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Review

Bioactive Peptides from *Lupinus* spp. Seed Proteins-State-of-the-Art and Perspectives

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Abstract: Nowadays, the search for food-suitable plant proteins is a great challenge. In addition to their sustainability and nutritional value, the focus is more and more on possible positive interactions with human health. To date, the presence of bioactive peptides encrypted in the structure of protein opens new perspectives, addressing the food industry's request for new ingredients with technological properties and also the nutraceutical and pharmaceutical sectors based on multifunctional health applications. *Lupinus* is a sustainable genus of the legume family *Fabaceae*, and the lupin seed-derived bioactive peptides have demonstrated different effects including anti-inflammatory, antidiabetic, antioxidant, antibacterial, hypocholesterolemic, and antihypertensive activities. This review aims to discuss the current knowledge on lupin protein and their bioactive peptides, highlighting the documented health claims, but also the possibility of allergenicity and the work to be done for the development of new functional products.

Keywords: lupin; food proteins; bioactive peptides; functional food; perspectives

Citation: Garmidolova, A.; Desseva, I.; Mihaylova, D.; Lante, A. Bioactive Peptides from *Lupinus* spp. Seed Proteins-State-of-the-Art and Perspectives. *Appl. Sci.* **2022**, *12*, 3766. https://doi.org/10.3390/app12083766

Academic Editors: António José Madeira Nogueira and Andrea Luísa Fernandes Afonso

Received: 5 March 2022
Accepted: 5 April 2022
Published: 8 April 2022

1. Introduction

Bioactive peptides are defined as specific protein fragments having a positive influence on human health [1,2]. They usually contain 2–20 amino acid residues per molecule, but sometimes may comprise over 20 amino acids and a molecular mass of less than 6000 Da [3]. As reported by Karami and Akbari-Adergani [4], the biological activity of these peptides is determined by the amino acid sequence after their release from the parent protein where they are encrypted.

Every protein can be a potential source of bioactive peptides. Of particular interest, however, are edible non-animal proteins. The seeds of various plants seem to be suitable in this regard. Therefore, the aim of the present review paper is to summarize the available information of bioactive peptides derived from *Lupinus* spp. seeds as a good nutritional and functional alternative to the animal proteins.

The search for bioactive peptides in food proteins can follow either the classical or a bioinformatic approach [5]. The latter is an in silico approach that uses available databases to obtain protein sequences and to further simulate the potential hydrolysis and chance to release bioactive peptides [6]. Nevertheless, confirmation with classical methods is needed every time. As regards the classical approach, there are three principal methods for peptide release. They are all based on enzymatic hydrolysis of the whole protein molecule (Figure 1). Each of these methods has its advantages and disadvantages that are discussed below in a concise way [7].

