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Pleiotropic biological effects of *Lupinus* spp. protein hydrolysates

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ABSTRACT

Background: Over the last two decades, the demand for meat-free food has increased for health, environmental, and animal welfare reasons. Thus, research on the value of vegetable compounds as beneficial agents for human health has gained much attention. In particular, great interest has been shown in protein hydrolysation since this food technology facilitates the release of encrypted peptides with biological activities. In this sense, during the last 10 years, the biological activities of *Lupinus* spp. protein hydrolysates (LPHs) have been extensively studied.
Scope and approach: The aim of this review is to address all studies (*in silico, in vitro,* and *in vivo*) in which the biological activities (antioxidant, anti-inflammatory, etc.) of LPHs were described, both the whole hydrolysate and the derived peptides. Moreover, the physicochemical characteristics of LPH peptides are evaluated, and challenges and future perspectives for their fast application have been discussed.
Key findings and conclusions: LPHs exert many important biological effects in both the *in vitro* and *in vivo* systems. The main activities of LPHs described are antioxidant, immunomodulatory, hypotensive, hypoglycemic, and hypolipidemic. These findings point to LPHs as a possible new nutraceutical for human health, capable of preventing or treating some chronic diseases such as diabetes, dyslipidemia, and atherosclerosis. Finally, the development of a new generation of nanonutraceuticals to improve the metabolic stability and bioactivity of LPHs has been discussed.

1. Introduction

In recent decades, plant products and their derivatives have gained great popularity in nutrition due to the exponential increase in meat-free food demand, usually motivated by health, environmental, and animal welfare reasons. The increased consumption of these products has generated great interest in the study of the beneficial properties of their macro- and micronutrients, and their natural derived compounds. In this context, plant peptides have shown several health effects beyond their basic nutritional functions (Görgüç, Gençdağ, & Yılmaz, 2020; Nong & Hsu, 2022).

Nowadays, there is a global increase in the prevalence of the main chronic diseases, such as cardiovascular disease (CVD), cancer, respiratory disease, diabetes, and obesity, leading to a great burden of disease, including a large number of deaths, comorbidities, and healthcare costs. Currently, the main chronic diseases cause 32.4 million deaths each year due to their negative effects on the cardiovascular, antioxidant, and immune systems, as well as their disturbance in lipid and glycemic metabolism (World Health Organization, 2020).

Diet is the first exogenous risk factor for chronic diseases when there is a high sugar and fat intake. Consequently, diet changes are recommended as the first line of treatment, and functional foods intake is increasingly used as a complementary strategy for the prevention of these conditions. According to the last definition in 2014, functional foods are defined as "*natural or processed foods that contain biologically active compounds, which, in defined, effective, and non-toxic amounts,*

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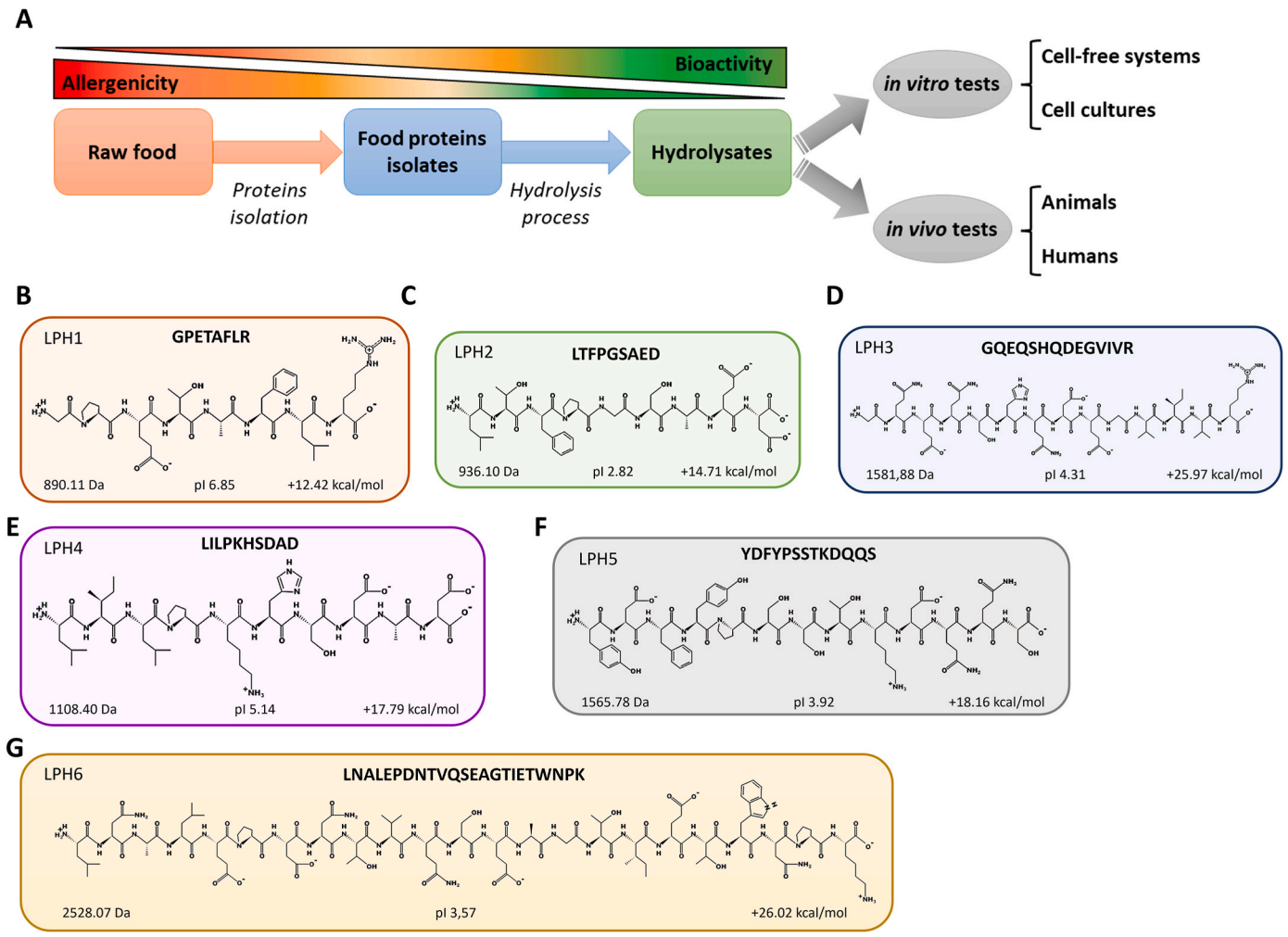

