resolution 4 cm^{-1}, 64 scans for each spectrum). Background correction and spectral normalization were performed and the nonlinear peak fitting of the amide I band was applied based on the Levenberg–Marquardt algorithm. Hidden peaks were determined by the second-derivative method, while the Savitzky–Golay and Voigt functions were used for peak smoothing and fitting [63]. Pumpkin seeds were ground to a powder in a blender. Characteristic spectrums of the powders were recorded with a Jasco FT-IR-4100 spectrophotometer (Oklahoma City, OK United States), using the KBr pellet method, a sample was prepared by condensing 3 mg of the pumpkin seed powder and 200 mg of potassium bromide under 10 tons of pressure for 2 min, and the mixture was pressed in 15 mm dies. Spectral data were later processed with the Origin 6.0 software application [64].

Standard presentation of FTIR spectroscopy was managed to know the molecular interactions and structural characteristics of pumpkin seed proteins. FTIR data suggested the presence of hydroxyl and carboxyl groups in pumpkin seed extracts, which made pumpkin seed extracts effective in synthesizing ZnO nanoparticles by acting as reducing, stabilizing, and capping agents [65]. FTIR has also shown sensitivity to refractive indices of the secondary structure of proteins, which serves as an indicator of protein aggregation and folding [66]. FTIR is an appropriate method for determining the structural property of proteins due to its accuracy, precision, simplicity to operate, speed, sensitivity, and non-destructive nature. This interferometer collects infrared radiation and converts it to signals that reveal the signature absorption properties of individual molecular bonds [67]. Additionally, FTIR can be used in the synergetic assessment of microalgal proteins, polyphenols, and carbohydrates in biofabrication (demonstrating that FTIR is versatile both in food science and material development [68].

3.2. SDS-PAGE

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is widely used to evaluate the molecular properties of proteins (such as proteins from pumpkin seeds). In this case, the protein was extracted according to the Osborne method, which pulls different fractions of protein based on solubility. The major protein fraction was the alkali-soluble fraction, which was profiled with SDS-PAGE. The gels were cast with a 15% separating gel and a 5% stacking gel in gel casting plates (1 mm thick; 8 cm × 10 cm for the separating gel and 7 cm × 10 cm for the stacking gel.) Then, electrophoresis was performed at constant voltage of 68 V until the dye travelled to the end of the gel. Following this, the gel was stained with Coomassie Brilliant Blue R-250 (Merck, Darmstadt, Germany), and destained with a destaining solution to increase band clarity. The molecular weight was estimated by comparing it with a protein ladder [69,70].

The SDS-PAGE profile was an important clue for determining the structural and biochemical properties of pumpkin seed proteins. The pumpkin protein isolate (PPI) disclosed several protein bands ranging from 50 to 7 kDa and the analysis of amino acid profiles confirmed the presence of all essential amino acids, while lysine and threonine were noted as deficient [71]. The heterodimeric structure of some of the enzymes and their instability over a pH range from 2 to 10, with optimum activities at pH 4 was confirmed [72].

In addition, this method was applied to study protein–polyphenol interaction. Conjugation of pumpkin seed protein isolate (PSPI) and polyphenols at pH 9 were studied at molecular weight shifts that signify molecular cross-linking along with improved thermal stability and antioxidant properties [73,74]. Post-sonication, the patterns of these proteins remained consistent, as observed by prominent and similar band profiles [75]. On the gel, however, the bands faded, manifesting structural changes due to thermal treatments [76].

SDS-PAGE is a great method for assessing protein sizes and purity in addition to performing some characterization of biochemical properties, as shown in Pakistan using 15% polyacrylamide gel which was able to resolve about 7.5% of pumpkin proteins [77]. In summary, SDS-PAGE is one of the prime methods available to acquire the structural attributes and functional potential of proteins [78] that find various applications in the food and pharmaceutical industries' needs.

