

Review

Valorization Potential of Oilseed Press Cakes from Pumpkin (*Cucurbita pepo*) and Hemp (*Cannabis sativa*)

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Abstract

The use of non-traditional oilseeds for food purposes has increased significantly in recent years. Due to the high content of unsaturated fatty acids, the production of cold-pressed oils from pumpkin and hemp seeds is growing at a steady pace. This results in the accumulation of a by-product (oilseed press cake), from which high-quality proteins can be derived. Protein hydrolysates based on these raw materials offer further potential for valorization. This review summarizes classical and modern methods for obtaining protein isolates from pumpkin and industrial hemp oilseed cakes, as well as possible approaches for their modification to enhance their nutritional and functional properties. Furthermore, it provides an overview of the methods for producing protein hydrolysates rich in bioactive peptides. The presence of peptides with antioxidant, antihypertensive, or hypoglycemic properties makes hydrolysates a valuable source of nutraceutical components with potential applications in the development of functional foods or dietary supplements. However, most studies to date have been conducted using *in vitro* systems. Further research involving animal models or clinical observations is required to determine dosage regimens and applications of these hydrolysates. Regarding protein isolates, the challenges for their broader use in the food industry are related to increasing solubility, removing anti-nutritional factors, and improving techno-functional properties.

Keywords: plant protein isolates; enzymatic hydrolysis; protein hydrolysates; bioactive peptides; functional foods; valorization

1. Introduction

One of the primary challenges facing modern agriculture and food production is the search for solutions that promote the sustainable use of natural resources and the transition toward a circular bioeconomy. The concept of a circular economy necessitates the adoption of closed-loop systems aimed at enhancing both economic and environmental sustainability. Ensuring the proper and efficient recovery and recycling of agricultural waste, as well as by-products and co-products generated throughout the entire food supply chain, is a critical component of a sustainable food system that utilizes innovative technologies for total resource recovery. The analysis and evaluation of protein-rich by-products and waste derived from the food industry provide a solid foundation for the development of new functional products with increased economic returns.

As a result of the increased interest in health foods, the consumption of cold-pressed vegetable oils from non-traditional sources is growing at a steady pace worldwide, includ-



Academic Editor: Dag Ekeberg

Received: 30 March 2026

Revised: 30 July 2026

Accepted: 17 August 2026

Published: 20 August 2026

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ing in Bulgaria. Their production generates a by-product with high protein content—such as in pumpkin seeds (53%), chia (29.5%), rapeseed (24.4%), sesame bran (15%), canola (33.9%), and coconut pulp (25.2%) [1], as well as in hemp seeds (31.62%) [2].

These by-products possess significant potential for the extraction of high-quality proteins and other valuable compounds; however, a large portion is still discarded, used as animal feed, or composted [3]. The processing of oilseed press cakes to obtain high-value-added products offers numerous advantages of a practical, economic, and environmental nature.

Pumpkin (*Cucurbita pepo*; *C. maxima*; *C. moschata*) and industrial hemp (*Cannabis sativa*) are traditional crops with diverse applications, including serving as sources of valuable vegetable oils. On a global scale, the production of cold-pressed oils from pumpkin and hemp seeds is steadily increasing. In 2024, the global market size for pumpkin and hemp oil was estimated at 1.14 and 0.162 billion US dollars, respectively. Although China is the main producer, Europe also has a significant share. The utilization of the protein-rich residue (oilseed cake) resulting from the production of these oils holds significant potential for valorization. Due to their high protein content, accessibility, and low cost, pumpkin and hemp seed press cakes serve as valuable alternative raw materials for obtaining proteins with high biological value, protein hydrolysates, and bioactive compounds (primarily oligopeptides), which possess a wide range of dietary and therapeutic functions. This creates opportunities for their application in the formulation of functional foods and dietary supplements with beneficial effects on human health and well-being.

The relevance of the issue and the increased interest from the scientific community are confirmed by the high number of publications over the last 20 years. A screening of documents on the topic in the scientific databases—Scopus and Web of Science (Core Collection)—was conducted using the keywords “pumpkin seed protein”, “pumpkin seed protein hydrolysate”, “pumpkin seed cake” and “hemp seed protein”, “hemp seed protein hydrolysate”, “hemp seed cake” for the period from 2005 to 2025. The results were filtered by “Search in article title, abstract, keywords”. According to Scopus and Web of Science, 686 and 641 documents, respectively, related to the utilization of pumpkin seed proteins were published during this period (Figure 1).

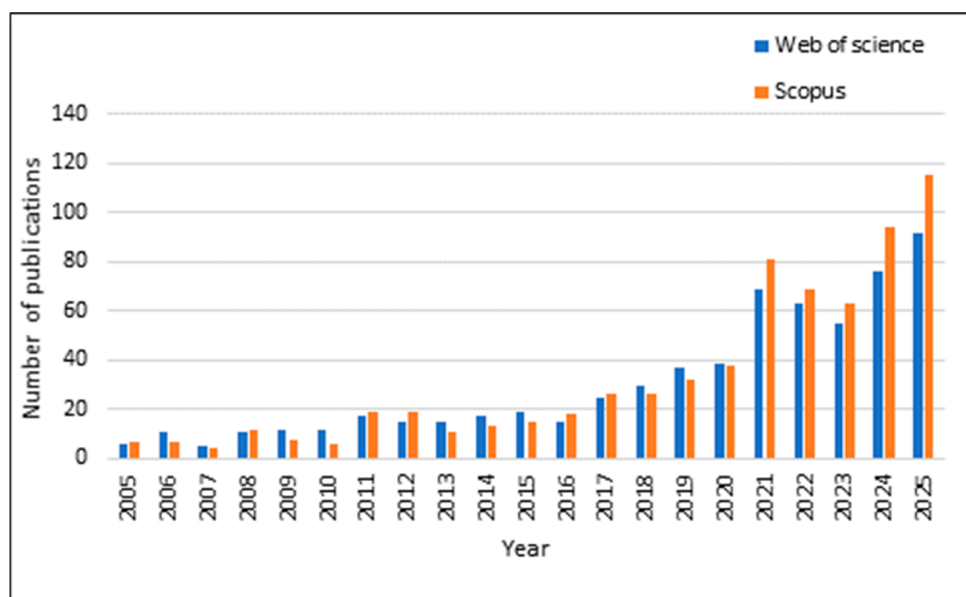


Figure 1. Number of publications related to pumpkin seed protein in the last two decades.

The literature review revealed a significant predominance of research articles, accounting for 85.65% (Scopus) and 88.46% (Web of Science), respectively. A substantial growth in publications has been observed over the last 5 years (approximately 60% of the documents), with main topics including “alternative food sources”, “functional characteristics”, “bioactive peptides in pumpkin seed protein hydrolysates”, “nanotechnology”, “sustainable food systems”, and “circular bioeconomy”.

Regarding articles related to hemp seed proteins and their utilization, the literature analysis identified 721 documents in Scopus and 585 in Web of Science. Research articles represent 85% of the peer-reviewed documents in both databases. Research activity has been particularly intensive during the last 5 years, with over 68% of the articles published in this period (Figure 2).

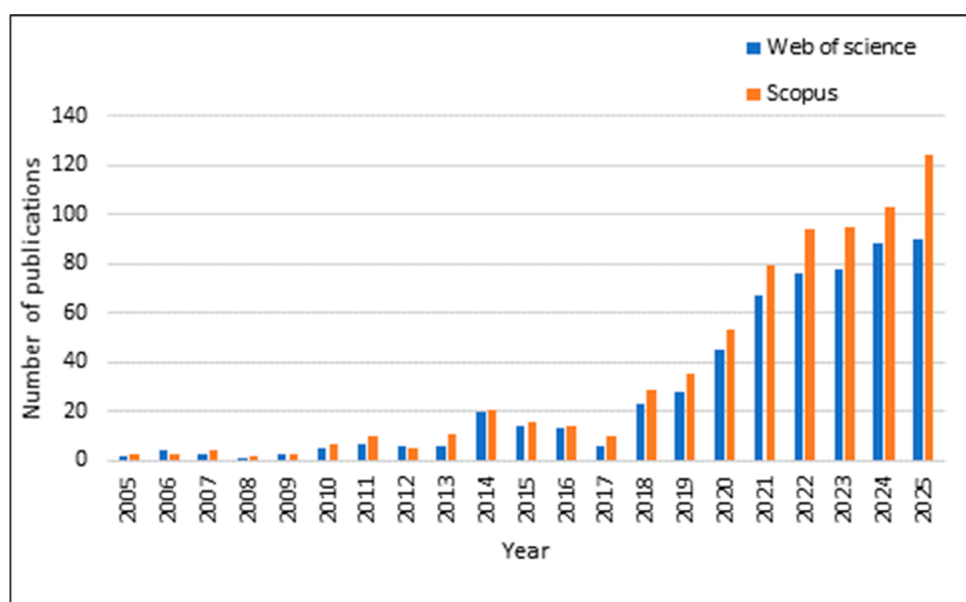


Figure 2. Number of publications related to hemp seed protein in the last two decades.

Similarly, the main topics are related to improving the functional and structural characteristics of hemp proteins, their nutritional applications, and sensory qualities within the context of sustainable development and waste utilization. Regarding hemp bioactive peptides, research focus is directed toward identifying amino acid sequences and the potential health benefits of their application.

Most available reviews are either too broad (encompassing all by-products of the oil industry, which blurs the details) or too specific (focusing on only one species). A comparative analysis between these two specific sources, which share a similar macronutrient profile but differ significantly in antinutrient composition and protein isolate functionality, is lacking. The existing literature often omits a direct comparison of the efficiency of enzymatic hydrolysis on the two substrates under identical conditions. Consequently, the aim of this review article is to analyze the information in the scientific literature from the perspective of utilizing pumpkin and hemp seed meals. Classic and modern methods for protein extraction from the raw materials are included, as well as possible approaches for their modification to enhance their nutritional and functional properties. Furthermore, methods for producing protein hydrolysates rich in bioactive peptides and their potential applications are described, along with future perspectives and challenges.