Fig. 1. Schematic representation of the different steps to obtain the protein hydrolysates and their biological application *in vivo* and *in vitro* tests (A). Primary structure of the six most studied lupin-derived peptides and their physicochemical properties (mass, isoelectric point -pI-, and hydrophobicity) (B–G).

provide a clinically proven and documented health benefit utilizing specific biomarkers for the prevention, management, or treatment of chronic disease or its symptoms" (Baker, Lu, Parrella, & Leggette, 2022). In this context, protein hydrolysates can be considered a lead compound for the development of protein-based functional foods with beneficial activities because enzymatic hydrolysis allows the generation of physiologically unobtainable neo-peptides, encrypted in the original proteins, beyond the intrinsic proteins of the food matrix. In fact, the bioactivity of these peptides is completely dependent on the method of enzymatic hydrolysis used. For this reason, protein hydrolysates exert multiple beneficial biological functions on human health unlike the original matrix of food (Chakrabarti, Guha, & Majumder, 2018). In this regard, numerous studies have shown the beneficial effects of vegetable-derived peptides on several biological processes; soy (Kim, Yang, & Kim, 2021), hemp (Santos-Sánchez, Álvarez-López et al., 2022), amaranth, quinoa, and chia, among others (Orona-Tamayo, Valverde, & Paredes-López, 2019; Valenzuela Zamudio & Segura Campos, 2022).

In particular, numerous investigations have shown the multifunctional effects of lupin-derived peptides related to the main components of chronic diseases (Millan-Linares et al., 2018; Santos-Sánchez, Cruz-Chamorro et al., 2021; Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022; Santos-Sánchez, Ponce-España et al., 2022). For this reason, along with the fact that lupin seeds have a high protein content, lupin-derived biopeptides are promising ingredients to consider for future functional foods and nutraceuticals.

2. *Lupinus* spp.

Lupinus is a genus of plants belonging to the Fabaceae family that is characterized by a shrub growth pattern and the presence of inflorescences of different colors above the height of the leaves (Wolko, Clements, Naganowska, & Nelson, 2011). It is cultivated worldwide (1,006,842 tonnes in 2019), being Australia (474,629 tonnes in 2019) and Europe (393,146 tonnes in 2019) the main producers (Food and Agriculture Organization, 2022).

Although there are approximately 600 species, only four, *L. albus*, *L. angustifolius*, *L. luteus*, and *L. mutabilis* are grown on a commercially. They are commonly known as white, blue, yellow, and Andean lupine, respectively, due to the color of their flowers or their geographic location. Their seeds, which are of different sizes and colors, are used principally for animal and human consumption. The nutritional properties of lupin have led to its widespread use in the Mediterranean diet, and, more recently, in the vegetarian diet. Moreover, it is widely used as an emulsifier in bakery. Due to possible allergic reactions, lupin has been added to the list of ingredients to be specified on food labeling, according to the European Regulation 2006/142/EC of December 22, 2006.

2.1. *Lupin composition*

Lupin seeds, like other legumes, are nutritionally high in protein, fiber, and micronutrients, and low in isoflavones and other antinutrients

Table 1
Biological effects of *Lupinus albus* hydrolysates.

Lupinus albus hydrolysates									
Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
Cell-free system									
Pepsin †	18 h	37	2			↑ACE-inhibitory activity			(Boschin et al., 2014a; 2014b)
Trypsin †	18 h	37	8			↑ACE-inhibitory activity			Boschin et al. (2014b)
Chymotrypsin †	18 h	37	8						
Corolase PP †	18 h	37	8						
Umamizyme †	24 h	60	7						
Flavourzyme †	24 h	45	7						
Pepsin (P) + Trypsin (T) †	4 h (P) + 24 h (T)	37	2 (P) + 8 (T)						
FVPY (hydrolysis with neutrase)	2 h	40	6.5	↓Lipid peroxidation ↑ABTS radical scavenging activity ↑DPPH radical scavenging activity ↑Superoxide anion scavenging ↑Hydroxyl radical scavenging					Babini et al. (2017)
LTFPGSAED (hydrolysis with pepsin)	18 h	37	2			↑DPP-IV activity inhibition ↑ACE-inhibitory activity		Encapsulation into nanogel (Pugliese et al., 2019; Pugliese et al., 2022)	Lammi et al. (2016)
LNALEPDNTVQSEAGTIETWNPK (hydrolysis with trypsin)	18 h	37	8	↑DPPH radical scavenging activity				Peptide included in a hydrogel	Pugliese et al. (2021)
Pepsin	2 h	37	2					↑bile-acid binding	Yoshie-Stark and Wäsche (2004)
Pepsin + pancreatin	2 h	37	2						
Alcalase (A) + Trypsin (T) + Flavourzyme (F)	7 h (A) + 4 h	50	8 (A + T)			↑DPP-IV activity inhibition		*in silico* identification of NPLL peptide as inhibitor of DPP-IV activity	Rivero-Pino et al. (2021)

(*continued on next page*)

Table 1 (*continued*)