3.3. Amino Acid Profiling

Quantitative information is needed for nutrient content. Dried pumpkin seeds were collected and their contents of essential amino acids, minerals, trace elements, and fatty acids were determined or analyzed. There was 58.8% protein and 29.81% fat that was determined on a dry basis; it contained some amount of linoleic acid, potassium, copper, magnesium, zinc, manganese, molybdenum, iron, selenium, but calcium and iron were found in low amounts [79]. Sulfur amino acids were present in the pumpkin seeds [80]. An amino acid analyzer was used [81]. Up to 45% of protein content was present in the raw pumpkin seeds when evaluated, and IVPD (in vitro protein digestibility) was 86%. While

processing, IVPD increased up to 96% under conditions of a 97.8 °C temperature, a pH of 8, and a 37 min time duration [82]. On a wet basis, the protein and fat contents were 40 mg per 100 g and 35.53 mg per 100 g, respectively [83]. A biostimulant product made from pumpkin seed protein hydrolysate (PH) contains many amino acids, antioxidant activities, and peptides [84]. First, 200 mg of dried and defatted pumpkin seeds were hydrolyzed with 7 mL of 6N HCl at a temperature of 105 °C for a period of 22 h. The hydrolysate was filtered. The filtrate was then evaporated in a water bath (40 °C), and the residue used distilled water. Amino acids were determined using a PTH amino acid analyzer (model 120A) [85]. After acid hydrolysis, the total amino acids were determined using AccQ Tag (Waters, Milford, MA, USA) and LC/fluorescence [86].

In vitro protein digestibility and amino acid profiles are responsible for protein quality. It was found that pumpkin seeds had 90% in vitro protein digestibility, and in vitro protein digestibility of defatted pumpkin seed flour was 77.91% [87]. The fat can help whole seeds hold on to some of their fat-soluble vitamins (like vitamin E), and other bioactive components which all together might have a positive effect. In the full seed, the protein is in a biophysical context that modifies its behavior with respect to other item parts during digestion and may immobilize matrixed fat containing factors of satiety (pulse proteins). With fat removed, this matrix recently turned out to be adjusted, and proteins likely take up less space against one another, restricting the capacity of stomach related catalysts to follow up on them. Studies on amino acids have shown that pumpkins have a high concentration of glutamic acid, ranging from 33.03 to 34.76 g/100 g of protein [88]. Using TLC, the chromatographic profile of the amino acid portion of domestic pumpkin seed samples was examined. The locations of the histidine, aspartic acid, glutamic acid, glycine, and leucine zones, as well as an olive-colored zone that stands out from the other amino acid zones and may represent a cucurbitin zone, were all identified in the chromatograms of the test solutions from all samples. Based on the findings of the qualitative inquiry, the developed UV technique yielded quantitative content of the total amino acid content. This technique involves removing the fatty oil, extracting the material again using saturated alcohol, choosing aliquots for the reaction with ninhydrin solution, and measuring the absorbance of the test solution and glutamic acid solution at 400 nm. It has been shown that the amount of glutamic acid, or the sum of all amino acids, in household samples is around 2 nm. The possibility of standardizing pumpkin seeds based on their amino acid composition was investigated. Using TLC technology, the chromatographic profile of the amino acid portion of domestic pumpkin seed samples was examined. Research was done on the quantitative content of the total amino acid sum was determined using immersion spectrophotometry. Established strategies will be suggested for expansion in the national monograph "Pumpkin seeds" draft [89].

4. Modification of Functional Properties

4.1. Heat Treatment and Denaturation

Heat treatment is a widely used technique for protein modification [90]. Stress, including primary processing, such as cooking, roasting, drying, and high-temperature extrusion, is called heat energy. The thermal mobility of peptide chains leads to intermolecular and intramolecular interactions when disulfide, electrostatic, and hydrogen bonds are formed above the denaturation of protein; initially, unfolding occurs, but at reversible high temperatures, this unfolding becomes permanent. As a result, there is a loss of the secondary and tertiary structures of protein molecules because of permanent denaturation [91]. This causes exposure of the hydrophobic core of the protein and a new cross-linking formed via hydrophobic interactions, disulfide bonding, and hydrogen bonding. Therefore, modification must be controlled by controlling the heating temperature, heating rate, ionic concentration, and pH of the protein solution [92].