2. Protein Extraction from Oilseed Cakes

The growing awareness among nutritionists and consumers regarding the importance of high-quality dietary proteins is driving the demand for eco-friendly and sustainable protein sources. The trend of replacing animal proteins with alternative plant-based sources has led to the development of suitable technologies for their cost-effective extraction and incorporation as nutritional or functional food additives. The methods for obtaining proteins from plant raw materials can be summarized as follows: chemical extraction, enzyme-assisted extraction, and physical extraction techniques [4]. The method employed has a significant impact on the composition and functional properties of the extracted plant proteins, such as solubility, antioxidant activity, as well as emulsifying, foaming, and gelling properties [5,6].

2.1. Chemical Protein Extraction Methods

Among the chemical methods, aqueous extraction is one of the most commonly used, although in some cases, extraction with aqueous–alcoholic mixtures or other solvents is applied. The primary parameters influencing the yield and characteristics of the resulting plant proteins are the pH, ionic strength, temperature, solid-to-liquid ratio, and extraction time [7–9].

The selection of an appropriate extraction method and regime depends primarily on the nature of the target protein—its structure, isoelectric point (pI), and solubility under specific conditions [10].

Alkaline extraction is a conventional method for obtaining proteins from plant sources. The solubility of albumins and globulins increases at $\text{pH} > 7.0$ due to the ionization of acidic and neutral amino acids at high pH levels [4]. High pH also leads to the cleavage of some disulfide bonds within the protein molecules, which in turn enhances solubility and yield [11]. Solutions of NaOH or KOH in concentrations ranging from 0.01 M to 1.0 M are typically used to maintain alkaline pH. The extraction itself proceeds as a two- or three-stage process involving protein solubilization, centrifugation to remove undissolved particles, and lowering the pH to the isoelectric point, at which the proteins precipitate [12–15]. In this extraction method, temperature and pH play a decisive role. Optimal temperatures vary from 30 to 70 °C depending on the plant raw material [12,16,17]. Zhang et al. investigated the influence of four factors during protein extraction from defatted cottonseed and established the best results using 0.1 M KOH as the extractant, a temperature of 60 °C, pH 12.5, and a duration of 40 min [12]. In a study by Hou et al., protein isolates were obtained from rice residues, which are by-products of the industrial production of sugars, fructose syrup, and maltodextrin. The authors evaluated the nutritional properties and safety of the protein isolates extracted with alkaline solutions of varying concentrations at 35 °C. They found that as the concentration increased from 0.03 M to 0.15 M, the solubility, emulsifying, and foaming properties of the resulting protein initially increased and then decreased. When the concentration exceeds 0.03 M, it may cause serious issues regarding the nutritional quality or safety of the resulting protein isolate [16]. Higher temperatures increase the extraction rate and yield; however, exceeding certain limits leads to denaturation, protein hydrolysis, and/or degradation of the functional and biological properties of the protein isolate. Generally, extraction under alkaline conditions provides higher yields but may denature the proteins, causing unfolding and polymerization [18].

Extraction using acids or at neutral pH (e.g., via hydrothermal treatment) typically results in lower yields, although this depends on the type of biomass and the operating parameters. This is demonstrated by the results of the study by Qin et al. on brewery by-products. Experiments conducted with various extraction variants showed that a single-

stage pretreatment with dilute sulfuric acid at high temperature ensures the recovery of 90% of the total protein available in the raw material [19].

Extraction with deep eutectic solvents (DESs) is a modern method for recovering bioactive substances that offers several advantages. The extractant is a fluid formed by combining a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), which self-associate into a eutectic mixture with a melting point significantly lower than that of each individual component [20,21]. In most cases, DESs are prepared by mixing a quaternary ammonium salt with a hydrogen bond donor [22].

2.2. Enzyme-Assisted Extraction

Enzyme-assisted extraction is another modern approach for obtaining high-quality proteins from plant raw materials [23]. The specific activity of pectolytic, xylanolytic, and cellulolytic enzyme preparations leads to the degradation of the cell wall, facilitating the release and extraction of intracellular proteins from oilseeds and cereal crops [24,25]. Controlled treatment of the raw material with proteases increases the yield by facilitating the release of proteins from the polysaccharide matrix. Furthermore, proteases fractionate high-molecular-weight proteins into smaller, more soluble fragments, thereby providing favorable conditions for extraction. Another advantage of enzyme-assisted extraction is the milder operating conditions (pH and temperature), which prevent protein denaturation [26]. Disadvantages of this method include longer process durations and the potential for microbial growth within the reaction medium.

2.3. Physical Methods for Protein Extraction

Physical techniques for the extraction of plant proteins include ultrasound-assisted extraction, a pulsed electric field, microwave-assisted extraction, and high-pressure processing. These techniques are typically used as supplements to alkaline extraction, either individually or in combination. Ochoa-Rivas et al. investigated the effects of applying microwaves and/or ultrasound to assist the alkaline extraction of proteins from peanut meal. Both treatments showed an increase in extracted protein compared to the control. Regarding the functional properties of the isolates, improvements were reported in water absorption, foaming, and emulsifying capacity, as well as *in vitro* digestibility (specifically for microwave extraction) [27].

Optimization of ultrasound-assisted extraction of protein from sunflower meal was conducted based on yield and energy consumption. It was established that ultrasonic treatment facilitates better protein recovery with favorable functional properties—solubility, emulsifying capacity, and emulsion stability—rendering the resulting product suitable for use in salad dressings and meat products [28]. High-intensity ultrasound treatment has also been employed to improve the functional properties of protein isolates from sunflower meal [29].

Pulsed electric field (PEF) assisted extraction involves subjecting the plant raw material to high-intensity electric field pulses in the range of 10–80 kV/cm, with durations spanning from microseconds to milliseconds. During this process, the cell membranes in the material become permeable to ions and large molecules, thereby facilitating their extraction [30]. The effects of pretreatment with PEF and high-voltage electrical discharges (HVED) on the extraction of proteins and polyphenols from sesame meal have been studied. The results indicate that the application of PEF or HVED leads to increased diffusion of the target compounds and reduces both extraction temperature and time [31].

The HVED method has been employed to enhance the extraction of proteins, polyphenols, and isothiocyanates from rapeseed and rapeseed meal. For proteins, the optimal

extraction conditions were found to be 240 kJ/kg with a solid-to-liquid ratio of 1:20, providing a protein yield of 9.41 g/100 g and 15.76 g/100 g, respectively [32].

High-pressure processing (HPP) is an innovative technology capable of improving both the yield and the functional properties of extracted proteins. HPP extraction is performed by mixing the plant material with an extraction medium and placing it in a pressure vessel. The fluid pressure typically ranges from 100 to 1000 MPa, allowing bioactive components to dissolve directly into the solvent. It has been established that protein extraction from rice bran is effective at 600–800 MPa [33]. Peanut protein isolate has also been obtained using high-pressure homogenization (HPH). It was found that HPH treatment improves the extraction of peanut protein, with the yield increasing from 16.84% (at atmospheric pressure) to 30.65% (40 MPa) and 39.86% (80 MPa) [34].

3. Protein Extraction from Pumpkin and Hemp Press Cakes

Methods for protein extraction from pumpkin and hemp seed press cakes (Figure 3) range from conventional solvent-based techniques to their combination with physical methods or enzymatic assistance [35–37].

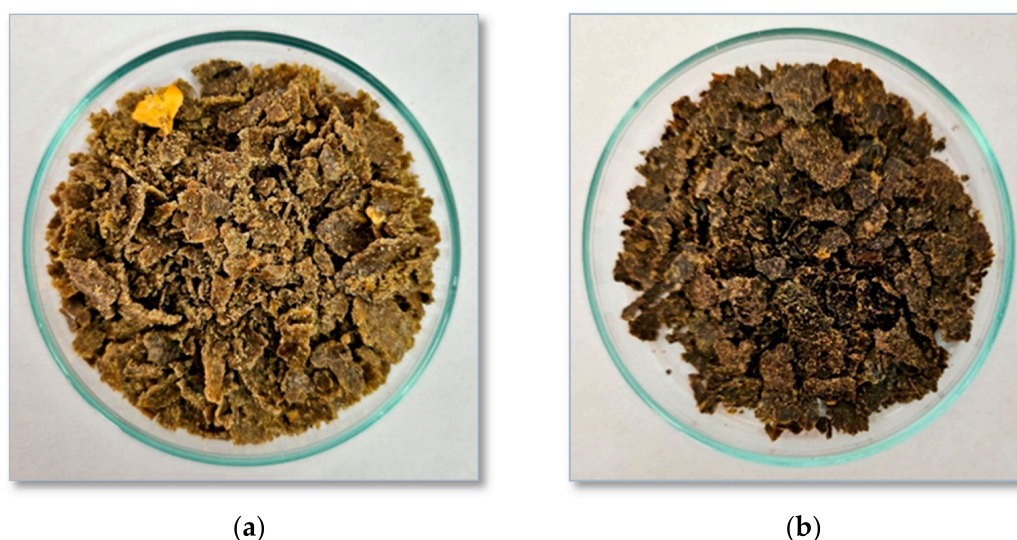


Figure 3. Appearance of the pumpkin and hemp oilseed press cakes: (a) Pumpkin seed press cake; (b) Hemp seed press cake.

3.1. Extraction of Pumpkin Seed Protein

A pumpkin seed protein isolate (PPI), obtained via alkaline extraction (pH 10.0) followed by isoelectric precipitation, reached a protein content of 94.3 g/100 g [38]. Rahemizadeh and Gholami applied alkaline extraction to sesame, hazelnut, almond, and pumpkin meals while varying the pH (from 8.0 to 12.0) and also found the highest yield at pH 10.0. In pumpkin meals, the protein yield was $26.83 \pm 0.13\%$, and the essential amino acid (EAA) content was $27.22 \pm 0.19\%$. According to the authors, all resulting protein isolates possess a favorable amino acid profile and can meet the growing demand for plant-based protein sources to replace animal proteins [39]. Another study compared the physicochemical, techno-functional, and antioxidant properties of protein isolates from flaxseed and pumpkin seed, establishing that both products have potential as alternative plant proteins. Furthermore, the PPI exhibited significant antioxidant properties [40].