Lupinus albus hydrolysates									
Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
	(T) + 2 h (F)		+ 6 (F)						
HepG2 cell line									
Pepsin Trypsin	18 h 18 h	37 37	2 8				↓HMG-CoAR activity ↓PCSK9 ↓PCSK9-LDL-R binding ↓HNF-1 ↑HMG-CoAR protein ↑pHMG-CoAR protein ↑SREBP2 ↑LDL-R ↑LDL-C Uptake ↑activation of PI3K/Akt/GSK3β kinases	Hydrolysates able to cross a monolayer of differentiated human intestinal cells (Caco-2 cell line)	(Lammi et al., 2014; Lammi et al., 2016; Lammi et al., 2016; Lammi et al., 2016)
GQEQSHQDEGVIVR (hydrolysis with pepsin)	18 h	37	2				↓PCSK9-LDL-R binding ↓$PCSK9^{D374Y}$ ↓$PCSK9^{D374Y}$-LDL-R binding ↓HMG-CoAR protein ↓HMG-CoAR activity ↓HNF-1 ↑LDL-R ↑LDL-C uptake ↑SREBP2	GQEQSHQDEGVIVR-derived mutated peptides showed to improve its effects (Lammi, Sgrignani, et al., 2018)	(Grazioso et al., 2018; Lammi et al., 2019)
YDFYPSSTKDQQS (hydrolysis with pepsin)	18 h	37	2				↓HMG-CoAR activity ↑LDL-R ↑LDL-C uptake ↑SREBP-1		Lammi, Zanoni, et al. (2018)
LILPKHSDAD (hydrolysis with pepsin)	18 h	37	2				↓HMG-CoAR activity ↓PCSK9 ↓HNF-1 ↓PCSK9-LDL-R binding ↑HMG-CoA-R protein	Its metabolite, LPKHSDAD, and its analogs, LYLPKHSDRD, LILPKASDAD, and LILPKHADAD, showed the same/ similar effects.	(Lammi et al., 2021; Lammi et al., 2022; Zanoni et al., 2017)

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Table 1 (*continued*)

Lupinus albus hydrolysates									
Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
							↑pHMG-CoA-R protein ↑pAMPK ↑LDL-R ↑LDL-C uptake ↑SREBP-2		
LTFPGSAED (hydrolysis with pepsin)	18 h	37	2			↑ACE-inhibitory activity	↓HMG-CoAR activity ↑HMG-CoAR protein ↑pHMG-CoAR protein ↑pAMPK ↑LDL-R ↑LDL-C uptake ↑SREBP-2		(Lammi et al., 2020; Zanoni et al., 2017)
Caco-2 cell line									
Pepsin Trypsin	18 h 18 h	37 37	2 8				↑HMG-CoAR protein ↑pHMG-CoAR protein ↑SREBP2 ↑LDL-R		Lammi et al. (2016)
LTFPGSAED (hydrolysis with pepsin)	18 h	37	2			↑DPP-IV activity inhibition ↑ACE-inhibitory activity		↑Circulating DPP-IV activity inhibition from human serum. Encapsulation into nanogel (Pugliese et al., 2019)	(Lammi et al., 2020; Lammi, Bollati, et al., 2018)
LTFPG (hydrolysis with pepsin)	18 h	37	2			↑ACE-inhibitory activity	↓HMG-CoAR activity	It is able to cross a monolayer of differentiated human intestinal cells (Caco-2 cell line)	(Lammi et al., 2020)
HK-2 cell line									
LTFPGSAED (hydrolysis with pepsin)	18 h	37	2			↑ACE-inhibitory activity			(Lammi et al., 2020)
RAW 264.7 cell line									
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 2 h (Pa)	37	3 (P) + 7 (Pa)	↓NO	↓IL-1β, IL-6, TNF-α ↓MCP-1 ↓TLR-4 ↓CCR2				(Gao et al., 2020)[37]
IQDKEGIPPDQQR [hydrolysis with pepsin (P) + pancreatin (Pa)]	2 h (P) + 2 h (Pa)	37	3 (P) + 7 (Pa)	↓NO	↓IL-1β, IL-6, TNF-α ↓MCP-1 ↓TLR-4 ↓CCR2 ↓p38 MAPK pathway				Gao et al. (2020)

Biological effects observed in *Lupinus albus* protein hydrolysates in the different *in vitro* models. ↑, increase; ↓, decrease. † Biological effects observed also in *L. luteus*.

Table 2
Biological effects of *Lupinus angustifolius* hydrolysates.

Lupinus angustifolius hydrolysates									
Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
Cell-free system									
Pepsin	18 h	37	2			↑ACE-inhibitory activity			Boschin et al. (2014b)
Trypsin	18 h	37	8						
Chymotrypsin	18 h	37	8						
Corolase PP	18 h	37	8						
Umamizyme	24 h	60	7						
Flavourzyme	24 h	45	7						
Pepsin (P) + Trypsin (T)	4 h (P) + 24 h (T)	37	2 (P) + 8 (T)						
Izyme	1 h	50	10		↓Thrombin activity ↓Transglutaminase ↓PLA2 ↓COX				Millan-Linares, Yust, et al. (2014)
Alcalase	1 h	50	8						
Izyme (I) + Alcalase (A)	1 h	50	10 (I) + 8 (A)						
HepG2 cell line									
Alcalase	4 h	50	10	↓ROS levels ↑SOD activity ↑GCL, SLC7A11, SRXN1, GPx, and SOD gene expression ↑FRAP ↑ABTS radical scavenging activity ↑DPPH radical scavenging activity		↑ACE-inhibitory activity		↓Cell death H_2O_2-induced	Guo et al. (2018)
Trypsin	4 h	37	7.5	↑FRAP ↑ABTS radical scavenging activity ↑DPPH radical scavenging activity		↑ACE-inhibitory activity			Guo et al. (2018)
Pepsin	4 h	37	2						
THP-1 cell line									
Alcalase	1 h	50	8	↓NO	↓TNF-α, IL-6, IL-1β ↓CCR2 ↓THP1 migration index ↑CCL18				Millan-Linares, Bermúdez, et al. (2014)
Izyme (I) + Alcalase (A)	1 h	50	10 (I) + 8 (A)						
GPETAFLR (hydrolysis with Alcalase)	15min.	50	8	↓NO	↓TNF-α, IL-6 ↓CCR2,CCL2 ↑CCL18 ↑IL-10				Millan-Linares et al. (2015)
Alcalase	1 h	50	8	↓ROS ↓NO	↓TNF-α, IL-6, IL-1β ↑IL-10				(Montserrat-de la Paz et al., 2021)
ARPE-19 cell line									
GPETAFLR (hydrolysis with Alcalase)	15min.	50	8	↓ROS ↓NO ↓iNOS ↑GSH	↓IL-1β, IL-6, IFN-γ, TNF-α, VEGF				Millan-Linares et al. (2019)
Monocytes-differentiated osteoclasts									