Denaturation, which involves the disruption of the protein's native structure, is known to play an important role in changing the functional properties of pumpkin seed proteins during heat treatment. Denatured proteins are characterized by the exposure of hydropho-

bic regions, so this process can contribute to high solubility and effective emulsification capacity because the interactions with water and lipids become easier [75,93]). Moreover, thermal treatment can also improve foaming properties since the denatured proteins could stabilize the air bubbles formed during whipping [94]. Also, high doses of heat can induce aggregation and lose their bioactive properties, meaning that temperature settings should be carefully controlled for the best functional results [73,77].

4.2. Enzyme Modification

One popular method of protein modification using enzymes is through their incorporation into the food system [95]. Under mild conditions, these modifications can be achieved [96]. Enzymatic modification preserves the initial protein composition compared with the chemical composition. Another advantage is the fast reaction time and specificity of enzyme modification [97]. Enzyme hydrolysis and cross-linking methods were used for this modification. Transglutaminase is used, and laccase catalyzes the acyl transfer reaction (glutamine and lysine) that forms the latter in the enzymatic cross-linking method. During enzyme hydrolysis, peptide bonds are broken using certain enzymes. Different enzymes have different properties and functionalities [98]. Bacteria such as *Streptoverticillium mobarainse* are used for the isolation of transglutaminase [99].

Hydrolysis-generated smaller peptides and cleaved peptide bonds perturb the protein structure through enzymatic modification of pumpkin seed proteins to create available sites and crosslinks, which are effective in breaking down the use-related functional properties. This process enhances solubility and bioactivity making the proteins more food friendly [71,100]. Plenty of enzymes like proteases could improve emulsifying and foaming capacity by changing the protein structure which in turn contributes to interfacial properties [76]. In addition, due to their possible enhanced antioxidant activity, enzyme-modified proteins will also contribute to the health benefits of functional food products [63,74].

4.3. pH Adjustment

A key factor is the pH of the liquid matrix in which the protein is dissolved. The functional properties of proteins and their structures can be triggered by acidic or alkaline treatments [101]. Increasing the basicity of pH values causes denaturing and unfolding of proteins, which leads to the exposure of sulfhydryl and hydrophobic patches that open new protein interactions [102]. Chemicals such as NaOH and NH_4OH/urea were added to achieve alkaline conditions [103]. The use of NaOH to adjust the pH to basic values resulted in molecular and structural (secondary) changes. This improves extensibility and tensile properties [104]. The protein is introduced at a low pH value during acidic treatment. This promoted unfolding, followed by adjusting the pH again. The most used acid is HCl [105].

Changing the pH of solutions of pumpkin seed protein has a very great impact on their functional properties, in particular solubility and gelation. Proteins possess unique isoelectric points (pI) at which they have the least solubility; therefore, changing to an optimal pH may increase protein solubility, emulsion, and gelling properties [106,107] (Kim et al., 2021). For example, solubility can be significantly improved around the pI, thereby improving incorporation into food matrices [108]. Protein–protein interactions can also be impacted by pH adjustments to promote denser gel structures with desirable characteristics for many food applications [109,110] (Win et al., 2021).

4.4. Chemical Modification

Chemical modification techniques are being used in terms of improving the applications of pumpkin seed proteins as functional bio-ingredients, especially for food industrial uses. These changes can enhance solubility, emulsification, foaming capability, gelation and other properties which in turn makes them more suitable for industrial purposes [71]. In the glycosylation process, carbohydrates (sugars) are attached to proteins by covalent bonding that may occur via the Maillard reaction or enzymatic methods. A structural change in the protein occurs with this kind of conjugation reaction. Enhanced water hold-