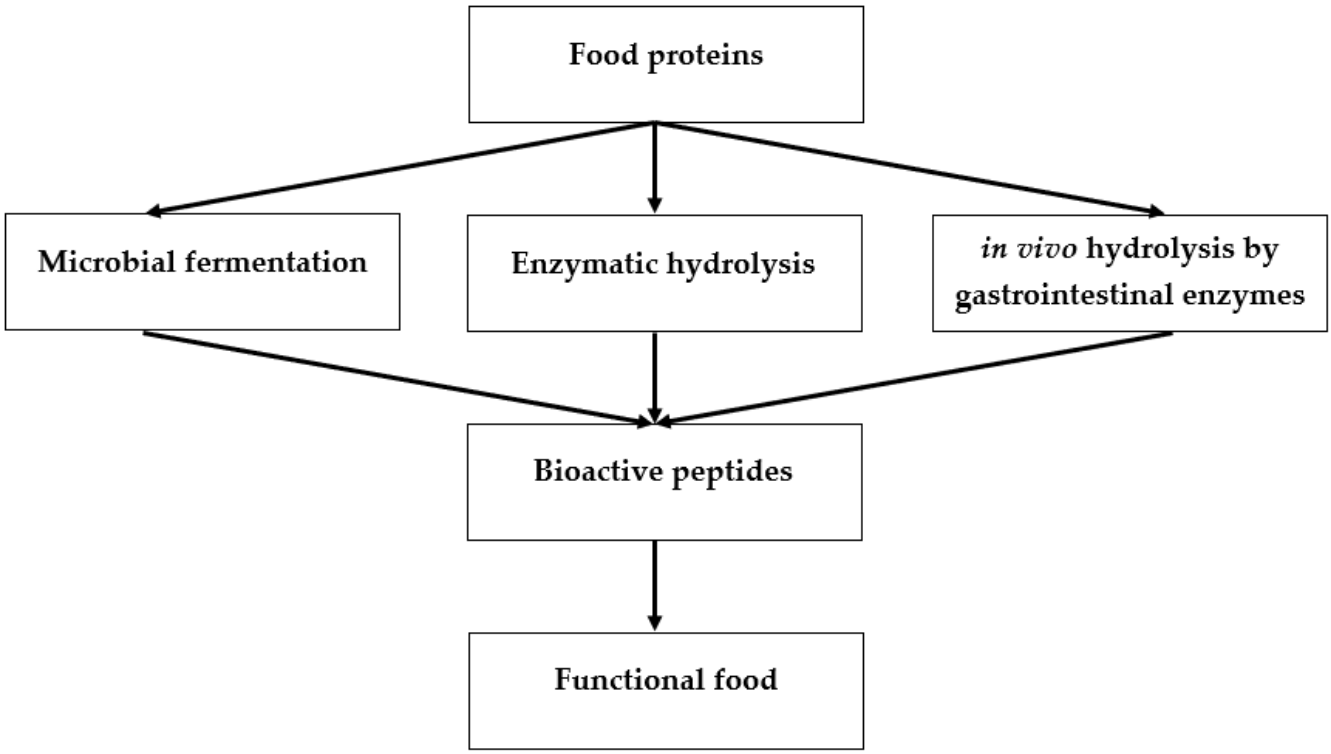

Figure 1. Production of bioactive peptides [1].

1.1. Enzymatic Hydrolysis

Pure proteases can be used for controlled protein breakdown. Such enzymes may be of animal, plant, or microbial origin. Depending on the wanted peptide length, endo- or exo-peptidase can be used [8]. The main advantages of this method are: moderate conditions; no loss of amino acids; better precision and control on the degree of peptide bond hydrolysis; and the protein is easily isolated thanks to the ability to deactivate small amounts of enzymes [9]. There is a possibility for the introduction of immobilized enzymes that can be recycled and thus avoid secondary metabolites from the autolysis of the protease [10]. The main disadvantage is the high cost of the purified enzymes.

1.2. Microbial Fermentation

The process relies on culturing bacteria or yeast on protein substrates. The proteolytic enzymes are produced by these microorganisms to potentially release peptides from the protein substrate of interest [11]. This method is less costly, and the use of purification techniques is not obligatory [12]. A successful example in this regard is lactic acid fermentation of milk by different probiotic strains [12]. When properly designed, during microbial fermentation, peptides and free amino acids are released [9]. The main disadvantage of this method is related with variation in the metabolite production of microorganisms under different culturing conditions, including Bioactive peptide release.

1.3. Gastrointestinal Digestion

The action of certain proteases, such as pepsin and trypsin, play a key role in the digestion process. The influence of factors such as gastric and intestinal pH, endogenous secretions, type of proteins, and the activity of digestive enzymes is strongly related to the digestion of food proteins [13]. Hereafter, peptides are released through the digestion of food in the gastrointestinal tract. Some of them possess a suitable structure for enhancing and modulating enzymes and processes in the organism and acting this way as beneficial agents. Fernández-Tomé and Hernández-Ledesma [14] reviewed the transformations that

proteins undergo during digestion to release biologically active peptides, as well as the mechanisms of action that these peptides are involved in.

The obtained hydrolysates can be tested for assessing their biological activity in vitro and in vivo. After that, the hydrolysates with good performances are considered a starting point for the functional food designing.

2. Lupins and Their Proteins

Lupin belongs to the genus *Lupinus* and the family *Fabaceae* whose role in agriculture dates back to more than 4000 years [15]. Since ancient times, lupin has mainly been used as animal feed (for forage and silage) and for soil improvement, especially as nitrogen fixers [16], and over the last decade it has increasingly been used as a functional ingredient and protein supplement. There are more than 400 species described at present, and the most popular ones are the sweet lupin species such as white lupin (*Lupinus albus*), blue lupin (*Lupinus angustifolius*), yellow lupin (*Lupinus luteus*), and pearl lupin (*Lupinus mutabilis*) [17]. The plant grows in a very wide range of environments and regions—the Mediterranean, Northern and Eastern Africa, North and Central America, Andean South America, and Atlantic South America [15]. Australia is the world's largest producer and exporter of lupin seeds, accounting for 80–85% of the global lupin production [15]. Blue lupin (*Lupinus angustifolius* L.) is a multipurpose legume, one of the 200 species of lupins, and is an alternative to soybean due to its high protein content. Beyond these qualities, lupin represents a good source of bioactive compounds including oligosaccharides and dietary fibre [18]. Lupin has higher content in fibre than soybean [19], and the oil content in *L. angustifolius* is 5 to 6%. Moreover, lupin seeds contain polyphenols, carotenoids, and phytosterols, as highlighted by Boukid and Pasqualone [20].