In a study by Sert et al., protein isolation from pumpkin seed press cakes was conducted using alkaline extraction (AE), high-pressure homogenization (HPH), and isoelectric precipitation, evaluating the effect of HPH on process efficiency [41]. The highest protein yield was observed with AE + 100 MPa HPH ($61.92 \pm 1.06\%$). The solubility, foaming

capacity, emulsification index, and denaturation enthalpy of the isolated protein were also increased, indicating that high-pressure treatment improves techno-functional properties. Another study by the same authors compared the yield and functional properties of pumpkin meal protein during ultrasound-assisted alkaline extraction. Following ultrasonic treatment, an increase in protein yield and an improvement in most functional properties were achieved compared to non-sonicated samples [42].

Tas et al. investigated the effects of microwave (MH) and conventional (CH) preheating methods, combined with alkaline (AE), enzyme-assisted (EA-AE), or salt (SE) extraction, on the yield and functional properties of pumpkin seed protein isolate [6]. For EA-AE, the enzyme used was Alcalase 2.4 L at a concentration of 2% (*w/w*) of the sample mass. Overall, alkaline extraction combined with microwave preheating treatment yielded the best results. Compared to the untreated samples, the PPI yield increased from $34.1 \pm 0.05\%$ to $55.2 \pm 0.21\%$ (MH-AE), with protein content increasing from 59.42 ± 0.11 g/100 g to 76.95 ± 0.52 g/100 g (MH-AE), respectively. In the samples obtained via MH-AE, an increase in protein solubility (up to $15.99 \pm 0.04\%$) and WSI (3.45 ± 0.005 *w/w*) was also observed. Furthermore, the emulsion activity and stability, as well as the foaming properties, were enhanced. The authors established that microwave-assisted alkaline extraction holds great potential as an innovative method for pumpkin seed protein extraction, as the resulting product is characterized by increased yield and enhanced functionality [6].

Good results have been observed in the enzyme-assisted (EA) and ultrasound-assisted (US) extraction of albumin fraction from defatted pumpkin seeds. The extraction rate is higher under ultrasonic treatment, whereas the maximum yield of albumin is obtained through enzymatic extraction using hydrolases (Viscozyme[®] L), which degrade the cell wall of the plant material. With both methods, the albumin yield is enhanced by more than 50% compared to conventional extraction, specifically by 68% (EA) and 50.1% (US). The functional properties of the albumin concentrate obtained via enzyme-assisted extraction remain unchanged, while ultrasound-assisted extraction leads to an improvement in water-holding, oil-holding, and emulsifying capacities, alongside a slight decrease in foaming capacity and the stability of both the emulsion and the foam [43].

Improvement of certain functional properties of PPI has been achieved through partial hydrolysis with pepsin. The study of solubility, interfacial, and emulsifying properties at various pH levels (3.0–8.0) and ionic strengths (0–1.0 M NaCl) established that enzyme treatment increases PPI solubility across the entire range of tested pH and ionic strengths, especially at pH values close to the isoelectric point (pI) of PPI (up to 100%). A reduction in surface and interfacial tension was observed, indicating that the protein adsorbs at the air/solution and oil/solution interfaces. Unlike the control sample, the hydrolyzed PPI stabilized emulsions against coalescence at all tested pH and ionic strength values. All emulsions were, however, susceptible to creaming instability [44].

A novel approach for synergistic ultrasound–microwave-assisted extraction (UMSE) of pumpkin seed protein has been developed using polyethylene glycol (PEG 200)-based DES. The results indicate that PEG 200 as a hydrogen bond donor, combined with choline chloride as a typical hydrogen bond acceptor, exhibits the highest efficiency. The optimal extraction parameters were the DES concentration (28% *w/w*); solid-to-liquid ratio (1:28); microwave power (140 W); and temperature (43 °C). Under these optimal conditions, the actual yield reached $93.95 \pm 0.23\%$ [45].

Table 1 summarizes the extraction methods, yield and some characteristics of the obtained protein isolates from pumpkin oilseed cakes.

Table 1. Extraction techniques for obtaining protein isolates from pumpkin oilseed cakes.

Plant Material (By-Product)	Extraction Methods	Optimal Extraction Conditions	Yield/Protein Content (PC)	Characteristic of Protein Isolates	Ref.
Defatted pumpkin cake	AE	pH 10.0 (with 1.0 M NaOH); room temperature; 30 min. Isoelectric precipitation (pH 5.0), drying.	Yield: $36.5 \pm 0.2\%$ PC: 94.3 g/100 g	Protein solubility—max at pH 8.0 (68%); min. at pH 5.0 (11%) Stable emulsions at pH 8.0 and 3.0.	[38]
Pumpkin, sesame, hazelnut and almond oilseed meals	AE	pH 10.0; S/L (1:20); room temperature; 1 h. Acidic precipitation (pH 4.0), drying.	Yield: $26.83 \pm 0.13\%$	PPI: total AA ($77.67 \pm 1.14\%$) EAA ($27.22 \pm 0.19\%$)	[39]
Flaxseed and pumpkin seed oilcakes	AE	Pumpkin—extraction at pH 8.5 (with 1.0 M NaOH); S/L—(1:20); 25 °C; 65 min. Acidic precipitation (pH 4.5), freeze—drying.	PC: 85.45%	PPI: All EAAs (44.44%) except histidine; WSI (6.05 ± 0.05); EC ($58.51 \pm 0.19\%$); ES ($43.44 \pm 0.51\%$); FC ($28.33 \pm 1.53\%$); FS ($46.56 \pm 0.51\%$); RSA-DPPH ($37.28 \pm 0.25\%$); RSA-ABTS ($19.46 \pm 0.28\%$)	[40]
Pumpkin seed press cake	HPH + AE; AE + HPH; AE	AE + 100 MPa HPH; pH 11.0; S/L (1:29); 1 h; room temperature. Isoelectric precipitation (pH 4.5), freeze—drying.	Yield: $61.92 \pm 1.06\%$. PC: 97 g/100 g	PPI: Protein solubility at pH 7.0 ($30.21 \pm 0.93\%$); EI ($51.14 \pm 1.16\%$); FC ($119.17 \pm 9.17\%$); Conserved electrophoretic pattern (main band—35 kDa)	[41]
Pumpkin seed press cake	UE + AE; UAE; AE + US	AE followed by US; pH 11; S/L (1:29); 1 h; room temperature.	Yield: $57.8 \pm 2.0\%$. PC: $94.04 \pm 0.77\%$	PPI: Protein solubility at pH 9.0 ($46.93 \pm 0.61\%$); increase in FC and FS.	[42]
Defatted pumpkin seed flour	Pre-treatment (MW; CH); AE; EA-AE; SE.	MW preheating followed by AE, pH 11; S/L (1:10); 1 h. Isoelectric precipitation (pH 5.0).	Yield: $55.2 \pm 0.21\%$ PC: 76.95 ± 0.52 g/100 g	PPI: Protein solubility ($15.99 \pm 0.04\%$); WSI (3.45 ± 0.005 w/w); increase in EA, ES, FC and FS.	[6]
Defatted pumpkin seed powder	US and EAE of albumin fraction.	USE: 20 W/g; 3 min, 40 min; pH 5.0, 30 °C. EAE: Viscozyme L; pH 5.0; 50 °C; 60 min.	USE—albumin yield: 17.0%; PC: 80–82%. EAE—albumin yield: 19.1%; PC: 80–82%.	Pumpkin albumin (10.5 kDa) USE: EI (0.23 ± 0.02); ES (16.3 ± 0.1 min); FC ($47.5 \pm 0.6\%$); FS (66.6 ± 0.5). EAE: EI (0.41 ± 0.04); ES (15.1 ± 0.1 min); FC ($45.0 \pm 0.7\%$); FS (64.0 ± 1.0).	[43]

Table 1. Cont.

Plant Material (By-Product)	Extraction Methods	Optimal Extraction Conditions	Yield/Protein Content (PC)	Characteristic of Protein Isolates	Ref.
Pumpkin seed oil cake	AE; partial enzymatic hydrolysis (pepsin)	AE: pH 10.0; 30 min; room temperature. Pepsin: 0.02 g/1 g; pH 3.0; 90 min; 37 °C.	PC: 94.3 g/100 g	Hydrolyzed PPI: Increased solubility (pH from 3.0 to 8.0); decrease in surface and interfacial tension.	[44]
Pumpkin seeds	US-MW extraction with aqueous PEG 200-based DES (optimization)	DES concentration (28% w/w); S/L (1:28); MW (140 W); US (240 W); 43 °C; 4 min.	Yield: 93.95 ± 0.23%	High extraction efficiency, environmental friendliness	[45]

AE—alkaline extraction; SE—salt extraction; EA-AE—enzyme-assisted alkaline extraction; HPH—high-pressure homogenization; US—ultrasound treatment; USE—ultrasonic extraction; EAE—enzyme-assisted extraction; CH—conventional heating; MW—microwave treatment; S/L—solid to liquid ratio; EAA—essential amino acid; WSI—water solubility index; EI—emulsification index; EC—Emulsion capacity; ES—Emulsion stability; FC—Foaming capacity; FS—Foaming stability; RSA—radical scavenging activity.

3.2. Extraction of Hemp Seed Protein

Alkaline extraction combined with isoelectric precipitation is also the most commonly used method for obtaining protein isolates from hempseed cakes [36,46–48]. Extraction is typically performed at pH 10.0 and temperatures ranging from 25 to 35 °C, followed by centrifugation and precipitation of the proteins in the supernatant at pH 4.3 [46] or pH 5.0 [47]. The reported protein yield usually ranges from 30% to 50% of the total seed content. A higher yield (80%) has been achieved using strongly alkaline solutions (KOH or NaOH, pH 13.0); however, these conditions result in structural damage to the proteins, which impairs their functional properties [48]. Eckhardt et al., however, obtained a yield of 87.3% at pH 11.0, which is explained by the extraction technique used. They used previously dehulled hemp seeds and applied a two-step alkaline extraction of the raw material followed by isoelectric precipitation (pH 5.0) [36].