ing and lipid–molecule interactions increase protein solubility, emulsification capacity, and thermal stability. In addition, glycosylation markedly enhances the antioxidant activity of pumpkin seed proteins. The mechanism involves introducing acetyl groups (-$COCH_3$), or acetylation, into the protein of interest (they are normally added to amine groups in a target protein) [111]. Acetylation has the effect of reducing protein–protein interactions and thus improves solubility due to a higher emulsifying capacity. It also decreases the bitterness of hydrolysates and thereby balances its taste. In the succinylation process, "to modify" means to add succinic anhydride which gives a protein more negative surface charges, resulting in amino groups being primarily modified. This increases the net charge on protein molecules, thus increasing their solubility and emulsifying properties, and enhancing their dispersibility in aqueous systems [112]. A crosslinking process refers to the covalent bonding between protein molecules, mediated with agents such as transglutaminase or chemical crosslinkers like glutaraldehyde. This process enhances gelation properties to add structure in food applications, such as plant-based meat or dairy analogs. This protein from pumpkin seeds is also ionically crosslinked to improve its thermal stability. The process of phosphorylation is the process by which phosphate groups are added to a protein backbone, typically via phosphoric acid or phosphorylating agents. Proteins can become more hydrophilic and exhibit increased water-binding when they are phosphorylated, which may lead to greater solution/concentration, and emulsification capacity, as well as pH stability [113].

4.5. Ultrasonication

Ultrasonication is an advanced method that uses high-frequency sound waves to disrupt biological structures and serves as a useful treatment for improving functional characteristics of pumpkin seed proteins. These ultrasonic waves create localized high-energy zones in the solution which are called cavitation bubbles that collapse, creating extreme shear forces to disrupt cell walls and protein structures. Cavitation is helpful in disrupting protein aggregates, allowing for increased surface area availability and facilitating the release of intracellular proteins, thus increasing extraction efficiency [114,115] Zisu et al., 2017). Ultrasonication is also known to increase the solubility of pumpkin seed protein. Sonication mechanically disrupts hydrogen bonds and unfolds protein molecules while exposing polar groups that render the proteins highly soluble in aqueous environments [116]. Furthermore, increased surface hydrophobicity after sonication may enhance the emulsification property so that proteins are better able to stabilize the oil–water interface. This process is especially important when considering the use of emulsifying agents in food formulations [117], for example, in the preparation of sauces or dressings.

Ultrasonication may even enhance water-holding capacity, which is significant for the texture and stability of food. Exposing polar amino acid residues makes proteins more effective in binding water molecules, which helps retain water in food products, improving food juiciness and texture [118]. For plant–protein applications, changes in functional properties, like emulsification, foaming, and water-holding capacity with ultrasonication, are of considerable importance due to the limited functionality of plant versus animal proteins [119]. It is a low-energy green technology that preserves the bioactivity and nutritional quality of proteins due to the low-temperature processing. Thus, it provides a potential tool for implementation into industrial settings, particularly for sustainability in food processing [120]. Thus, ultrasonication not only improves functional properties but also provides a scalable process to process plant-based proteins for functional food applications.

4.6. High Pressure Processing (HPP)

High Pressure Processing (HPP) is a non-thermal method that utilizes very high-pressure (300–600 MPa) to induce changes in protein structures without high-temperature treatment. This technique has great potential for utilization in the preservation of nutritional and bioactive properties of plant-based proteins, like pumpkin seed-derived proteins, while improving functional attributes. The secondary, tertiary, and quaternary structures of proteins are altered under high pressure, causing structural unfolding and increased

exposure of hydrophilic and hydrophobic groups. Such alteration improves the solubility of protein, a desirable trait for food applications with a requirement for stable protein solutions [121,122]. HPP can also enhance the gelation property of pumpkin seed proteins, which is an important functional property in terms of textural application of food products. Protein molecules lose their original conformation and aggregate into a gelling matrix under high pressure. This change is helpful in plant-based foods that need gel-state structures, e.g., meat substitutes and desserts, as it mimics the texture offered by meat peptides [123]. In addition, HPP promotes the water-holding capacity of proteins due to the structural changes in proteins, which can cause them to hold more water. This property enhances the juiciness and consistency of products, making it a convenient property for its application in bakery and meat substitute products [124]. An additional advantage of HPP is that it can retain the bioactivity of heat-sensitive protein compounds. Since HPP works at lower temperatures, it avoids the thermal denaturation which destroys heat labile bioactive compounds (e.g., particular amino acids and bioactive peptides with antioxidant properties) [114].