Over recent decades, new genetic varieties of lupin appeared with improved protein content and thinner tastes [21]. The seed protein, which may correspond to 35–40% of the dry weight, is composed mostly of albumins and globulins in an approximate 1 to 9 ratio [22]. δ-conglutin is the most represented albumin protein—5% of the total seed proteins—and it is involved in plant defence against pathogens [23]. The protein content depends on location, climatic variations, and time of sowing [16]. In *L. mutabilis*, the highest protein content was found (44%), followed by *L. luteus*, *L. albus,* and *L. angustifolius* [16]. It was observed that the protein content increased after dehulling, and in whole lupin seeds it is as high or higher than that of soybeans. Its quality depends mainly on its amino acid composition and protein digestibility. In Table 1, the amino acid profile of the four sweet lupins species *L. albus, L. angustifolius, L. luteus*, and *L. mutabilis* is represented. The plant has a low content of sulphur amino acid (methionine and cysteine). Their content is more than three times lower than the suggested pattern of amino acid requirements [17]. The other essential amino acids (histidine, threonine, valine, isoleucine, tryptophan, leucine, lysine), however, cover or exceed these requirements. One study proved that lupin protein is high in glutamic, aspartic acid, and arginine [24]. Carvajal-Larenas et al. [16] suggested that the variation in the amino acid content in raw lupin species is imperceptible.

The functional and technological properties of lupin proteins (solubility, emulsifying, and foaming ability, water holding capacity) represent a great interest in the food industry. Due to these properties, lupin flour can successfully replace butter and eggs and can participate in the preparation of pasta, bread, biscuits, muffins, and cakes [19,25], as well as fried sweets or salty snacks. As a result, a better flavour, texture, water binding capacity, and shelf-life is reported, and it is recommended in low cholesterol diets [25]. Lupin does not contain gluten and can be used as a functional ingredient in gluten-free foods [19,26,27]. The yellow colour of lupin flour has its value in the preparation of pasta and noodle dishes, but can be removed easily, if necessary. This can be done by the addition of an aqueous 1.0% citric acid solution as described by [28]. However, the presence of allergenic peptides is an important issue [19]. Nevertheless, if the preventing role of lupin-based foods is proved in health issues, such as obesity, metabolic syndrome, diabetes, and cardiovascular diseases, this can be an important advantage over other plant proteins used in the food industry.

The properties listed below explain the fact that lupin offers new opportunities. Exploring more about the seed components and improving knowledge on their properties can lead to the development of new products for food and non-food industries.

Table 1. Essential amino acid content of different lupin species (g/100 g).

Amino Acid	*L. albus*	*L. angustifolius*	*L. luteus*	*L. mutabilis*	FAO [a]
Essential amino acid					
Histidine	2.0	2.6	3.1	3.5	1.6
Threonine	3.4	3.4	3.0	3.5	2.5
Valine	3.8	3.7	3.4	3.8	4.0
Methionine	0.7	0.7	0.6	0.8	2.3 [b]
Isoleucine	4.1	4.0	3.6	4.2	3.0
Tryptophan	0.9	0.9	0.9	0.8	0.7
Leucine	6.8	6.9	7.8	7.0	6.1
Lysine	4.5	4.6	4.5	5.8	4.8
Phenylalanine	3.4	3.7	3.7	3.5	4.1 [c]
Conditionally essential amino acids					
Cystine [d]	1.5	1.6	2.4	1.6	NA
Tyrosine	4.8	3.4	2.9	4.0	4.1 [c]
Arginine	12.4	12.0	9.1	10.2	NA
Glutamine	NA *	NA	NA	24.3	NA
Glycine	NA	NA	NA	3.8	NA
Proline	NA	NA	NA	3.8	NA

Source: Adopted from [16]. [a] Suggested pattern of amino acid requirements (FAO/WHO/UNU 2007) [17]. [b] Met + Cys; [c] Phe + Tyr; [d] Equivalent to 3.168 g/100 g cysteine; * NA—not available.

3. Lupin Bioactive Peptides and Their Properties

Table 2 shows the reported peptide sequences obtained by *Lupin* spp. and their predicted biological activities in purified fractions. These include lipid-lowering, anti-diabetic, anti-cancer, ACE-inhibitory, anti-inflammatory, anti-amnesic, antimicrobial, and antioxidant activities in a variety of experimental models. Moreover, Okagu et al. [29] reviewed the transepithelial transport, biostability, bioavailability, and safety concerns of lupin-derived peptides.