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Table 2 (*continued*)

Lupinus angustifolius hydrolysates									
Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
GPETAFLR (hydrolysis with Alcalase)	15min.	50	8		↓TNF-α, IL-6, IL-1β ↑IL-10, IL-4			↓Osteoclastogenesis (TRAP, OSCAR, RANK, and CATHK) ↑Osteoblastogenesis (OPG)	Millan-Linares et al. (2018)
BV2 Cell Line									
GPETAFLR (hydrolysis with Alcalase)	15min.	50	8	↓iNOS	↓IL-1β, IL-6, TNF-α ↓CCR7 ↑IL-10 ↑Arg1, Ym-1				Lemus-Conejo, Millan-Linares, et al. (2020)
Ex vivo (human monocytes)									
GPETAFLR (hydrolysis with Alcalase)	15min.	50	8		↓M1-monocytes ↓CD64, CD80 ↓CCR2, CCL2 ↓IL-1β, IL-6, TNF-α ↑M2-monocytes ↑CD200R, MRC-1 ↑IL-10				(Montserrat-de la Paz et al., 2019)
Ex vivo (human PBMCs)									
Alcalase	15min.	50	8	↑SOD and CAT activities ↑TAC	↓IL-2, IL-12, IFN-γ, TNF-α, IL-17, IL-9, IL-13 ↑Th2/Th1 ratios ↑IL-10 gene expression			↓Cell death H_2O_2-induced	Cruz-Chamorro et al. (2019)

Biological effects observed in *Lupinus angustifolius* protein hydrolysates in the different *in vitro* and *in vivo* models. ↑, increase; ↓, decrease.

(Supplementary Table S1) (Arnoldi, Zanoni, Lammi, & Boschin, 2015; Erbaş, Certel, & Uslu, 2005; Rybiński et al., 2018; Uzun, Arslan, Karhan, & Toker, 2007). The average percentage of lupin seed protein is 38%, being the *L. albus* the species with the highest content. Compared to other legumes, lupin seeds have a higher percentage of protein than lentils (33%) or chickpeas (25%), only surpassed by soy (45%). Furthermore, the essential amino acid profile is slightly lower than that of soy. Lupin seeds are also a good source of valuable oil with a balanced profile of fatty acid composition, with 84.5% of unsaturated ones. The main proteins in lupin seeds are albumin and globulins (ratio 1:9). Globulins are classified into four groups: α, β, γ, and δ conglutins. Although the exact proteins responsible for the allergic reaction to lupin are unknown, it seems that conglutins could be responsible. Within conglutins, β-conglutin might be the one with the highest allergenic activity (Villa, Costa, & Mafra, 2020). Conglutins are not only storage proteins in lupin seeds, but also contain bioactive peptides with numerous beneficial health properties (Lima-Cabello et al., 2017). These peptides, with an amino acids length range between 2 and 50, remain inactive in the intact protein; however, once released by proteases during the digestion process or by external hydrolysis, they are absorbed by the intestinal tract, reaching the organs, modulating the target of interest, and exerting their biological activities. Thus, although the nutritional and biofunctional properties of lupin proteins have been widely studied (Lo, Kasapis, & Farahnaky, 2021), in the past decade there has arisen a great interest about the production, characterization, and applications of biopeptides and protein hydrolysates from lupin.

3. Protein hydrolysates

Protein hydrolysates are defined as a mixture of peptides and/or free amino acids that are produced by partial or extensive hydrolysis. The normal process for obtaining them begins with the isolation from raw food. The next step is the hydrolysis process, in which protein hydrolysates can be obtained through chemical or enzymatic hydrolysis (including fermentation) processes (Clemente, 2000). Chemical hydrolysis, nevertheless, has some drawbacks, as the use of high-temperature and strong acid solutions that deteriorate the nutritional quality of the final peptides, even creating anti-nutritional secondary products (Lys - Ala). From an ecological point of view, this hydrolysis is unsuitable. On the contrary, the main disadvantage of enzymatic hydrolysis is the cost of production, essentially derived from the price of the enzymes and the low efficiency of fermentation by microorganisms. Thus, enzymatic hydrolysis is the most widely used technique, as it is carried out under low aggressive experimental conditions, and has a great efficiency in the generation of peptides (Clemente, 2000). The first and easier step to identify any possible biological activity of a hydrolysate is the *in vitro* approach, including both cell-free or cultured cells (primary cultures or cell lines) systems. More complex systems include *in vivo* studies, which are carried out in experimental animals or, directly, in humans (Fig. 1A).

It is important to note that the profile of the hydrolysates generated (net charge, hydrophobicity grade, molecular weight, etc.), as well as their biological effects, depend on the protein substrate, the enzyme used (Alcalase, papain, Izyme, trypsin, pepsin, among others) and the conditions under which the hydrolysis process is carried out (pH, temperature, and time) (Chakrabarti et al., 2018). In this context, Alcalase, trypsin, and pepsin are the most widely used enzymes in the generation of bioactive peptides.

In addition to the nutritional values and biological activities of protein hydrolysates, the hydrolysis process might cleave those epitopes implicated in allergic processes, generating hypoallergenic products, suitable for allergic people. In this regard, wheat or milk hydrolysates are less allergenic due to the breakage of gluten, β-lactoglobulin, and α-lactalbumin proteins during this process (El Mecherfi et al., 2021). In the case of plant protein, it has been observed a low antigenicity in the hydrolysis of soybean β-conglutin through alkaline protease from *Bacillus subtilis,* and a reduction in antigenicity (>90%) was obtained in extensively hydrolyzed chickpea proteins by sequential treatment with endo- and exopeptidase.