4.7. Significance of Substrates

During the protein extraction process from pumpkin seeds, several substrates like acids, bases, and inorganic salts, as well as organic solvents have been used to alter or enhance the functional properties of proteins. In both extraction and post-extraction modification of extracts, these substances are essential. Most of them contain acids (organic acid—acetic, citrate) that are used because of their organic chemicals, such as for protein extraction from pumpkin seeds. Conformational change can remain incomplete, as it helps denature proteins by exposing hydrophobic groups, enhancing protein solubility. Disassembling the cell structure of complex proteins into simpler components makes it possible to easily separate and extract the protein fraction, leaving other seed mass. It is also possible to change the function properties, e.g., improve emulsifying and gelling proprieties of pumpkin seed proteins [125]. The only downside to this is that the use of acids can present a risk for acid traceback residue, which must be monitored closely. Too much acid residue presents risks to taste, safety, and shelf life of the end-product protein. Analytical methods of detection are limited by nature and involve titration or chromatographic analysis (such as GC-MS) to ensure that residues do not exceed permissible levels.

During extraction, the solubility of the protein is increased by applying bases like sodium hydroxide (NaOH). Also, alkalinization supports the plausibility of degradation/destabilizing of protein composition on seed cell wall structures. For the isolation/enrichment of the protein isolate from de-oiling pumpkin kernel powder, we want to increase functions such as gelling/emulsification. Alkaline material with any residue of a highly alkaline substance like NaOH can be detrimental if not fully neutralized. Alkaline residues are typically detected by means of pH measurements or specific ion detection to determine the safety for food use [126].

The use of inorganic salts, mainly sodium chloride (NaCl) and calcium chloride ($CaCl_2$), are used to precipitates proteins from a solution. Modifying protein structures to increase water-holding capacity is essential for food texture. Post-treatment it is even more important to confirm that any residual salt in the protein product does not exist above required levels as this could impact functionality and sensory properties. Conductive probes and ion-selective electrodes are used to detect residues for inorganic salts, i.e., conduct measurement techniques tell operators how much residual salt is left at the end of a process loop or control at what level of concentration is to be in the final product. Before protein extraction, lipids may be removed from pumpkin seeds using organic solvents such as ethanol or acetone. These solvents serve to defeat the seed material, thus rendering it more suitable for protein extraction [127].