Table 2. Peptide sequences obtained from *Lupin* spp. and their predicted biological activities in purified fractions.

Predicted Bioactivity/Source	Protein/Peptide	Purification Method	Main Outcomes	Methods of Obtention	Reference
Osteoprotective/anti-inflammatory *L. angustifolius* L.	GPETAFLR	Ultrafiltration; chromatographic techniques	Prevention development and progression of osteoclast-related diseases, (anti-osteoclastogenic activity in human blood monocyte-derived osteoclasts), prevent the pro-inflammatory activation of microglial cells in cultures	in vitro hydrolysis	[30]
Anti-inflammatory *L. albus*	AKIQDKEGIPPDQQR; LIFAGKQLEDGR; LDDALRAEK; IQDKEGIPPDQQR; RRAIGK; RDDAASCLVR (10 μM).	ultrafiltration	Inhibition the lipopolysaccharide (LPS) which induce overproduction of pro-inflammatory mediators	in vitro-simulating gastrointestinal digestion	[31]
ACE-inhibitory activity *L. angustifolius* *L. albus* *L. luteus*	Unidentified peptides, IC50 = from 136 μg/mL (*L. luteus*) with chymotrypsin to 1053 μg/mL (*L. albus*) treated with umamizyme	Ultrafiltration	Lowering blood pressure, preventing/treating hypertension by inhibiting the angiotensin-converting enzyme (ACE)	Enzymatic Hydrolysis (pepsin, trypsin, chymotrypsin, flavourzym, umamizyme, and corolase PP)	[32]
L. mutabilis	purified fractions of conglutin-gamma RLGN; VNEGA; SEIGGA; SAPRST; GALGLGH; PQNLDL; AGGPQQR; PSELSGAAH; LPKHSDAD; LTFPGSAD	anion and cation FPLC analysis		Enzymatic digestion (pancreatin, pepsin)	[33]
Antioxidant activity *L. mutabilis*	PSELSGAAH	anion and cation FPLC analysis	Reduce the formation of oxidative products along with the induction of antioxidant enzymes in vivo	Enzymatic digestion (pancreatin, pepsin)	[33]

Table 2. *Cont.*

Predicted Bioactivity/Source	Protein/Peptide	Purification Method	Main Outcomes	Methods of Obtention	Reference
L. albus (DPPH) assay; (ABTS) assay; (FRAP) assay; Superoxide anion scavenging, Hydroxyl radical scavenging	FVPY	size exclusion chromatography (SEC)		Enzymatic hydrolysis-alcalase, chymotrypsin, pancreatin, pepsin, neutrase, thermolysin; Microbial hydrolysis (*Lactobacillus* spp.)-enzymes from bacterial and fungal sources	[34]
Cholesterol-lowering activity *L. albus* *L. albus*	E.LTFPGSAED.I (IC = 68.4 μM) GQEQSHQDEGVIVR (IC= 99.5 ± 0.56 μM), YDFYPSSTKDQQS (IC = 70 μM), LILPKHSDAD (IC = 147.2 μM)	Ultrafiltration	Modulate cholesterol metabolism in HepG2 cells; prevention of hypercholesterolemia	In vitro experiments using human recombinant HMGCoAR in silico molecular model and scoring approach; Enzymatic hydrolysis (pepsin)	[35–37]
Anti-amnesic activity *L. albus*	R.AVNELTFPGSAEDIER.L; K.ELTFPGSAEDIER.L; A.IPPGIPYWT.Y; E.LTFPGSAED.I;	Chromatographic methods Ultrafiltration	Potential inhibitors of prolyl endopeptidase (PEP) activity; applications in the prevention and treatment of mental disorders.	In vitro	[36]
	AGGPQQR	anion and cation FPLC analysis		Enzymatic hydrolysis (pancreatin; pepsin)	[33]
Antidiabetic activity *L. albus* L.	LTFPGSAED (IC = 228 μM)-LILPKHSDAD; GQEQSHQDEGVIVR;	Chromatographic methods	sources of DPP-IV inhibitory peptides useful for the prevention of type 2 diabetes	In vitro—enzymatic hydrolysis	[38]
Lupinus angustifolius *L. mutabilis*	RLGN; NVLSQL; LPKHSDAD; LTFPGSAD; AGGPQQR	anion and cation FPLC analysis		Enzymatic hydrolysis	[33]
L. albus *L. mutabilis*	VNEGA; SEIGGA; NPDDC; SAPRST; GALGLGH; VVVVDE; LPKHSDAD; PQNLDL; PSELSGAAH;				