Various techniques have been applied to purify hemp protein isolate (HPI). Due to the high concentration of phenolic substances and pigments in hemp seeds, the extracted protein often exhibits unsatisfactory solubility and color. Hadnadev et al. compared two treatment methods for hemp meal: alkaline extraction with isoelectric precipitation and micellization (salt extraction). Although the resulting protein isolates showed similar electrophoretic and amino acid profiles, differences were observed in yield, color, solubility and thermal characteristics. The reported yield in alkaline extraction is 50.60%. While micellization resulted in lower yield (40.17%), it produced protein isolates with higher purity, lower total phenolic content, lighter color and preserved native state. The solubility of AE-HPI is highest at pH 6.0–9.0 and of SE-HPI at pH 3.0–5.0. AE-HPI exhibits a higher water-holding capacity—1.59 g/g, which is due to the partial denaturation of proteins during alkaline extraction and exposure of polar amino acid side chains [47].

The combination of salt extraction with ultrafiltration is another suitable method for the production and purification of hemp seed protein isolates; however, the yield remains relatively low, and the resulting product shows unsatisfactory solubility at neutral pH [36,49].

To enhance protein recovery from hemp press cakes, two physical techniques have been employed: high-pressure pretreatment (0–600 MPa; 4–8 min) and ultrasound-assisted

extraction [37]. Compared to conventional extraction, these methods allow for a high yield of protein isolates while preserving the amino acid profile. Table 2 summarizes the extraction methods, yield and some characteristics of the obtained protein isolates from hemp oilseed cakes.

Table 2. Extraction techniques for obtaining protein isolates from hemp oilseed cakes.

Plant Material (By-Product)	Extraction Methods	Optimal Extraction Conditions	Yield/Protein Content (PC)	Characteristic of Protein Isolates	Ref.
Dehulled and defatted hemp seed	AE with isoelectric precipitation; SE with ultrafiltration.	two-step AE: pH 11.0; S/L (1:20); 1 h; room temperature. two-step SE: 0.75 M NaCl; S/L (1:20); 1 h; 50 °C.	AE: Yield: 87.3%; PC: 88.1%. SE: Yield: 81.6%; PC: 86.6%.	Protein solubility: AE-HPI: 15.5% (pH 7.0) and 57.2% (pH 3.2). SE-HPI: 22.0% (pH 7.0) and 55.0% (pH 3.2). In vitro digestibility: 90.9% (AE-HPI); 87.0% (SE-HPI)	[36]
Hemp seed press cakes (7 hemp cultivars)	AE with isoelectric precipitation	pH 10.0; S/L (1:10); 35 °C; 1 h; isoelectric precipitation (pH 5.0).	PC: 96.66–99.22%.	Protein solubility: 14.50–17.50% (pH 7.0); FC (52.9–84.9%); FS (68.1–89.4%); WHC (0.83–1.05 g/g)	[46]
Hemp meal, fraction < 250 µm	AE with isoelectric precipitation; SE with ultrafiltration	AE: pH 10.0; 35 °C; 2 h; S/L (1:20); isoelectric precipitation (pH 5.0); SE: 0.8 M NaCl; 35 °C; 2 h; S/L (1:10).	AE: Yield: 50.60% PC: 91.44% SE: Yield: 40.17% PC: 98.87%.	AE-HPI: WHC (0.80 g/g); higher solubility at pH 6.0–9.0; TPC (139.89 mg GAE/100 g); SE-HPI: higher purity, lighter color; WHC (1.59 g/g); higher solubility at pH 3.0–5.0; TPC (101.28 mg GAE/100 g).	[47]
Defatted hempseed meal	AE or SE	Solutions of NaOH, KOH, NaCl; S/L (1:20); 24 h; room temperature.	AE: Yield: 60–78%. SE: Yield: 37%.	Extraction with water and 0.25 M NaOH or KOH results protein fraction rich in EAAs. 0.75 M NaCl is suitable solvent for high-molecular-weight proteins.	[48]
Defatted hempseed meal	SE	0.5 M NaCl; S/L (1:10); 1 h; 24 °C; fractionation.	Albumin fraction—Yield: 6.33% Globulin fraction—Yield: 6.07%	Globulin fraction—higher level of hydrophobic amino acids. Albumin fraction—higher solubility and FC (80–100%); ES (80–100%) for both fractions.	[49]

Table 2. Cont.

Plant Material (By-Product)	Extraction Methods	Optimal Extraction Conditions	Yield/Protein Content (PC)	Characteristic of Protein Isolates	Ref.
Defatted hempseed cake	HPP, AE or UAE	HPP pre-treatments (200 MPa; 8 min) UAE: 100% power for 10 min); S/L (1:20); 0.25 M NaOH.	Yield: 62% PC: 46%	EAA/TAA (30.45–32.86%)	[37]

AE—alkaline extraction; SE—salt extraction; HPP—high-pressure processing; UAE—ultrasound-assisted extraction; S/L—solid to liquid ratio; EAAs—essential amino acids; TAA—total amino acids; TPC—total phenol content; WHC—water holding capacity; ES—Emulsion stability; FC—Foaming capacity; FS—Foaming stability.

For both types of raw materials, conventional alkaline extraction followed by isoelectric precipitation is still the most commonly applied method. Although this method provides a relatively high protein yield, it has several drawbacks, such as long processing times, potential protein denaturation, amino acid loss, high solvent and energy consumption, and environmental concerns. Extreme alkaline conditions (pH above 10) disrupt the tertiary and quaternary structures due to the cleavage of hydrogen bonds. At the isoelectric precipitation stage, the loss of surface charge can lead to the formation of dense aggregates and impede rehydration. To avoid additional thermal denaturation of the proteins, alkaline extraction is most commonly carried out at room temperature. Salt extraction is another classic method for obtaining protein from pumpkin and hemp seeds, but the yield is lower, and high salt concentrations can cause denaturation. Another disadvantage is the high water consumption and the lower solubility of the resulting protein. To increase extraction efficiency, emerging technologies such as enzyme-assisted, ultrasound-assisted, microwave-assisted, and high-pressure processing have been tested. Among these, ultrasound-assisted extraction has shown the most promising results for improving speed and yield. However, excessive cavitation can also induce denaturation. The impact of the extraction method on the techno-functional properties of the resulting protein isolates is most frequently associated with changes in solubility. Other functional properties that can be affected include emulsifying ability, foaming capacity, water/oil holding capacity, and gelation.

4. Application of Protein Isolates from Pumpkin and Hemp Press Cakes

The protein component of pumpkin seeds consists of albumin, globulin, glutelin, and prolamin. The primary storage protein is 12S globulin, also known as cucurbitin, which is compared in terms of nutritional and functional properties to glycinin in soybeans, legumin in peas, and cruciferin in rapeseed. Pumpkin seed protein exhibits high nutritional quality and contains nine essential amino acids, including histidine, which is essential for infants [35]. Another advantage of pumpkin seed protein isolate (PPI) is its low allergenicity, particularly when compared to soy and sesame [50].

The functional properties of protein isolates are of great importance when used as technological ingredients in food production. The term “functionality” refers to the properties of food components beyond their nutritional value, including solubility, water and oil binding capacity, thermal stability, gelation, foaming, emulsification, and film formation [51]. These properties are related to the physicochemical characteristics of the protein (e.g., molecular weight, structure, hydrophilicity, and hydrophobicity) but are also influenced by external factors such as pH, temperature, and ionic strength [35]. Solubility is a

fundamental property that dictates the other functionalities of pumpkin seed protein isolate and its potential for use in food systems. The solubility of PPI is lowest at the isoelectric point (pH 5.0) and increases with changes in pH, a phenomenon also observed in other plant proteins.

Vinayashree and Vasu compared the dissolution profile of PPI with soy protein isolate and found an increase in solubility in both acidic and alkaline pH ranges. The good solubility at pH 2.0–3.0 suggests the potential application of PPI in fruit juices and other low-pH beverages. They also investigated other functional properties of pumpkin seed protein and concluded that they are comparable to those of soy protein isolate [52].

On the other hand, various factors such as surface hydrophobic–hydrophilic balance, changes in protein conformation, extraction conditions, and the surrounding environment can significantly affect the solubility of proteins isolated from plant raw materials, including PPI [53].

It has been shown that high-intensity ultrasound treatment improves the solubility of plant proteins [29]. PPI obtained through ultrasound-assisted alkaline extraction, or through alkaline extraction followed by ultrasonic treatment, exhibits significantly higher solubility compared to control samples (alkaline extraction only). This is likely due to the fact that proteins form aggregates in their native state, and cavitation acts as a physical factor that reduces the hydrophobic interactions necessary for the intermolecular association of protein molecules [42].

The dietary and functional properties of PPI provide significant potential for diverse food applications, including its use as a carrier for bioactive compounds, a protein supplement in bakery products, meat analogues, and biodegradable packaging [35,53]. The primary drawbacks limiting its large-scale industrial application are mainly associated with naturally occurring antinutritional factors (trypsin inhibitors, phytates, tannins, oxalates). These factors impair digestibility and may reduce the bioavailability of certain essential macro- and microelements, such as calcium, iron, and zinc. Ongoing intensive research and development are focused on improving digestibility, as well as techno-functional and biological properties. In this regard, various PPI modification techniques are being explored, including enzymatic treatment, ultrasound and microwave processing, and fermentation [53]. Giami employed fermentation technology for seed pretreatment prior to the extraction of protein fractions, resulting in reduced concentrations of polyphenols and phytic acid, and improved *in vitro* digestibility [54]. This biochemical approach not only restructures insoluble prolamins and glutelins to enhance their digestibility but also triggers the degradation of antinutritional compounds. The advantages and disadvantages of pumpkin seed protein isolates are summarized in Figure 4.