5. Applications in Food Industry

5.1. Meat Industry

The pumpkin seed kernel (PSK) has physiochemical properties and is now used as a replacement for fat in meatballs. There were formulations of meatballs that were prepared with 0%, 3%, 6%, 9%, and 12% of beef fat replaced with PSK flour. As a result, it was found out that PSK flour incorporated into meat balls had reduced fat content of approximately 9–27% and decreased the sensory scores. Therefore, it is estimated that 3% PSK flour can be used for fat replacement in meat or beef balls [128]. Pumpkin seed is an agro-waste with a moisture content of 5–26%, protein of 24.46%, ash of 3.2%, fat of 38.5%, and crude fiber of 14.77%. It is also a great source of dietary fiber, omega-3 fatty acid, etc. The addition of pumpkin seeds to meat products results in an increase in cooking yield, enhanced stability, and increased water-holding capacity. It provides safe meat products [129]. It has been established that the bologna-type link combination batters and the properties of the end products are significantly impacted by the employment of PSO in some locations as a backup source of creature fat. Compared to the links made with beef fat, those made with PSO were slightly softer. Compared to links made with beef fat, those made with PSO had softer and less red colours. During the storage period, the TBARS readings of the links containing PSO increased more than those containing beef fat, although this increase was still within the acceptable upper limits. The obtained results indicate that more research is required on this matter and that pre-emulsified PSO will produce more favourable physicochemical and textural outcomes [130]. This analysis demonstrated that including pumpkins in an animal's diet improves productivity. Furthermore, some of the qualities of meat, milk, and eggs are improved owing to the high concentration of antioxidants and adipose acids found in fruit and seeds alone, which supports natural access to a better diet. The objective is to employ fruits that are unfit for human consumption, or the trash left over after harvest or post-processing, rather than growing pumpkins for animal feed. Therefore, the negative effects of creature output on the environment are mitigated, supporting the sustainability of creature output systems and meeting the need for food originating from creatures, which affects the natural being's internal and external health [131]. A mixture of pumpkin seed flour and turkey flesh was added, and the technology of hams was studied using mathematical modulation. Ion exchange chromatography was used to determine the amino acid composition. Turke carcasses of the first and second classes have a major percentage of muscle tissue, ranging from 44 to 47%, while the skin content with subcutaneous fat is between 13 and 22%. It was shown that oilseeds have a protein composition similar to that of raw meat, making them a rich source of vegetarian protein (19.4–34.2). Three experimental methods for restructured gammon were developed using pumpkin seed flour in the amounts of 5, 10, and 15% aqueous in a 1:2 ratio and turkey skin in place of 10 turkey meat. According to acceptable nutrition, physicochemical investigations have revealed that gammon prepared with pumpkin seed flour and turkey skin has a slightly modified protein content and a more balanced 1:1 ratio of protein to fat. Galenko et al. [132] have demonstrated the good quality of these statements developed for the product of restructured ham, in which 10% of turkey skin and 5–15% of pumpkin seed flour are mixed.

5.2. Bakery Industry

Pumpkin seed flour is a potential raw ingredient for the production of coloured baked goods. Its amino acid profile is rich, and its birth value is high. The protein structure undergoes conformational changes during the technical process of bread manufacturing. Using near-infrared reflection spectroscopy, this study sought to ascertain how pumpkin seed flour affects structural changes in the protein components of dough and bread made from wheat flour. When 10 or more grams of wheat flour were substituted, the protein profile changed to complete because the score for all amino acids was greater than 100. The practical section shows that all the protein samples had the same amino acid balance. The quantity of amino acids utilized by an organism for anabolic functions decreases as

the likelihood of replenishment increases, and they are used less often [133]. A study was carried out to investigate the potential of pumpkin seeds in sponge cake production. With the use of 5% and 10% pumpkin seed powder in sponge cake, physical and sensory characterization was carried out with those of a controlled sponge cake sample. It was found that the crust color of both the control sample and the pumpkin seed powder-incorporated cake was similar. Pumpkin seed-incorporated sponge cakes have good qualitative characteristics [134]. Three different crackers were formulated as control crackers prepared with 100 chickpea flours and crackers containing 20 and 35 of the cold-pressed pumpkin seed cake flour as a replacement for chickpea flour. The proximate composition of the crackers gained is a function of the step-by-step replacement of chickpea flour with pumpkin seed cake flour, with the presence of pumpkin seed cake flour affecting the improved content of proteins, fats, and ash in crackers while reducing the total carbohydrate content. Due to the high dietary fiber and protein content of the used raw stuff, the crackers may have the claims "high in fiber" and "source of protein". The incorporation of pumpkin seed press cake flours resulted in progressive increases in total phenolic content and antioxidant activity. All tested crackers had a moderate glycemic index, but substituting pumpkin seed press cake flour for chickpea flour at both ranks (20 and 35) significantly reduced the crackers' glycemic index. Sensory evaluation of crackers showed that all examined samples had satisfactory sensory properties, indicating that the presence of pumpkin seed press cake flour did not decrease, but rather enhanced some sensory attributes, such as taste and flavor. The present study revealed that this by-product could be exploited in gluten-free cracker production, assuring multiple benefits, such as building up the nutrient quality of the final product, enhancing total phenolic content and antioxidant exercise, and reducing the glycemic index of bakery foods, thereby concomitantly supporting the concept of man-made symbiosis and revaluing by-products of the food industry [135].