Table 2. *Cont.*

Predicted Bioactivity/Source	Protein/Peptide	Purification Method	Main Outcomes	Methods of Obtention	Reference
Chemopreventive/Anticancer *L. albus* *L. mutalis* *L. montanus* *L. campestris*	Lunasin/lunasin-like peptides	-	Inhibition of HT-29 cells and MMP-9 gelatinolytic activity; Prevents breast cancer induced by chemical carcinogens	in vitro and in vivo assays	[39]
L. albus	Albumin and globulin fractions		Antiproliferative effects on HT-29 human colorectal cancer cells and affect MMP-9 gelatinolytic activity		
L. mutabilis. *L. albus*	VVVVDE	anion and cation FPLC analysis		Enzymatic hydrolysis	[33]
Antimicrobial activity of alkaloid extracts of *Pseudomonas aeruginosa* (from skin) *Klebsiella pneumoniae* (from inguinal skin) *L. allbus*	Unidentified peptides *P. aeruginosa*—MIC = 67 μM *K. pneumoniae*—MIC = 67 μM		Application in food preservation; therapeutic purpose in health care (Antibiotics)	Enzymatic hydrolysis by alcalase; chemical modification (esterification)	[40]
Antimicrobial activity against *Gram-positive bacteria* (*S. aureus* and *B. subtilis*) and *Gram-negative bacteria* (*P. aeruginosa* and *E. coli*) by *conventional well-diffusion assay* *L. angustifolius* L.	Lupin Protein Hydrolysates			Digestive proteases, microbial and plant proteolytic enzymes (alcalase is a bacterial extract from *Bacillus licheniformis*)	[41]
Allergen activity *L. albus*	conglutin A	Anion-exchange fast liquid chromatography	Potential allergen	in vitro IgE-binding studies	[42]

Table 2. *Cont.*

Predicted Bioactivity/Source	Protein/Peptide	Purification Method	Main Outcomes	Methods of Obtention	Reference
L. angustifolius	conglutin B		Lupin major allergen	Enzymatic hydrolysis (Papain, Alcalase and Pepsin) and lactic acid fermentation (*Lactobacillus sakei* ssp. carnosus, *Lactobacillus amylolyticus* and *Lactobacillus helveticus*) proteomic analysis	[43]
L. angustifolius *L. luteus*	Peptides with antigenetic properties (conglutin b) R.TNRLENLQNYR.I; R.IIEFQSKPNTLILPK.H; K.HSDADFILVVLNGR.A; R.ATITIVNPDK.R; R.LPAGTTSYILNPDDNQNLR.V; K.LAIPINNPGK.L; K.DQQSYFSGFSK.N; K.NTLEATFNTR.Y; R.GQEQSHQDEGVIVR.V; R.LLGFGINANENQR.N; R.TNRLENLQNYR.I; R.IVEFQSKPNTLILPK.H; K.HSDADYILVVLNGR.A; R.ATITIVNPDK; R.QAYNLEHGDALRLPAGTTSYILN; PDDNQNLR.V; R.LPAGTTSYILNPDDNQNLR.V; K.LAIPINNPGNFYDFYPSSTK.D; R.NTLEATFNTR.Y; R.NTLEATFNTRYEEIQR.I; R.YEEIQR.I; R.NFLAGSEDNVIR.Q	Peptides with antigenetic properties (conglutin b)	In vitro (simulating digestion taking place in digestive track), specific hydrolysis by trypsin	[44]

Abbreviations: A = alanine, R = arginine, N = asparagine, D = aspartic acid, C = cysteine, E = glutamic acid, Q = glutamine, G = glycine, H = histidine, I = isoleucine, L = leucine, K = lysine, F = phenylalanine, P = proline, S = serine, T = threonine, W = tryptophan, Y = tyrosine, V = valine; LPS-lipopolysaccharide, DPPIV: dipeptidyl peptidase IV; DPPH assay: 2,2-Diphenyl-1-picrylhydrazyl; ABTS assay: 2,2,-Azino-bis (3-ethylbenz-thiazoline-6-sulfonic) acid; FRAP assay: Ferric reducing antioxidant power; MIC—Minimum inhibitory concentration; HT-29—human colon cancer cell line; MMP-Matrix metalloproteinases; ACE—angiotensin converting enzyme; PEP-prolyl endopeptidase; FPLC—fast protein liquid chromatography.

3.1. Osteoprotective and Anti-Inflammatory Peptides

Inflammation is an immune response to the body that involves different immune system cells, releasing various substances (inflammatory mediators) that help to identify and kill pathogens, aging, and tumour cells. Excessive and uncontrolled inflammatory changes often lead to chronic diseases. It is not a coincidence that nutrition and immunity are strongly related [45]. Plant and animal sources were already reported to possess immunomodulatory peptides released during the digestion process affecting in this way the cellular functions and immunological responses [46].