Hemp seed protein isolate contains globulin and albumin fractions. The primary globulin, known as edestin, accounts for approximately 60–80% of the total protein content, while the albumin component constitutes the majority of the remaining proteins. Albumin exhibits higher solubility and foaming capacity compared to globulin due to its greater molecular flexibility and ordered secondary structure, whereas globulin possesses a more compact or aggregated structure [49].

The nutritional value of hemp seed protein in terms of essential amino acid content is comparable to that of egg white and soy. Hemp seed protein contains all essential amino acids with limited content of lysine and the proportion of essential amino acids to the total amino acids is significantly higher than that of soy protein isolate [50,55]. Despite its nutritional benefits, HPI also contains certain antinutritional factors, although in smaller quantities compared to other plant-based protein isolates [55]. Hemp seeds naturally contain condensed tannins, trypsin inhibitors, phytic acid, and saponins, which can be reduced through various techniques such as thermal treatment or fermentation [55,56].

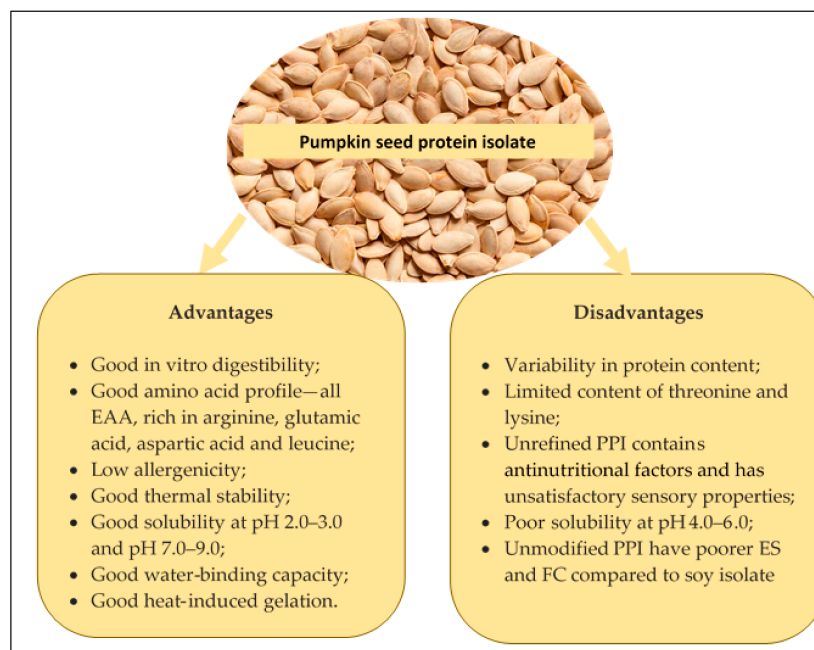


Figure 4. Advantages and disadvantages of pumpkin protein isolate.

A primary disadvantage of HPI is its poor solubility at pH levels below 8.0, which subsequently affects its techno-functional properties and its potential for inclusion in various food systems. The emulsifying activity index, emulsion stability index, foaming capacity, and water-holding capacity of hemp seed proteins are lower than those of soy protein, while the oil adsorption capacity is similar [50]. To overcome this limitation, attempts have been made to apply innovative methods such as high-pressure processing, supercritical fluid extrusion, tribomechanical activation, ultrafiltration, as well as ultrasound, microwave or cold atmospheric plasma treatment [57].

The advantages and disadvantages of hemp seed protein isolates are summarized in Figure 5.

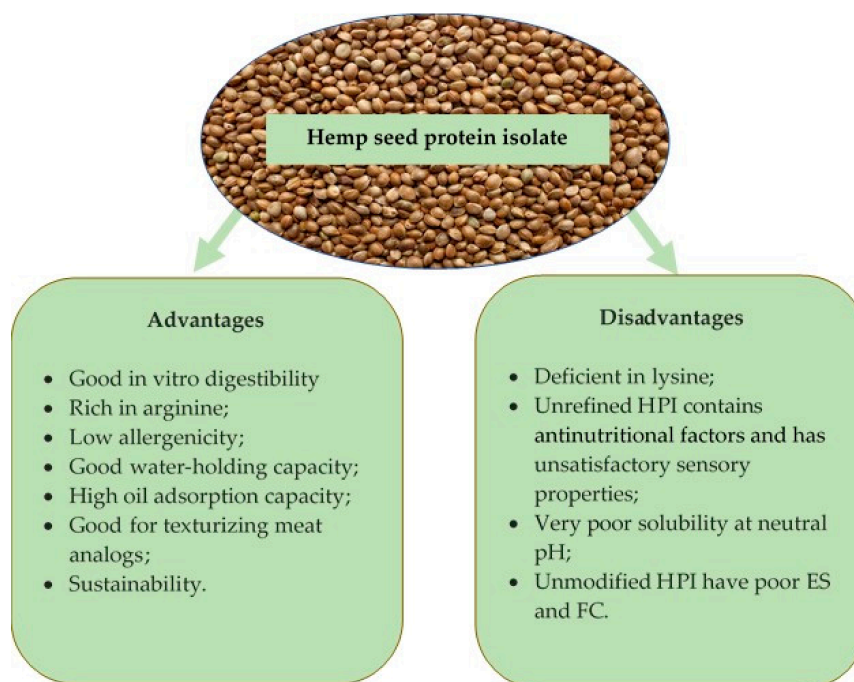


Figure 5. Advantages and disadvantages of hemp protein isolate.

Protein isolates from pumpkin and hemp seed press cakes possess favorable nutritional and functional properties, which can be further enhanced through appropriate extraction and modification methods. They can not only replace a portion of animal proteins in the human diet but also address the issue of waste management in oil production while simultaneously providing additional income for producers and processors. Furthermore, protein hydrolysates derived from these same raw materials offer additional potential for valorization and the production of goods with high bioactive compound content.

5. Production and Biological Effects of Protein Hydrolysates from Pumpkin and Hemp Press Cakes

Protein hydrolysates from pumpkin and hemp seeds press cakes are most commonly produced by treating either the cake itself or the respective protein isolates with proteolytic enzymes. The identification of bioactive peptides within these hydrolysates and their potential biological effects are currently subjects of intensive research [58,59].

Bioactive peptides are defined as specific protein fragments that exert positive effects on the body's physiological and metabolic functions, providing beneficial impacts on human health [59,60]. These peptides typically contain 2 to 20 amino acids and have molecular masses less than 6000 Da. The small size and low molecular weight allow for easy passage through the intestinal barrier and rapid diffusion across cell membranes, while the biological activity is determined by the amino acid sequence and structural properties. Bioactive peptides exhibit diverse biological activities, such as opioid agonists and antagonists, metal-binding agents, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, hypoglycemic, and antihypertensive functions [1,59–64].

Within the primary structure of protein molecules, these encoded peptides remain latent and manifest their biological activity only after being released during the degradation of the parent protein [65,66]. Generally, the release of bioactive peptides from protein sources occurs through three primary pathways: via digestive enzymes within the body, through microbial hydrolysis (fermentation), or by the action of exogenous proteolytic enzymes [67–70]. In all types of hydrolysis, the peptide bonds within the proteins are cleaved, releasing peptides of varying sizes and free amino acids [71].

Enzymatic hydrolysis is the most widely used method for producing bioactive peptides. The enzymatic reaction typically proceeds under mild conditions—pH (6.0–8.0) and temperature (30–60 °C), while the type and extent of the treatment can be controlled due to the specificity of different proteases. The most commonly used enzymes of animal origin include chymotrypsin, trypsin, and pepsin, while those of plant origin include papain, bromelain, and ficin. Among proteases produced by fungi or microorganisms, various individual or combined enzyme preparations are widely applied under commercial names such as Flavourzyme[®], Alcalase[®], Protamex[®], Neutrase[®], Corolase[®], and Protease P [63,64,71,72].

Enzymatic hydrolysis can be supplemented with physical techniques such as high hydrostatic pressure [70], ultrasound [73], or pulsed electric fields [74]. In recent years, the application of these non-thermal technologies as pretreatment methods for protein raw materials prior to enzymatic hydrolysis has attracted significant attention due to their ability to increase both the yield and the bioactivity of the resulting peptides. The primary mechanisms of action for these techniques are attributed to the induction of conformational changes in proteins, thereby enhancing their susceptibility to protease cleavage and subsequent proteolysis [72].

Many factors influence the peptide composition of hydrolysates, as well as their bioactive and functional properties. In addition to the type of protein substrate, the enzyme used, the enzyme–substrate ratio, pH, temperature, and hydrolysis time are also of significance.

Consequently, hydrolysates obtained through different enzymatic processes may exhibit distinct peptide profiles and, accordingly, different potential applications [59,75].

Figure 6 illustrates the biological activities of protein hydrolysates derived from pumpkin and hemp seeds.

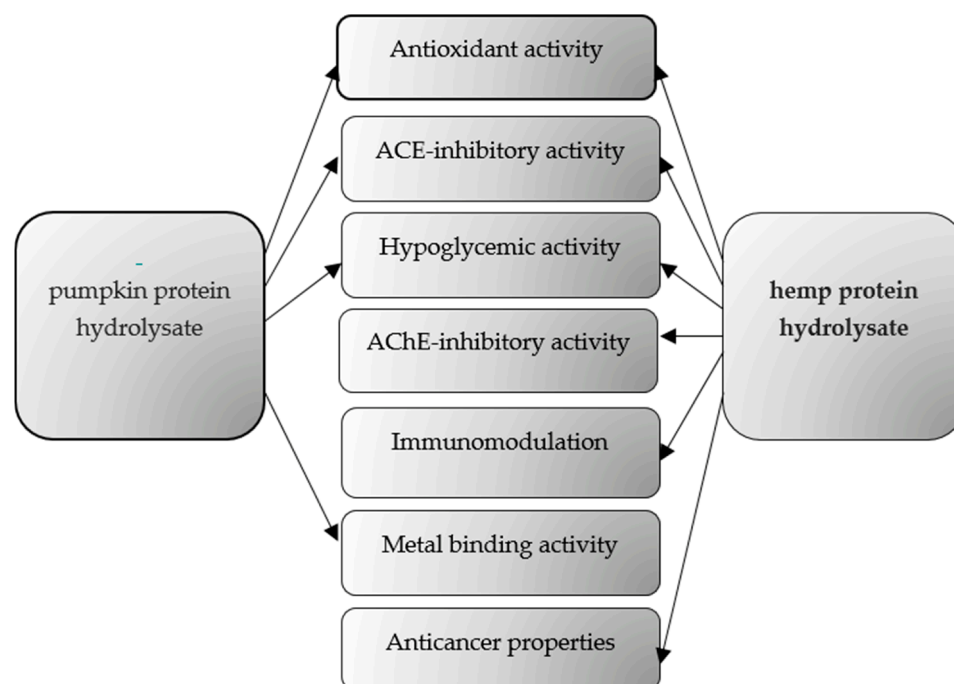


Figure 6. Biological activities of pumpkin and hemp protein hydrolysates.