5.3. Dairy Industry

The purpose of this study was to examine how ruminal fermentation, lactation efficiency, milk adipose acid, and ruminal bacterial populations were affected when high-oil pumpkin seed cake (HOPSC) was used instead of soybean feed. Six Chinese Holstein cows, having a mean milk production of 105.50 $\pm$ 5.24 days and a milk output of 36.63 $\pm$ 0.74 kg/d, were randomly assigned to three healthy treatments using a 3 $\times$ 3 Latin square design. The treatments included the replacement of the soybean diet with HOPSC. Group 2 consisted of a 50 substitution of soybean meal with HOPSC and dried distillers' grains with solubles (DDGS; 50HOPSC); Group 3 consisted of a 100 substitution of soybean meal with HOPSC and DDGS (100HOPSC); Group 1 was the necessary diet with no HOPSC (0HOPSC). We found no differences between the three treatment groups in terms of milk production amounts or composition. The productivity of the feed tended to increase linearly with the addition of HOPSC. Furthermore, the substitution of HOPSC and DDGS for soybean meal did not affect rumen fermentation; however, it did modify the relative abundance of ruminal bacteria across the phylum and genus ranks. Additionally, we observed that the relative abundance of Firmicutes and Tenericutes grew linearly with increasing HOPSC supplementation, whereas Bacteroidetes superabundance decreased. However, the relative superabundance of Prevotella had a straight-line decreasing tendency and the relative superabundance of Ruminococcus decreased linearly at the rank status in the rumen with increasing HOPSC supplementation. The amount of adipose acid in milk was not significantly affected by changes in beneficial content or rumen microorganisms. Therefore, our findings suggest that high-yielding dairy cows can achieve their nutritional needs without experiencing a decline in milk output or quality when soybean mess is substituted with a blend of HOPSC and DDGS. However, rumen bacterial composition may need to be adjusted. Further research is needed to determine how long-term HOPSC feeding affects dairy cow performance and rumen fermentation [100]. According to the information presented above, combining PSM with camel milk improves its chemical,

antioxidant, consistency, and sensory qualities. These improvements were proportionate to a mixing ratio of up to 50, which improved the technological issues related to camel milk's weak texture and salty taste, and increased the number of phenolic components and antioxidants, which strengthened the milk's nutritional value and health benefits. In rats with oxidative stress, the levels of malondialdehyde (MDA), low-consistency lipoprotein (LDL), cholesterol (CL), triglycerides (TGs), AST, ALT, creatinine, and urea were significantly reduced upon ingestion of fermented camel milk containing 50 PSM. Compared to the untreated group, it increased the total protein, albumin, and high-consistency lipoprotein (HDL) levels. These results imply that further in-depth research on PSM is necessary. It might be beneficial to add it to dairy products, especially non-traditional milk, as it has an off flavor [136]. Table 2 explains the functional properties of various food systems.

Table 2. Protein functional properties in different food systems.

Functional Properties	Food System	References
Solubility	Beverages Protein concentrates/isolates	[137]
Water—binding and holding capacity	Muscle foods Cheese Yogurt	[24]
Gelation	Muscle foods Eggs Yogurt Gelation Tofu Baked goods	[23]
Emulsification	Salad dressing Mayonnaise Ice cream Gravy	[107]
Foaming	Meringues Whipped toppings Angel cake Marshmallows	[138]

6. Future Perspective

As a result of the above, the approaches used to alter the functional characteristics of pumpkin seed proteins are highly adaptable and show great promise for further research and application. First, the field facilitates the creation of new procedures as well as further refinement of existing modification techniques. To accomplish the intended functional increase in protein solubility, emulsification, and gelation, additional modifications of the enzymatic hydrolysis process may be required. Furthermore, new opportunities for simpler and more effective protein modifications have been presented through developments in biocatalysis and enzyme engineering. These alterations may expand the overall adaptability and use of modified proteins across various sectors. Second, the sector is full of applications for cutting-edge new technologies, such as biopolymer engineering and nanotechnology. As previously shown, modified pumpkin seed proteins can be used to create nanoparticles and nanocomposites that provide intriguing new possibilities for tissue engineering, medication delivery, and food packaging. Owing to their distinctive physicochemical behaviour, conjugated nanoparticles with certain ligands are ideal for producing functional materials with high levels of bioactivity, stability, and biocompatibility. Third, studies might look at changes in the potential bioactivity of proteins. By binding to receptors, enzymatically hydrolysed peptides exhibit superior bioactivity and metabolic regulatory functions. These hydrolysates have been shown to include antioxidant, antihypertensive, antidiabetic, anti-inflammatory, and potent antibacterial properties. Lastly, the topic of