Different studies have demonstrated the anti-inflammatory effects of lupin protein hydrolysates, obtained after enzymatic hydrolysis. An octapeptide, GPETAFLR (isolated from *L. angustifolius* L.), was observed to have osteoprotective and anti-inflammatory activity, evaluated in a human monocytic cell line [47] and THP-1-derived macrophages [48]. LPS have shown to reduce the gene expression of osteoclastogenic cytokines—TNF-α, IL-1β, and IL-6, and GPETAFLR—to raise anti-osteoclastogenic cytokine (IL-4, and IL-10) gene expression [30].

Non-alcoholic fatty liver disease (NAFLD) is a health problem associated with obesity and dyslipidaemias, which affects the world's population. It is caused by an accumulation of fat in the liver, and in this regard, the lupin peptide's role was investigated in the prevention of NAFLD in the hepatic tissues of mice [49].

Possessing the two activities, osteoprotective and anti-inflammatory, GPETAFLR is used also in the prevention of osteoclast-related diseases and chronic inflammation.

Besides GPETAFLR, several more lupin peptides (from *L. albus*) have shown their anti-inflammatory properties: AKIQDKEGIPPDQQR, LIFAGKQLEDGR, LDDALRAEK, IQDKEGIPPDQQR, RRAIGK, and RDDAASCLVR (isolated via in vitro gastrointestinal digestion).

A recent study also observed the anti-inflammatory activity of lupin peptides, focusing on the effects of p38 MAPK on TNF-mediated inflammatory responses and the pro-inflammatory cytokine production [49].

3.2. ACE-Inhibitory Peptides

Cardiovascular diseases are a very common problem worldwide. The interest in antihypertensive peptides increases as they are thought to be a safer alternative to the commercial drugs. These peptides are able to inhibit the angiotensin-converting enzyme (ACE) which converts decapeptide angiotensin I into octapeptide angiotensin II [32], a substance that causes blood pressure to rise. Peptides with ACE-inhibitory activity can be isolated from a wide range of plant and animal sources. Angiotensin II also have the ability to bind to LDL and to form modified lipoprotein which can slow down plaque formation and delay the development of atherosclerosis [50].

Many studies claimed that lupin protein-derived hydrolysates possess an inhibitory activity towards ACE. A study identified 10 peptides (from *L. mutabilis*), derived via enzymatic hydrolysis, possessing an ACE-inhibitory activity—RLGN, VNEGA SEIGGA, SAPRST, GALGLGH, PQNLDL, AGGPQQR, PSELSGAAH, LPKHSDAD, and LTFPGSAD [33]. Pepsin hydrolysates of *L. albus*, *L. angustifolius*, and *L. luteus* proved to be more efficient, resulting in a mean IC_{50} value of 186 ± 10 μg/mL [32]. Fractions in the molecular weight range of 2–3 and 3–5 kDa of blue lupin proteins also possessed ACE-inhibitory activity (IC_{50} values from 450 to 600 μg/mL) [51]. Alcalase hydrolysates showed ACE inhibitory activities with IC_{50} values ranging from 0.10 to 0.21 mg/mL [52].

3.3. Antioxidant Peptides

The cell damage can be slowed down or prevented by antioxidants. Free radicals are highly unstable molecules that are naturally formed when the body converts food into energy or when environmental and other pressures appear causing oxidative stress. It is caused by an imbalance between the production and removal of oxygen reactive species (ROS) in cells and tissue and can cause health problems including cancer, cardiovascular

diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration [53]. ROS are formed during normal aerobic cellular mechanism and can exert several different physiological roles (cell signalling) [54]. The expression of the beneficial effects of these peptides depends on their absorption. Their mechanisms of action could be defined in two types: hydrogen transfer and electron donation. The first one is more valued in the context of chain-breaking reactions and the second one (e.g., ABTS and DPPH) is related to the change of maximum absorbance due to the presence of an antioxidant compound [55].

Antioxidant peptides, produced by lupin proteins, can reduce the formation of oxidative products along with the induction of antioxidant enzymes in vivo [55]. The antioxidant activity depends mainly on the peptides' property, type of protease, and duration of hydrolysis. Two peptides are found in *Lupin* spp., PSELSGAAH (from *L. mutabilis*), derived from enzymatic hydrolysis, and FVPY (*L. albus*) through enzymatic/microbial hydrolysis, using a DPPH assay, ABTS assay, FRAP assay, superoxide anion scavenging, and Hydroxyl radical scavenging [33,34]. Moreover, it was demonstrated that lupin antioxidant and anti-inflammatory peptides are potentially resistant to the gastrointestinal tract and may reach the bloodstream to exert their beneficial effects [56].