5.1. Antioxidant Properties

In a significant number of research articles, the antioxidant potential of pumpkin or hemp hydrolysates is evaluated using various *in vitro* assays, including DPPH and ABTS radical scavenging activity, Fe^{3+} reducing power, total antioxidant activity, and metal chelating activity. These methods are widely utilized to demonstrate the antioxidant action of bioactive substances from various natural sources. Only a few studies discuss the antioxidant effects of the resulting peptides *in vivo* or in cell cultures. Protein hydrolysates from globulin fractions of *C. moschata* were administered orally to rats once daily for 15 days, while ethanol (50%) was simultaneously introduced to the test animals. Ethanol induces a severe increase in reactive oxygen species (ROS) levels in plasma and tissues, elevates malondialdehyde (MDA) levels in serum and liver, and disrupts the cellular antioxidant defense mechanism. The globulin hydrolysates significantly reduced MDA levels, demonstrated protective effects against ROS, and could serve as a novel source for mitigating the risk of oxidative stress [76].

In a study by Han et al., peptides derived from PPI significantly increased the survival rate of H_2O_2 -treated Caco-2 cells by enhancing the activity of superoxide dismutase (SOD) and catalase (CAT) while reducing ROS and MDA levels [77]. These findings corroborate the high *in vitro* antioxidant activity demonstrated via the DPPH assay.

Hemp seed protein hydrolysate (HSH), obtained through sequential treatment with pepsin and pancreatin, exhibited significant intracellular antioxidant activity in the Caco-2 cell line. The protein isolate itself also showed antioxidant effects, albeit to a lesser extent. A specific pattern was observed regarding HSH peptide fractions of different molecular weights: higher molecular weight peptides may act with high specificity at low doses, whereas smaller peptides (<1 kDa) more easily penetrate cells and scavenge radicals in a dose-dependent manner [78].

Hydrolysates with high antioxidant activity (determined by DPPH and ABTS assays) were obtained by treating pumpkin seed proteins with pepsin [79] or a combination of Alcalase[®] and pepsin [80]. Nourmohammadi et al. compared the antioxidant activity of PPI hydrolyzed with Alcalase[®] and trypsin, finding higher activity in samples treated with Alcalase[®] [81]. The authors noted that the antioxidant activity of hydrolysates depends on the type of enzyme and its specificity. This observation was further confirmed by Tang et al. in an experiment involving the hydrolysis of hemp seed protein isolate using six proteases (Alcalase[®], Flavourzyme[®], Neutrase[®], Protamex[®], pepsin, and trypsin) [82]. Results indicated that the hydrolysates exhibited varying DPPH radical scavenging activities, as well as differing reducing power and Fe²⁺-chelating capacity. Overall, samples obtained through hydrolysis with Protamex[®] and Neutrase[®] showed the highest activity.

Lu et al. hydrolyzed hempseed protein with Alcalase[®] and found that the resulting hydrolysate possessed radical scavenging activity [83]. Following further fractionation via gel filtration and reversed-phase high-performance liquid chromatography (RP-HPLC), the most active fraction was isolated, yielding two purified peptides containing histidine and 50% hydrophobic amino acids. Protective effects of these peptides against cell death and oxidative apoptosis were observed in rat pheochromocytoma line PC12 cells.

Other authors have produced hemp protein hydrolysates with radical scavenging activity through treatment with pepsin + pancreatin [84] and actinidin, zingibain, Protease G, and two types of neutrases [85].

Beyond the type of enzyme used, the antioxidant activity of hydrolysates also depends on the process parameters. The Response Surface Methodology (RSM) statistical technique was applied to optimize the enzymatic hydrolysis of defatted pumpkin seed cake with papain [86] and defatted hemp seed cake with bromelain [87], resulting in hydrolysates with high DPPH and ABTS radical scavenging activity.

In recent years, experiments have focused on using ultrasound to enhance enzymatic reactions and increase hydrolysates yield. A comparative study by Pacheco et al. evaluated the effect of ultrasound-assisted (38 W/L, 40 kHz) versus conventional hydrolysis of PPI for 45 and 180 min at 25 °C, 40 °C, and optimal temperatures for each enzyme [88]. Three commercial proteases were used: Brauzyne[®] (papain), Flavourzyme[®], and Neutrase[®]. Notably, for all three enzymes, ultrasound-assisted hydrolysis yielded products with higher antioxidant activity. This effect was most pronounced in hydrolysates produced using Flavourzyme[®].

5.2. ACE: Inhibition Effects

The renin-angiotensin system (RAS) plays a key role in regulating blood pressure and fluid balance within the body. Renin, released by renal cells, cleaves angiotensinogen to release angiotensin I. Under the action of the angiotensin-converting enzyme (ACE), angiotensin I is converted into the highly active peptide hormone angiotensin II, which induces vasoconstriction, aldosterone synthesis, and the inhibition of bradykinin activity. Hypertension therapy related to the RAS is directed toward blocking various components of this system. ACE inhibition is a successful therapeutic approach, and ACE inhibitors are among the most common antihypertensive agents [89].

Bioactive peptides derived from food proteins exhibit ACE-inhibitory activity and can serve as alternative or complementary therapies for hypertension. Currently, despite the presence of many potential candidates, a relatively small number of dietary supplements containing bioactive peptides with a blood pressure-regulating effect are available on the market, such as Vasotensin[®] (derived from Bonito fish), Calpis (derived from fermented milk), and Valturon (derived from sardine). Experiments have been conducted to obtain ACE-inhibitory peptides from pumpkin seed meal following hydrolysis with Neutrase[®] 5.0

BG and purification via ultrafiltration, column chromatography, and RP-HPLC. The authors isolated and identified two peptides with significant ACE-inhibitory activity, which were also stable under simulated gastrointestinal digestion and regulated ACE2 activity [90].

Lin et al. produced PPI hydrolysates by treatment with papain, pepsin, and bromelain, finding that the pepsin hydrolysate exhibited the highest and most stable ACE-inhibitory activity. After ultrafiltration of the pepsin hydrolysate, it was found that the fraction below 1 kDa had the highest ACE-inhibitory activity [91].

Peptide hydrolysates from hemp seed obtained during simulated gastrointestinal digestion through the sequential action of pepsin and pancreatin demonstrated in vitro inhibition of both the angiotensin I-converting enzyme (ACE) and renin. These results were further confirmed in vivo in experiments with spontaneously hypertensive rats [92].

Malomo et al. expanded the scope of research by investigating the potential use of other commercially available proteolytic enzymes (Alcalase® and papain). They found that the hemp seed hydrolysate produced with 2% papain had the highest ACE-inhibitory activity, while other hydrolysates inhibited renin to a high degree. In animal studies, the fastest reduction in blood pressure was observed with the Alcalase®-derived hydrolysate, while the most long-lasting effects were seen with pepsin hydrolysates [93].

The previously cited study by Teh et al. demonstrates both antioxidant and ACE-inhibitory activities in hemp seed hydrolysates produced under the influence of six different proteases [85]. The authors noted that the inhibition percentage depends on the type of protease and the duration of the hydrolysis process.

In conclusion, enzymatic protein hydrolysates derived from pumpkin and hemp seeds have a multicomponent composition containing peptides of varying molecular weights. Generally, studies conclude that the highest ACE-inhibitory activity is possessed by small peptides with a molecular weight of <1 kDa or between 1 and 3 kDa. These typically contain aromatic or hydrophobic amino acids at the C-terminus and amino acids with aliphatic chains at the N-terminus [75,90,91,94]. Conversely, in the study of Girgih et al., the entire hydrolysate exhibited stronger hypotensive activity compared to the individual peptide fractions. The results of animal experiments on spontaneously hypertensive rats demonstrate that the oral administration of hemp protein hydrolysates led to significant reductions (−30 mmHg) in systolic blood pressure. The hypotensive effect of the individual peptide fractions (<1 and 1–3 kDa) is weaker (−15 mmHg). This suggests a synergistic effect of the peptides within the hydrolysate, a synergy that is lost during peptide fractionation [92].

5.3. Hypoglycemic Effects

Diabetes mellitus (DM) is the most prevalent chronic metabolic disease, affecting over 300 million people worldwide. It is a syndrome characterized by hyperglycemia and impaired lipid metabolism. In DM, there is either an absolute deficiency of the hormone insulin (resulting from damaged pancreatic β -cells) or defects in insulin secretion and action. Diabetes treatment aims to reduce blood glucose to near-normal levels and prevent micro- and macrovascular complications. Developing appropriate therapeutic strategies for individuals with diabetes presents several challenges. Bioactive peptides with antihyperglycemic effects can support treatment and improve the overall condition of patients. The bioactive peptides derived from various natural raw materials act through different mechanisms: as inhibitors of α -amylase, α -glucosidase, or dipeptidyl peptidase-IV (DPP-IV) [95,96]. The enzymes α -amylase and α -glucosidase break down dietary carbohydrates into absorbable monosaccharides. Their inhibition delays glucose release and absorption, leading to a reduction in postprandial blood glucose levels. Therefore, discovering new

drugs or natural compounds capable of reversibly inhibiting α -glucosidase and α -amylase activity could facilitate better diabetes management.