employing natural resources in the form of agro-industrial byproducts has become more pertinent, as environmental sustainability remains a century-defining concern. Thus, the problems of waste and resource consumption in protein processing may be addressed by advances in environmentally friendly extraction and waste valuation techniques as well as circular economy methods. Ultimately, these interdisciplinary methods present a wide range of opportunities for cooperation in sustainability, food science, and biotechnology.

7. Conclusions

This review evaluates some recent attempts at addressing the improvements of functional attributes for pumpkin seed proteins. It highlights several key strategies with considerable potential for advancements in the future within this area. Enzymatic hydrolysis appears to be a robust approach for the enhancement of protein functionality, mainly solubility, emulsification, and gelation properties. Enzymatic treatments for pumpkin seed proteins have been confirmed in various research studies, particularly when they are incorporated with ultrasound to enhance participation. In food formulations, breaking proteins into smaller peptides by proteases improves their bioavailability and ability to form stable gels and emulsions mainly through the formation of bonds or linkages. The second key strategy involves a high-tech approach to developing new functional foods with pumpkin seed proteins through nanotechnology. Nanoparticles and nanocomposites are increasingly used in a few fields, particularly tissue engineering (TE), drug loading systems or carriers for controlled release applications (CS), and food packaging, due to their enhanced biocompatibility, stability, and bioactivity compared with traditional materials. Pumpkin seeds are a novel raw material suitable for the industrial and medical sectors due to their extraordinary properties, which can lead to the use of biomass in the formation of new materials that take advantage of these technological advancements. Other important in vitro investigations, including bioactive foods produced by genetic modification of pumpkin seed proteins, have been highlighted in the current paper. Many bioactive peptides generated via the hydrolysis of proteins have demonstrated a varied and potentially broad range of outstanding bio functional properties, including antioxidant, antimicrobial, antihypertensive, and anti-inflammatory activities. These peptides may be used in the development of functional foods to enhance health and prevent disease. In addition, the review highlights sustainability potential and circular economic prospects of pumpkin seed protein applications. It calls for the adoption of green extraction systems along with zero waste processes in such a manner that does not place the environment or people's health at risk and which adapts to growing global interest on sustainable food production systems. Further studies could be conducted on those ecological strategies and applied in the preparation of other dishes using pumpkin seeds protein-based products.

Author Contributions: Conceptualization, V.K.P., K.S. and T.S.; methodology, S.S. and S.R.; software, A.M.S.; validation, V.K.P., S.S., K.S. and A.M.S.; formal analysis, D.U. and S.R.; investigation, A.M.S.; resources, B.K.; data curation, A.M.S.; writing—original draft preparation, V.K.P., K.S. and T.S.; writing—review and editing, A.M.S., D.U., S.R., S.S. and B.K.; visualization, A.M.S. and D.U.; supervision, B.K.; project administration, B.K.; funding acquisition, B.K. All authors have read and agreed to the published version of the manuscript.

Funding: Project No. TKP2021-NKTA-32 was implemented with support from the National Research, Development, and Innovation Fund of Hungary, financed by the TKP2021-NKTA funding scheme, and supported by the University of Debrecen Program for Scientific Publication.

Data Availability Statement: All data were collected from the published research papers.

Acknowledgments: The authors would like to convey thanks and express gratitude to all the partner universities and the University of Debrecen for collaborating on this project.

Conflicts of Interest: The authors declare no conflicts of interest.

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