3.4. Cholesterol-Lowering Peptides

Reducing LDL (low-density lipoprotein) is a key factor for the prevention of cardiovascular diseases. Hypocholesterolemic peptides were identified in lupin protein hydrolysates, one of which is LTFPGSAED obtained by the pepsin hydrolysis of beta-conglutin, known as inhibiting the HMGCoAR activity in vitro and having an IC_{50} value equal to 68.7 μM [35].

Lammi et al. [36] reported two other lupin peptides LILPKHSDAD (IC—147.2 ± 1.34 μM) [57] and GQEQSHQDEGVIVR (IC—99.5 ± 0.56 μM) [37] derived from *L. albus* as dipeptidyl-peptidase IV inhibitors decreasing the blood cholesterol. Cholesterol absorption was also suppressed by the action of YDFYPSSTKDQQS (IC—70 μM) in Caco-2 cells in vitro [34].

3.5. Potentially Anti-Amnesic Peptides

The mechanism of lupin peptides having an anti-amnesic activity is not revealed yet but several peptides, derived from purified fractions of conglutin-gamma (*L. mutabilis)*, are expected to have such bioactivity—AVNELTFPGSAEDIER, K.ELTFPGSAEDIER.L, A.IPPGIPYWT.Y, E.LTFPGSAED.I (obtained in vitro), and AGGPQQR (via enzymatic hydrolysis) [35]. They are potential inhibitors of prolyl endopeptidase (PEP) activity which has the potential to treat and prevent mental disorders.

3.6. Antidiabetic Peptides

Diabetes type 2 and insulin resistance are diseases related to hyperlipidaemia and obesity. Lupin is a medicinal food plant which can be used in the management of diabetes (high fibre and protein quantity). It is proved that that γ-conglutin treatment increased insulin gene expression at mRNA and protein levels [36]. LTFPGSAED (IC—228 Mm), LILPKHSDAD, and GQEQSHQDEGVIVR are known as dipeptidyl-peptidase IV inhibitors, which decrease the blood sugar levels using in vitro bioassay against human recombinant DPP-IV [38].

The lupin purified fractions, RLGN, NVLSQL, LPKHSDAD, LTFPGSAD AGGPQQR, VNEGA, SEIGGA, NPDDC, SAPRST, GALGLGH, VVVVDE LPKHSDAD, PQNLDL, and PSELSGAAH (from *L. angustifolius*, *L. mutabilis*, and *L. albus*), derived via enzymatic hydrolysis, are known to also have an antidiabetic activity [33].

3.7. Anticancer Peptides

Cancer is the leading cause of human death worldwide [58,59]. It is known as malignant neoplasm, a problem related to more than 100 different diseases, affecting various tissues and different types of cells [8]. It occurs when there is an abnormal cell growth and

when the cell division is difficult to control. Different size of peptides derived from different sources has been indicated to exert an anticancer effect in in vivo studies [60]. Two types of peptides with anti-cancer mechanisms are well known in the scientific world [8].

- Peptides with selective activity against carcinogenic cells. This type of peptides does not attack the normal cell; the action is direct to the carcinogenic cell.
- Peptides with nonselective activity. These are peptides with action to bacteria, carcinogenic cells, and against normal eukaryotic cells which mechanism is related to the permeabilization of the cell membrane mediated by electrostatic interaction. Key factors, related to the selectivity of peptides for carcinogenic cells, are electrostatic interactions between cationic peptides and anionic components of the cell membrane, surface area of the cell and membrane fluidity [8].

There are not many studies on lupin-derived anticancer peptides. However, there is some evidence for the presence of peptide lunasin [39]. It is a 45 amino acid residue peptide initially identified in the soybean with proved chemopreventive properties in vitro and in vivo.

Another study demonstrated the presence of the peptide "VVVVDE" expected to possess anticancer activity [33]. Meanwhile, Melke et al. indicated that protein peptides derived from lupin reduced cancer cell migration by inhibiting matrix metalloproteinase-9 (MMP-9) activity. The same authors warn that lupine-derived peptides modulatory effect contribute to cancer cell development. However, more studies are needed to identify the quantity of the active lupin peptides.

3.8. Antibacterial Peptides

The use of antimicrobial peptides in food preservation and for therapeutic purposes attracts the attention of manufacturers and scientists. The peptides can be found and isolated from different organisms-bacteria, fungi, plants, and animals and can be classified into numerous groups depending on their structure [46]. The physicochemical properties of the peptides such as solubility, size, charge, and hydrophobicity are strongly related to their antimicrobial functions [61]. A possible mechanism of action could be the attack of the membrane and/or cytoplasmic components of the microorganisms, the antimicrobial molecules can "change" their cellular functions and this can lead to cellular death [61]. Cell wall, nucleic acids, and proteins synthesis can be inhibited by the antimicrobial peptides by the action of several other enzymatic functions of the target cells [61].