Table 3
in vivo effects of *Lupinus albus* and *Lupinus angustifolius* hydrolysates.

Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
Wistar rats									
Alcalase (A) + Flavourzyme (F) (from *Lupinus albus*)	30min. (A) + 30min. (F)	50	9 (A) + 8.5 (F)			↓plasmatic and renal glucose	↓plasmatic TG ↓hepatic TC and TG	Renal function: ↓urinary volume ↓urea ↓creatinine ↓albumin ↓citrate ↓phosphorus ↓muscular tunic ↑fresh weight of caecum ↑plica mucosae length	Kapravelou et al. (2013)
ApoE$^{-/-}$ mice									
Alcalase (from *Lupinus angustifolius*)	15min.	50	8	↓OxLDL ↓iNOS ↑SOD and CAT activities ↑TAC	↓Lymphocytes aortic infiltration ↓IFN-γ, TNF-α, IL-1β, IL-18 ↓CXCL1, CXCL2, CCL2 ↓GM-CSF ↑Th2/Th1 ratio		↓LDL-C ↓TC ↓TG	↓CD36 ↓P-selectin ↓MMP2	Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al. (2022)
Alcalase (from *Lupinus angustifolius*)	15min.	50	8	↑SOD, GPx, and GR activities ↑FRAP			↓hepatic TC ↓hepatic TG ↓hepatic steatosis ↓CD36 ↓adipocyte hypertrophy ↓adipocytes ↑Adiponectin receptor		Santos-Sánchez, Cruz-Chamorro et al. (2021)
Alcalase (from *Lupinus angustifolius*)	15min.	50	8				↓HMG-CoAR protein ↓PCSK9 ↓HNF-1 ↓SREBP2 ↓LDL-R ↑pHMG-CoAR protein ↑pAMPK		Santos-Sánchez, Cruz-Chamorro, Bollati, et al. (2022)
Alcalase (from *Lupinus angustifolius*)	15min.	50	8					Anxiolytic-like effects	(Santos-Sánchez, Ponce-España et al., 2022)
Obese mice									
GPETAFLR (hydrolysis with Alcalase; from *Lupinus angustifolius*)	15min.	50	8		↓IL-1β, IL-6, TNF-α ↑IL-10		↓body weight ↓TG ↓hepatic steatosis ↓fatty acid synthase ↓AST, ALT, ALP, LDH ↓liver weight ↓leptin ↓leptin receptor ↑PPARα ↑UCP1		Lemus-Conejo, Grao-Cruces et al. (2020)
C57BL/6J mice									
	15min.	50	8						

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Table 3 (*continued*)

Enzyme	Time	T (°C)	pH	Antioxidant effects	Immunomodulatory effects	Antihypertensive and hypoglycemic effects	Hypolipidemic effects	Others effects	Reference
GPETAFLR (hydrolysis with Alcalase; from *Lupinus angustifolius*)					↓IL-1β, IL-6, TNF-α ↓CCR7 ↑IL-10 ↑Arg1, Ym-1			↓Total microglia ↓M1 microglia ↑M2 microglia	Lemus-Conejo, Millan-Linares, et al. (2020)
Clinical trial									
Alcalase (from *Lupinus angustifolius*)	15min.	50	8	↑TAC ↑ORAC	↓IL-2, IFN-γ, TNF-α ↑Th2/Th1 ratio		↓LDL-C/HDL-C ratio		Cruz-Chamorro et al. (2021)

Biological effects observed in *Lupinus angustifolius* protein hydrolysates in the different *in vitro* and *in vivo* models. ↑, increase; ↓, decrease.

4. Aim of this review

Although some interesting reviews about the lupin protein and derived peptides have recently been published (Garmidolova, Desseva, Mihaylova, & Lante, 2022; Okagu et al., 2021), little or no information has been provided about hydrolysates of lupin proteins. In fact, these reviews had focused on bioactive peptides but not the bioactivity of the hydrolysates, physicochemical characteristics and the methods of generation (types of enzymes, conditions, etc.), something of pivotal interest for future industrial commercialization. In this review, we report a comprehensive state-of-the-art of the lupin protein hydrolysates (LPHs) and their peptides, with special attention to their biological activity.

In particular, we focus on all scientific articles in which the biological activities (antioxidant, anti-inflammatory, etc.) of LPHs or LPHs-derived peptides were studied. The enzymatic hydrolysis conditions used for LPHs generation have also been reviewed. This review addresses *in silico*, *in vitro*, and *in vivo* (animal models and clinical trials) studies. Moreover, a critical description of the physicochemical characteristics and bioavailability of LPHs has been shown. Finally, future technological perspectives have been highlighted.

Therefore, to date, no previous review has focused on the study of the biological effects and their action mechanisms of protein hydrolysates or peptides from lupin. To our knowledge, this is the first review focused on the wide range of the biological effects of lupin protein hydrolysates or peptides and their action mechanisms, representing an important piece of knowledge in the field of food and technology sciences.

5. Biological activities of lupin protein hydrolysates

LPHs and lupin-derived bioactive peptides (in Fig. 1B–G, the primary structure and physicochemical properties of the six most studied lupin-derived peptidesare shown) have been shown to play important biological activities, including antioxidant, immunomodulatory, antihypertensive, hypoglycemic, and lipid-lowering effects in both *in vitro* (summarized in Tables 1 and 2) and *in vivo* (summarized in Table 3) studies.

5.1. Antioxidant effects

Reactive oxygen species (ROS) are the main molecules involved in the generation of oxidative stress, and they are highly reactive. The main ROS are superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The reaction of ROS with nitric oxide (NO) generates reactive nitrogen species (RNS), the main of which are peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2) that generate nitrosative stress. ROS and RNS can interact, giving rise to reactive oxygen-nitrogen species (RONS). Excessive generation of RONS, normally due to external stimuli such as smoke or radiation, causes tissue damage. For this reason, the organism has enzymatic and non-enzymatic antioxidant systems which restrain RONS overproduction. To remove the RONS, several antioxidant molecules are produced, such as glutathione (GSH), or the antioxidant system, a complex antioxidant biological machinery, is activated.