The enzyme DPP-IV is a protease involved in the degradation of glucagon-like peptide-1 (GLP-1), which is a vital insulinotropic hormone—i.e., it stimulates insulin release in response to nutrient intake. Thus, inhibiting DPP-IV leads to increased insulin production and facilitates postprandial glucose homeostasis.

Currently, there are only a few reports on the hypoglycemic activity of pumpkin and hemp protein hydrolysates. Vařag et al. identified α -amylase inhibitory activity in cucurbitin hydrolysates [97]. The type of protease used influences the inhibitory properties of the resulting hydrolysates, with samples obtained through the action of pepsin being more active than those treated with Alcalase®.

PPI was hydrolyzed with papain, pepsin, and bromelain and analyzed for DPP-IV inhibitory activity. As previously mentioned, the type of enzyme significantly impacts the bioactivity of the resulting hydrolysates. The sample hydrolyzed with papain showed the highest inhibitory activity, which was further increased after treatment with pepsin and pancreatin for simulated gastrointestinal digestion. Regarding the peptide fractions within the hydrolysate, the highest activity was found in the fraction with $M_w < 1$ kDa, which inhibited 79.55% of DPP-IV activity [91].

Samples of HPI hydrolyzed with Flavourzyme®, Protamex®, Neutrase®, papain, trypsin, and Alcalase® were investigated for their α -glucosidase inhibitory activity [98]. The best results were observed in Alcalase® hydrolysates. Two active oligopeptides were isolated from the fraction exhibiting the strongest inhibitory effect, and identified with the sequences Leu-Arg (287.2 Da) and Pro-Leu-Met-Leu-Pro (568.4 Da).

In an extensive study by Cai et al., hemp meal was hydrolyzed with alkaline protease, yielding a thermally and pH-stable hydrolysate with α -glucosidase inhibitory activity [99]. The hydrolysate was fractionated via ethanol precipitation, identifying four peptides with potential hypoglycemic activity. Animal tests demonstrated that these peptides modulate blood glucose and lipid levels in hyperglycemic rats.

Nongonierma and FitzGerald produced and evaluated 12 types of protein hydrolysates from hemp, pea, rice, and soy using three enzyme preparations: Corolase® L10, Promod® 144MG, and Protamex®. They were the first to report that protein hydrolysates from hemp inhibit the DPP-IV enzyme in vitro. Different enzymes generate hydrolysates with different peptide composition and correspondingly different inhibitory activity towards DPP-IV and the highest activity was found after treatment with Promod® 144MG (IC_{50} value of 2.10 mg/mL) [100]. Amigo-Benavent et al. also reported the DPP-IV inhibitory effect of hemp protein hydrolysates [101]. Enzymatic hydrolysis was performed using Alcalase® and Flavourzyme®, with the best results obtained through serial hydrolysis with both proteases. Peptides were isolated and identified using LC-TIMS-MS/MS and characterized in silico. The sequences TNGPQLIH and GKLDLVKPQ were identified as the most active. TNGPQLIH (released after Flavourzyme® treatment) showed an IC_{50} of 1.70 mg/mL. The DPP-IV inhibitory activity was generally maintained or even enhanced following simulated gastrointestinal digestion.

5.4. Other Biological Activities

Additional biological activities of pumpkin and hemp protein hydrolysates have been reported in several research papers. It has been established that bioactive peptides from pumpkin seeds, obtained via enzymatic hydrolysis with papain, possess a high Zn-binding capacity. These peptide–zinc complexes exhibit greater stability during simulated gastrointestinal digestion and higher bioavailability compared to inorganic zinc salts, suggesting their use as functional food ingredients or for dietary zinc fortification [102].

Hemp seed protein hydrolysates have shown inhibitory activity against acetylcholinesterase (AChE). Excessive AChE activity is linked to neurodegenerative diseases such as dementia and Alzheimer's disease; therefore, developing functional foods and dietary supplements with AChE-inhibitory effects is a significant therapeutic goal. Malomo and Aluko examined hydrolysates produced with six different proteases and observed the highest activity against AChE in samples treated with pepsin (IC_{50} —6 $\mu\text{g/mL}$) [103]. In another paper, the same authors reported that the most active fraction of the pepsin hydrolysate primarily contained low-molecular-weight peptides ranging from 244 Da to 1009 Da. The small size of these peptides may facilitate their absorption and potential crossing of the blood–brain barrier [104].

Rodriguez-Martin et al. reported the immunomodulatory effect of hemp seed hydrolysates obtained with Alcalase® and Flavourzyme® on lipopolysaccharide-activated primary human monocytes [105]. Both types of hydrolysates decreased pro-inflammatory mediators (TNF- α , IL-1 β and IL-6) and increased anti-inflammatory mediators (IL-10 and IL-4), allowing for their potential application as natural bioactive compounds in the prevention and treatment of inflammatory conditions. Additionally, peptides obtained through the enzymatic hydrolysis of hemp proteins have been investigated for anticancer properties [106] and have shown promising results as functional ingredients for improving physiological markers associated with hypobaric hypoxia [107].

A brief overview of the enzymatic hydrolysis conditions and potential bioactive properties of pumpkin and hemp protein hydrolysates is presented in Table 3.

Table 3. Enzymatic hydrolysis conditions and biological properties of pumpkin and hemp protein hydrolysates.

Substrate	Enzyme	Hydrolysis Conditions				Mw Distribution; Peptide Sequences	Properties	Ref.
		E:S	pH	T (°C)	Time			
Globulin fraction of <i>C. moschata</i> seeds	Trypsin	1:250	7.5	37 °C	4 h	Not reported	In vivo antioxidant properties; decrease MDA level, increase catalase level in mice	[76]
PPI	Pepsin	1, 1.5, 2% (w/w)	2.0	30, 35, 40 °C	2, 3.5 or 5 h	Not reported	In vitro antioxidant activity (DPPH)	[79]
PPI	Alcalase,	0.02 g/1 g	8.0	50 °C	0–150 min	22 and <12 kDa	In vitro antioxidant activity (ABTS, Fe-reducing power) for Alcalase and pepsin hydrolysates	[80]
	Flavourzyme		7.0	50 °C	0–150 min	33, 22, and <12 kDa		
	Pepsin		3.0	37 °C,	0–150 min	<12 kDa		
PPI	Alcalase	1, 1.5, 2%	9.0	45, 50 or, 55 °C;	2, 3.5 or 5 h	6.5 kDa	Antioxidant activity—max. in Alcalase samples (optimized parameters: 2% Alcalase, 50 °C, 3.5 h; 1% trypsin, 35 °C, 5 h)	[81]
	Trypsin	1, 1.5, 2%	8.0	35, 40 or 45 °C				
Defatted pumpkin seed cake	Papain (Optimization)	1, 2, or 3% (w/w protein basis)	6.5	20, 30 or 40 °C	60, 120 or 180 min	Not reported	In vitro antioxidant activity (DPPH, ABTS) (optimized parameters: 1.0%, 40 °C, 60 min)	[86]

Table 3. Cont.

Substrate	Enzyme	Hydrolysis Conditions				Mw Distribution; Peptide Sequences	Properties	Ref.
		E:S	pH	T (°C)	Time			
PPI	Brauzyn (Papain) Flavourzyme Neutrase	1:100	7.5	25, 40 or 60 °C 25, 40 or 55 °C 25, 40 or 50 °C	45 min or 180 min (US: 38 W/L, 40 kHz)	Not reported	In vitro antioxidant activity (max. in Brauzyn hydrolysates: 46 ± 2% (DPPH); 74 ± 4% (ABTS)	[88]
Pumpkin seed meal	Neutrase 5.0 BG	1% (w/w protein basis)	7.0	50 °C	2 h	bioactive peptide sequences: SNHANQLDFHP; PVQVLASAYR	In vitro ACE-inhibitory activity; protective function on EA.hy926 cells	[90]
PPI	Pepsin Papain Bromelain	1%, 2%, or 3% (w/w protein basis)	2.0 7.0 8.0	37 °C 55 °C 50 °C	1–10 h	fractions: <1 kDa, 1–5 kDa, 5–10 kDa	In vitro ACE-inhibitory activity (max. pepsin hydrolysate—70.26%); In vitro DPP-IV inhibitory activity (max. papain hydrolysate—58.62%)	[91]
Cucurbitin (pumpkin 12 S globulin)	Pepsin Alcalase	1:100	2.0 8.0	37 °C 50 °C	60 min	Not reported	In vitro antioxidant and ACE-inhibitory activity; α-amylase inhibitory activity (max. pepsin hydrolysate)	[97]
PPI	Bromelain Alcalase Flavourzyme Papain Trypsin	2% w/w	6.5 8.0 7.0 7.0 7.5	37 °C 50 °C 55 °C 55 °C 37 °C	3 h	mean Mw: 1.463 kDa; 1.022 kDa; 1.167 kDa; 3.312 kDa; 1.582 kDa.	Good Zn-binding capacity (max. papain hydrolysate: 76.3 ± 8.7%)	[102]
HPI	Sequentially Pepsin Pancreatin	4 U/1 g protein 4 U/1 g protein	2.0 7.5	37 °C 37 °C	1 h 2 h	fractions: 1–3 kDa <1 kDa	Antioxidant activity on cell lines	[77]
HPI	Alcalase, Flavourzyme, Neutrase, Protamex, Pepsin Trypsin	5% (w/w protein basis)	8.5 7.0 7.0 7.0 1.5 7.0	55 °C 50 °C 55 °C 50 °C 37 °C 37 °C	0–240 min	<14.4 kDa (pepsin)	In vivo antioxidant properties, max. at Neutrase and Protamex hydrolysates DPPH: IC ₅₀ —2.3 mg/mL (Neutrase, 4 h)	[82]
HPI from defatted hempseed meal	Alcalase	1/50	9.4	50 °C	1 h	Purified active peptide sequences: NHAV; HVRTALV	In vitro and in vivo antioxidant properties	[83]

Table 3. Cont.