Lupin protein hydrolysates, with potential as a bio-preserver, have shown antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) by a conventional well-diffusion assay [41]. The mode of action and effectiveness of antimicrobial peptides varies and depends on their structural characteristics. It was proved that the extracts from the lupin hull exhibit antibacterial activity against the indicator strains. The extracts of *L. luteus* have demonstrated the lowest activity, and the highest was represented by *L. albus*.

In another study, pancreatin hydrolysed peptide fractions showed the best antimicrobial activities with several fractions exhibiting $\geq$85% inhibition against *Bacillus cereus* and *Staphylococcus aureus* [51].

3.9. Allergen Peptides

Legume allergy is one of the most common. The EU Food Information Regulation declared that lupin is one of fourteen new food allergens [62]. The inhalation of the lupin flour (of *L. albus*) can be the cause of lupin allergy. Different methods were used to reduce the allergenicity of lupin, as the most effective proved to be that of Guillamónet al. [63], which destroys the allergen by autoclaving at 138 °C for 30 min and pressure drop for 3 min at 6 bar. As indicated in Table 2, conglutin A and conglutin B represent potential allergens. Conglutin B turns out to be the major one [64]. It was identified using proteomic analysis and recognized by serum IgE (from lupin allergic patient's sera matching with vicilin-like (7S-type) protein) [43]. The studied proteins from lupin hydrolysates bind IgE

antibodies, which molecular weight is in the range 43–45 kDa [65]. A subsequent study by Czubinski et al. [44] shows that hydrolysis can influence the decrease in the level of allergenicity in lupin seed proteins, and digestion carried out in vitro, the opposite.

R.TNRLENLQNYR.I, R.IIEFQSKPNTLILPK.H, K.HSDADFILVVLNGR.A, R.ATITIVNPDK.R, R.LPAGTTSYILNPDDNQNLR.V, K.LAIPINNPGK.L, K.DQQSYFSGFSK.N, K.NTLEATFNTR.Y, R.GQEQSHQDEGVIVR.V, R.LLGFGINANENQR.N, R.TNRLENLQNYR.I, R.IVEFQSKPNTLILPK.H, K.HSDADYILVVLNGR.A, R.ATITIVNPDK.R, R.QAYNLEHGDALRLPAGTTSYILN, PDDNQNLR.V, R.LPAGTTSYILNPDDNQNLR.V, K.LAIPINNPGNFYDFYPSSTK.D, R.NTLEATFNTR.Y, R.NTLEATFNTRYEEIQR.I, R.YEEIQR.I, and R.NFLAGSEDNVIR.Q are peptides (obtained from gamma conglutin) providing anti-genetic properties. A release of these peptides was observed after specific hydrolysis with trypsin and in vitro model simulating digestion taking place in the digestive tract [44].

Another study from Schlegel et al. [66] reported that the combination of enzymatic hydrolysis and fermentation of lupin protein hydrolysates has helped to reduce the major allergen lupin to a residual level of <0.5%. However, the inclusion of lupin in the European list of allergenic food components indicates that more intensive studies are necessary to investigate this type of allergy.

4. Perspectives

There is an increasing interest to identify more lupin multifunctional hydrolysates, and to study the mechanisms by which they provide their beneficial effect on the human body lowering LDL, blood pressure, improving glucose metabolism, and preventing and treating chronic diseases. In particular, the bioactive peptides, released through GRAS microbial fermentation, seem to be a good and affordable strategy. In addition, fermentation may be a strategy also for reducing legume allergy if the corresponding strain manages to break down these proteins. Due to their nutritional properties, food-derived peptides could be sold as nutraceuticals. Moreover, the high concentration of protein and the functional attributes such as foaming, emulsification, and adhesion offer an excellent motivation for incorporating lupin as an ingredient in different foods [23]. In addition to the benefits listed above, *Lupinus* spp. show a high adaptability to different climates and soil conditions and improve the soil quality due to their nitrogen fixation ability, reducing the need for chemical nitrogen fertilizers. In brief, the crop follows the European indications for a low-input agriculture model and can address the environmental issue for a more sustainable diet [67].

Author Contributions: Conceptualization, A.G. and I.D.; writing—original draft preparation A.G. and I.D.; writing—review and editing, all authors; visualization A.G. and I.D.; supervision, I.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Bulgarian Scientific Fund, grant No. KP-06-M36/2.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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