The principal antioxidant enzymes involved in the antioxidants defense are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). An increase in the gene expression of these enzymes, mediated by several transcription factors, such as nuclear factor erythroid 2-related factor 2, contributes to an increase in total antioxidant capacity. The principal assays which have studied total antioxidant capacity are the ferric reducing antioxidant power (FRAP) assay, the oxygen radical absorbance capacity (ORAC) assay, and the Trolox equivalent antioxidant capacity (TEAC) assay. Additionally, copper reducing assay (TAC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical method are used.

5.1.1. GSH-based antioxidant activity

GSH is one of the most important antioxidant scavengers that participate in many of the mechanisms which eliminate excessive RONS production. GSH is a tripeptide formed by glutamate, cysteine, and glycine, and its biosynthesis takes place in the cytosol obtained from the precursor glutamate, which is introduced into the cell by the cystine/glutamate transporter (SLC7A11) (Lewerenz et al., 2013). Subsequently, two other key enzymes participate in the synthesis of GSH: glutamate-cysteine ligase (GCL) and glutathione synthetase (Lewerenz et al., 2013). This means that the control of these steps can be crucial for the improvement of the GSH-mediated antioxidant defense.

There are two studies of the GSH-based antioxidant effects of LPHs in the human retinal pigment epithelial (RPE) cell line (ARPE-19) (Millan-Linares et al., 2019) and the human hepatocyte (HepG2) (Guo, Shang, Strappe, Zhou, & Blanchard, 2018) cell lines (Fig. 2A). A peptide obtained from Alcalase hydrolysis (LPH_{alc}), with the amino acids sequence: glycin-proline-glutamine-threonine-alanine-pheomelanin -leucine-arginine (GPETAFLR), abbreviated as LPH1, reverses the pro-oxidant status induced by H_2O_2 in ARPE-19 cell line (Millan-Linares et al., 2019). Treatment with 50 or 100 μg/mL LPH1 for 24 h reduces ROS and NO production, and increases the GSH content. Furthermore, LPH1-treatment decreases inducible nitric oxide synthase (iNOS) gene expression, the main NO producer, in lipopolysaccharide (LPS)-stimulated ARPE-19 cells (Millan-Linares et al., 2019). These results are of special interest because a RPE alteration can produce an irreversible damage to photoreceptors and, therefore, impair central vision. Moreover, transepithelial transported LPHs have been shown to reduce ROS and NO production in LPS-stimulated THP-1 cells (Montserrat-de la Paz et al., 2021).

In addition, LPHs from *L. angustifolius*, obtained by 4 h of Alcalase digestion at 50 °C and pH 8, has been shown to decrease ROS levels and increase the expression of the GCL and SLC7A11 genes in H_2O_2-stimulated HepG2 cells. Furthermore, LPHs-treatment (4, 6, and 8 mg/mL) for 24 h increases the viability of HepG2 cells incubated with 300 μM of H_2O_2 for 4 h (Guo et al., 2018). A similar result was obtained in human peripheral blood mononuclear cells (PBMCs), in which LPHs, obtained by Alcalase enzyme activity for 15 min at 50 °C and pH 8, decreased the

50 mM H_2O_2-induced cell death for 1 h (Cruz-Chamorro et al., 2019).

These findings show the antioxidant potential of LPHs mediated by the GSH system.

5.1.2. Enzymatic-based antioxidant activity

SOD catalyzes the dismutation of the highly toxic radical O_2^- into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), a dangerous substance, precursor of •OH, the most devastating for biological macromolecules ROS. In this sense, H_2O_2 must be quickly removed. There are three enzymes involved in the H_2O_2 detoxification process. On the one hand, the enzyme CAT converts millions of H_2O_2 molecules into H_2O and O_2 every second; on the other hand, the enzyme GPx reduces H_2O_2 to H_2O, using GSH as the electron donor. Oxidized GSH is subsequently reduced by GR to re-establish the antioxidant capacity; for this reason, the combination of GPx and GR enzymatic activities plays a key role in the maintenance of the GSH levels.

Besides, SOD, CAT, GPx, GR, and sulfiredoxin (SRXN) enzymes can also participate in ROS degradation by oxidation of peroxiredoxin (Prdx).

Hydrolysis of *L. angustifolius* proteins with Alcalase for 4 h at 50 °C and pH 10, generated peptides capable of increasing the antioxidant activity of SOD, as well as SRXN-1, GPx, and SOD gene expression in HepG2 cells (Guo et al., 2018). Similar results are observed when *L. angustifolius* proteins were hydrolysated with Alcalase for 15 min at 50 °C and pH 8. In this case, LPHs increase the activity of the SOD and CAT enzymes in *in vitro* cultured PBMCs. Moreover, a strong correlation between the TAC and SOD and CAT activities was observed (Cruz-Chamorro et al., 2019).

In addition, apolipoprotein E (ApoE) knockout ($ApoE^{-/-}$) mice fed with a Western diet (WD) and treated for twelve weeks with the same LPH_{alc} have also shown an increase in SOD and CAT plasma activities (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022), as well as an increase in liver of SOD, GPx, and GR activities (Santos-Sánchez, Cruz-Chamorro et al., 2021). Moreover, LPH_{alc} also decrease iNOS mRNA gene expression and levels of oxidized low-density lipoprotein (oxLDL), a major marker in atherogenesis process (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022).

Down-regulation of iNOS gene expression has also been observed in ARPE-19 (Millan-Linares et al., 2019) and BV2 microglia cell lines (Lemus-Conejo, Millan-Linares, et al., 2020) treated with LPH1.