Substrate	Enzyme	Hydrolysis Conditions				Mw Distribution; Peptide Sequences	Properties	Ref.
		E:S	pH	T (°C)	Time			
Decolorized HPI	Sequentially Pepsin	4% (w/w protein basis)	2.0	37 °C	2 h	fractions: <1 kDa 1–3 kDa, 3–5 kDa, 5–10 kDa.	In vitro antioxidant activity max. < 1 kDa (DPPH; OH [−]) and 5–10 kDa (ferric reducing power)	[84]
	Pancreatin		7.5	37 °C	4 h			
two types HPI	AFP 4000	5% (w/w protein basis)	6.5	45 °C	1, 2 or 4 h	Not reported	In vitro antioxidant and ACE-inhibitory activity max. in HT-hydrolysates 40.65 µM TE/g (ORAC) 76.82 ± 0.06% (ACE—inhibition)	[85]
	HT		6.5	45 °C				
	Protease G		8.0	45 °C				
	Actinidin		5.5	45 °C				
	Zingibain		6.0	65 °C				
Defatted hemp seed cake	Bromelain (Optimiza- tion)	1, 2, or 3% (w/w protein basis)	6.5	20, 30 or 40 °C	60, 120 or 180 min	Not reported	In vitro antioxidant activity: 6.227 mg TE/g (DPPH); 26.378 mg TE/g (ABTS) (optimized parameters: 3.0%, 40 °C, 60 min)	[87]
HPI	Sequentially Pepsin	4% (w/w protein basis)	2.0	37 °C	2 h	fractions: <1 kDa 1–3 kDa,	In vitro renin and ACE- inhibitory activity; In vivo hypotensive effect (higher in hydrolysate than in peptide fractions)	[92]
	Pancreatin		7.5	37 °C	4 h			
HPI	Alcalase	1:100	8.0	50 °C	4 h	peptide sizes: 0.3–9.59 kDa	In vitro renin and ACE- inhibitory activity In vivo hypotensive effect (max. for 1% Alcalase hydrolysate)	[93]
	Papain	2:100	6.0	65 °C	4 h			
	Pepsin	2/4:100	2.0	37 °C	2 h			
	Pancreatin	2:100	7.5	37 °C	4 h			
Hemp seed protein	Flavourzyme	3:100	7.0	50 °C	2.5, 3.0, 2.5, 2.0 and 3.0 h	bioactive peptide sequences: Leu-Arg (287.2 Da); Pro-Leu-Met-Leu- Pro (568.4 Da).	In vitro α-glucosidase inhibitory activity (max. inhibition rate 58.26% for Alcalase hydrolysate with DH 27.24%)	[98]
	Protamex	2.5:100	6.5	50 °C				
	Neutrase	2.2:100	7.0	50 °C				
	Papain	1.8:100	7.0	65 °C				
	Trypsin	2.0:100	8.0	37 °C				
Non- dehulled hemp seed meal	Alcalase	0.8:100	8.5	60 °C	6 h	bioactive peptide sequences: TGLGR; SPVI; FY; FR.	In vitro α-glucosidase inhibitory activity; In vivo hypoglycemic activity	[99]
	Alkaline protease	1% (w/w protein basis)	8.0	55 °C				
HPI	Corolase	2% (w/w protein basis)	7.0	50 °C	4 h	fractions: <1 kDa (65%) 1–5 kDa, 5–10 kDa,	In vitro DPP-IV-inhibitory activity (max. for Promod hydrolysate—IC ₅₀ 2.10 mg/mL)	[100]
	Promod		7.0	50 °C	4 h			
	Protamex		7.0	50 °C	4 h			

Table 3. Cont.

Substrate	Enzyme	Hydrolysis Conditions				Mw Distribution; Peptide Sequences	Properties	Ref.
		E:S	pH	T (°C)	Time			
HPI	Alcalase	0.3 AU/g protein	8.0	50 °C	20, 30, 45, 60 min	bioactive peptide sequences: TNGPQLIH; GKLDLVKPQ.	In vitro DPP-IV-inhibitory activity (max. after sequential treatment with Alcalase and Flavourzyme—IC ₅₀ 1.59 mg/mL)	[101]
	Alcalase + Flavourzyme	0.3 AU/g	8.0		60 min			
		60 LAPU/g	7.0	50 °C	15 min			
HPI	Alcalase		8.0	50 °C	4 h	bioactive peptides: 244 Da—1009 Da	In vitro AChE-inhibitory activity (max. for 1% pepsin hydrolysate—IC ₅₀ 0.6 µg/mL)	[103, 104]
	Papain	0.5, 1.0, 2.0,	6.0	65 °C	4 h			
	Pepsin	3.0 or 4.0%	2.0	37 °C	2 h			
	Thermoase	(w/w	8.0	50 °C	4 h			
	Flavourzyme	protein	8.0	50 °C	4 h			
	Pepsin + Pancreatin	basis)	2.0	37 °C	2 h			
HPI	Alcalase	1% (w/w	8.0	50 °C	20 min	Not reported	Ex vivo immune-modulatory and anti-inflammatory effects (decrease TNF-α, IL-1β, and IL-6; increase IL-10 and IL-4)	[105]
	Alcalase + Flavourzyme	protein basis)	8.0	50 °C	60 min +15 min			
HPI	Papain Neutral protease	0.4% 3.0% (protein basis)	5.0	70 °C	3 h	bioactive peptides: Mw < 400 Da	Ex vivo anticancer properties (increased apoptosis, reduced cell migration in Hep3B human liver cancer cell)	[106]
Edestin	Alcalase 2.4 L	0.4 mL/g	8.0	65 °C	5 h	325 peptide fragments (Mw 923–2655 Da)	Supplementation with hydrolysate improves hypoxia-related physiological indicators (animal and human trials)	[107]

6. Future Perspectives and Key Challenges

Although they do not belong to the main oilseed raw materials, pumpkin and hemp seeds are sources of oils valued for their specific taste and high concentration of beneficial nutrients. Their production and consumption have been growing at a steady pace in recent years. The waste generated after the production of cold-pressed pumpkin and hemp oils contains significant amounts of protein and has the potential to serve as a source for sustainable, high value-added products—protein isolates, protein concentrates, and protein hydrolysates. In terms of nutritional value, pumpkin and hemp proteins compare favourably with other plant proteins. Pumpkin protein contains all essential amino acids, although in not fully balanced proportions. The amino acid profile of hemp protein is rich in essential amino acids, even though lysine is present in small amounts. A key advantage is its high content of arginine—an amino acid that plays a positive role in regulating blood pressure and is important for cardiovascular health. Another advantage of both proteins is their low allergenicity, especially when compared to soy and sesame. The nutritional properties of pumpkin and hemp protein allow them to be used as alternative protein sources, including as meat analogs and for the fortification of dairy and bakery products.

The primary challenges to the broader use of pumpkin and hemp proteins and their inclusion in various food products are related to their low solubility in specific pH ranges: from 4.0 to 6.0 for pumpkin protein and below pH 8.0 for hemp protein. Poor solubility at pH values typical of most food systems negatively affects all other techno-functional properties, such as emulsifying and foaming properties, water-holding capacity, and gelling. Another drawback is the presence of antinutritional factors in pumpkin and hemp seeds, albeit to a lesser extent than in legumes. The specific organoleptic properties of PPI and HPI also complicate their broader application in novel food formulations and can negatively affect consumer acceptability. This applies to a greater extent to HPI, whose color characteristics may also prove unacceptable. Addressing these challenges begins with extraction techniques. Combining conventional and modern extraction methods, along with applying partial enzymatic hydrolysis, makes it possible to improve the solubility and other functional properties of the resulting protein isolates. Studies have also been conducted on potential non-food applications of PPI and HPI, such as the development of sustainable, biodegradable packaging and as carriers of active ingredients.

Protein hydrolysates from pumpkin and hemp press cakes represent additional potential for valorization, with the resulting products having potential applications in the development of functional foods and nutraceuticals. They demonstrate *in vitro* antioxidant, ACE-inhibitory, and hypoglycemic activities. Additionally, AChE-inhibitory activity, immunomodulatory, and even anticancer effects have been reported for hemp hydrolysates. To date, only a few *in vivo* studies have been reported. The stability of peptides and hydrolysates during endogenous digestion is essential for their absorption and bioavailability. Extensive *in vivo* studies are required to explore the full complexity of bioactive peptide delivery, efficacy and metabolism under real physiological conditions. Systematic studies on the interaction between peptides and the food matrix are lacking, which is necessary, especially when peptides are intended to enrich functional food formulations.

7. Conclusions

The intensive development of agriculture and the food industry has led to increased raw material consumption and, consequently, a higher generation of waste and by-products. This explains the growing interest in developing technologies that minimize environmental impact and enable total valorization of these resources. Pumpkin and hemp seed press cakes, which are by-products of cold-pressed oil production, are carriers of highly valuable substances and can be transformed into high-value-added products. Their high protein content makes them precious alternative raw materials for the production of proteins, protein hydrolysates, and bioactive compounds (mainly oligopeptides) that possess a wide range of dietary and therapeutic functions.

The trend of replacing animal proteins with alternative plant-based sources has intensified research focus on developing cost-effective technologies for the extraction and utilization of proteins from pumpkin and hemp meals. Proteins from these seeds have a balanced amino acid composition, high nutritional quality, and low allergenicity. The challenges for their broader application as food or functional additives are related to increasing their solubility, eliminating antinutritional factors, and improving their techno-functional and sensory properties.

Protein hydrolysates derived from these raw materials offer additional potential for valorization and the production of products with a high content of bioactive substances. The presence of bioactive peptides possessing antioxidant, antihypertensive, or hypoglycemic properties makes them a valuable source of nutraceutical components with potential application in the development of functional foods or dietary supplements with preventive and therapeutic effects against a number of socially significant diseases. However, to date,

most demonstrated effects have been reported in vitro and in cell systems. Further research involving animal models or clinical observations is necessary to determine dosage regimens and the potential applications of these hydrolysates.

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

Funding: This article was funded by the Bulgarian National Science Fund, grant number KP-06-N66/1 (granted to Iliana Nacheva).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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

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