Guo and collaborators have shown that the hydrolysis of *L. angustifolius* proteins with Alcalase, trypsin or pepsin can generate peptides that increase radical scavenging activity by FRAP, TEAC, and DPPH assays in HepG2 cells (Guo et al., 2018). Also, the hydrolysis of *L. albus* with neutrase for 2 h at 40 °C and pH 6.5 generates peptides with antioxidant capacity (Babini, Tagliazucchi, Martini, Dei Più, & Gianotti, 2017). In particular, this study identified the peptide FVPY (LPH2), which possesses a strong *in vitro* antioxidant activity, increasing radical scavenging activity. Authors associated these effects with the chemical composition of the peptide, indicating that the presence of tryptophan (W) or tyrosine (Y) at the C-terminus is essential to explain its biological activity. Moreover, the presence of phenylalanine (F) has been associated with the inhibitory role of LPH2 in lipid peroxidation (Babini et al., 2017).

Recently, a thixotropic lupin peptide hydrogel has been shown to possess intrinsic antioxidant activity, quantified by a DPPH assay (Pugliese, Arnoldi, & Lammi, 2021). In addition, *in silico* studies have shown the potential capacity of this hydrogel to inhibit the angiotensin-converting enzyme (ACE), the dipeptidyl peptidase (DPP) -IV, and alpha-glucosidase activities.

Moreover, LPHs obtained from *L. angustifolius* and using Alcalase enzyme for 15 min at 50 °C and pH 8 not only increase the antioxidant capacity in the plasma (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022) but also in *ex vivo* cultured PBMCs (Cruz-Chamorro et al., 2019), as well as in PBMCs from the participant in the clinical trial Lupine-1 who ingested a beverage based on LPH_{alc} during four week (Cruz-Chamorro et al., 2021).

These studies point out the great antioxidant potential of *L. angustifolius* protein hydrolysates, not only through *in vitro* approaches, but also in animal and human physiological systems.

5.2. Immunomodulatory effects

There are many studies on the anti-inflammatory properties of LPHs using *in vitro* approaches such as cell-free systems (Millan-Linares, Yust, Alcaide-Hidalgo, Millán, & Pedroche, 2014), cell lines (Lemus-Conejo, Millan-Linares, et al., 2020; Millan-Linares, Bermúdez, Yust, Millán, & Pedroche, 2014; Millan-Linares et al., 2019; Millan-Linares, Millán, Pedroche, & Yust, 2015) and primary human cells (Cruz-Chamorro et al., 2019), as well as *in vivo* animal models (Lemus-Conejo, Grao-Cruces et al., 2020; Lemus-Conejo, Millan-Linares, et al., 2020; Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022) and human studies (Cruz-Chamorro et al., 2021).

5.2.1. Antithrombin action

External stimuli can alter homeostasis of biological processes. The immune system is involved in the defense and maintenance of health status acting on the organism through molecules such as eicosanoids and cytokines, produced by several types of immune cells (Abdulkhaleq et al., 2018). Eicosanoids include different signaling molecules that come from the polyunsaturated fatty arachidonic acid (AA). Among eicosanoids, prostaglandins (PGs) are mediators of several pro-inflammatory processes, leading to cytokine production and cell proliferation. Cyclooxygenases (COX) are the main enzymes involved in the production of PGs from AA.

Thrombin (Thr) is a trypsin-like protease involved in the hemostatic process and thrombus formation through the transformation of fibrinogen into fibrin, the main protein involved in blood clotting, along with the coagulation factor XIIIa. Thr also participates in the stimulation of the transglutaminase activity that transforms the inactive form of factor XIII into the active form (XIIIa) (Fig. 2B). Thr also mediates the activation of phospholipase A2 (PLA2) by protease-activated receptors. PLA2 is involved in the release of AA, which is subsequently transformed by COX into PGs. In fact, a increase in PLA2 has been shown in several inflammatory diseases. Therefore, the control of these enzymes is considered a therapeutic target for the control of several inflammatory processes. In this context, it has been shown that hydrolysates of *L. angustifolius* protein, obtained with Alcalase and/or Izyme inhibit Thr activity, as well as decrease transglutaminase activity (Millan-Linares, Yust, et al., 2014) in cell-free *in vitro* systems. The decrease in fibrin and factor XIIIa actions induced by the inhibition of these activities can also prevent atherosclerotic events and thrombus formation. In addition, these peptides have shown a great capacity to decrease PLA2 activity, preventing the formation of AA, as well as inhibiting COX-2 activity, the inducible form of COX (Millan-Linares, Yust, et al., 2014).

Hence, the combined action of LPHs and the signaling mediated by PLA2 in reducing PGs generates a protector microenvironment characterized by a reduction in pro-inflammatory processes, leukocyte migration, oxidative stress, cell proliferation, and cytokine production. LPH_{alc} has shown greater effectiveness in reducing transglutaminase, PLA2, and COX-2 activities in comparison to the hydrolysis with Izyme (LPH_{izy}). Interestingly, the hydrolysates generated through a sequential hydrolysis with Izyme and Alcalase (LPH_{i+a}) have only the capacity to inhibit transglutaminase and PLA2 activities (Millan-Linares, Yust, et al., 2014). These results suggest that a second hydrolysis with a different enzyme can eliminate the biological peptides generated in the first one.

Taking all these results into account, LPHs could be considered an option for its use in nutritional antithrombotic therapies.

5.2.2. Anti-inflammatory actions

T helper cells (Th), type 1 (Th1) and type 2 (Th2), and the monocytes/macrophages (Mφ) are key cell populations involved in immune

regulation. Th1 cells mediate pro-inflammatory responses, releasing interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL) -1β, and IL-6; while Th2 cells mediate anti-inflammatory responses by means of IL-4, IL-13, and IL-10 releasing. Mφ also participate in inflammation. Two Mφ subtypes have widely been described: the pro-inflammatory M1 and the anti-inflammatory M2 phenotypes. M1 cells are characterized by iNOS and TNF-α production. In contrast, M2 phenotype is characterized by IL-10 and arginase (Arg) production. Therefore, TNF-α and IL-10 are key regulatory molecules for the immune status of the organism. In addition, IL-17 is another important pro-inflammatory cytokine produced by Th17 cells. Pro-inflammatory cytokines have been shown to increase in many diseases, such as atherosclerosis, neurodegeneration, and chronic diseases (Langhans, 2006); thus, many pharmacological treatments focus on inhibiting their expression or production.

LPHs obtained by hydrolysis with Alcalase for 1 h, or with the combination of this enzyme with Izyme, show anti-inflammatory properties in *in vitro* differentiated Mφ from the THP-1 cell line, a human monocytic cell line (Millan-Linares, Bermúdez, et al., 2014). These LPHs (at a concentration of 0.5 mg/mL) reduce IL-1β, IL-6, and TNF-α production, whereas increase the chemokine (C–C motif) ligand (CCL) 18, an anti-inflammatory marker gene of Mφ, and decrease NO production, an important molecule implicated in the inflammation and the immune reaction (Millan-Linares, Bermúdez, et al., 2014).

In addition to CCL18, the CCL2/chemokine (C–C motif) receptor (CCR) 2 axis is important in the regulation of monocyte extravasation to the inflammation site and in the chemotactic activity of Mφ (Bianconi, Sahebkar, Atkin, & Pirro, 2018). Interestingly, both LPH_{alc} and LPH_{i+a} reduce the expression of the CCR2 gene in THP-1-derived Mφ and decrease the migration index of the cells by 45–60% (Millan-Linares, Bermúdez, et al., 2014).

A recent study has shown that LPH_{alc} retains its anti-inflammatory activity after crossing the intestinal barrier by using the transepithelial human intestinal cell line (Caco-2 cells) barrier system. Caco-2 absorbed LPH_{alc} at 0.1 and 0.5 mg/mL are able to reduce IL-1β, IL-6, and TNF-α mRNA and proteins levels in THP-1 cells stimulated with LPS (Montserrat-de la Paz et al., 2021). Likewise, simulated gastroduodenal digestion of *L. albus* protein (pepsin + pancreatin digestion = LPH_{p+pa}) inhibits pro-inflammatory cytokine production in LPS-stimulated RAW 264.7 cells, a mouse Mφ cell line (Gao et al., 2020). In particular, six peptides were identified capable of inhibiting the production of TNF-α, IL-1β, IL-6, monocyte chemoattractant protein-1, and NO, as well as the production of cytokine receptors (toll-like receptor 4 and CCR2), the most active of which was IQDKEGIPPDQQR peptide (LPH3). In addition, transcriptome analysis showed that LPH3 down-regulates the p38 mitogen-activated protein kinase (MAPK) pathway, one of the most important metabolic pathways involved in the pro-inflammatory process.

On the other hand, the *ex vivo* treatment of PBMCs with LPH_{alc}, obtained by hydrolysis of *L. angustifolius* with Alcalase for 15 min, decreased the phytohemagglutinin (PHA)-stimulated pro-inflammatory cytokine production, such as IL-2, IL-9, IL-12, IL-17, IFN-γ, and TNF-α, and increased the IL-10 gene expression (Cruz-Chamorro et al., 2019). Therefore, LPH_{alc} promotes the skewing of Th1/Th2 responses towards an anti-inflammatory microenvironment (Cruz-Chamorro et al., 2019). Then, LPH_{alc} was incorporated into a functional beverage (with a final LPH_{alc} concentration of 1 g/day) that 33 healthy volunteers (Lupine-1 clinical trial) ingested for 28 days. After 28 days of LPHalc ingestion, a reduction in pro-inflammatory cytokines IL-2, IFN-γ, and TNF-α production was observed, without changing in IL-4 and IL-10 in the PBMCs from the volunteers. In addition, an increase in Th2/Th1 balance was detected, indicating the capacity of LPH_{alc} to improve the anti-inflammatory environment (Cruz-Chamorro et al., 2021). Another study from the same group, carried out in WD-induced hypercholesterolemic mice, has shown that LPH_{alc} treatment reduces aortic immune cell infiltration and decreases the production of IL-1β, IL-18, IFN-γ, and TNF-α, key cytokines involved in the inflammatory process underlying the atheroma plaque formation. In addition, an increase in Th2/Th1 balance was observed after 12 weeks of LPH_{alc} treatment (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022). Furthermore, LPH_{alc} decreases the levels of some chemokines involved in immune cell recruitment, such as the chemokine (C-X-C motif) ligand (CXCL) 1 and 2, CCL2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in the aorta of mice (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022). All these results related to the *in vivo* anti-inflammatory role of LPH_{alc} in arterial dysfunction point to LPHs as a new possible bioactive ingredient for the development of nutraceuticals to prevent atherosclerosis.

LPH1 has been *in vitro* tested in different cell systems (Millan-Linares et al., 2015). This octapeptide (at a final concentration of 1 mg/mL) reduces the mRNA expression of TNF-α, IL-6, CCL18, CCL2, and CCR2 in THP-1-derived Mφ, and it also increases IL-10 and decreases NO production (Lemus-Conejo, Grao-Cruces et al., 2020; Lemus-Conejo, Millan-Linares, et al., 2020; Millan-Linares et al., 2015; Millan-Linares et al., 2018; Millan-Linares et al., 2019; Montserrat-de la Paz et al., 2019). Moreover, LPH1 decreases pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, as well as the frequency of M1-monocytes, downregulating the CCR2-CCL2 axis in isolated human monocytes. Furthermore, LPH1 reduces the gene expression of cluster differentiation (CD) 64 and CD80, important markers of M1 Mφ. In contrast, LPH1 increases the frequency of M2 Mφ, up-regulating the expression of IL-10, CD200 receptor, and mannose receptor C-type 1 gene (Montserrat-de la Paz et al., 2019). These facts support the conclusion that the peptide LPH1 is capable of favoring monocyte differentiation to the protective phenotype M2.

Monocytes present in the central nervous system (CNS) are named microglia, and they represent 10–15% of total cells in the CNS. The main functions of microglia are to maintain homeostasis and act as antigen-presenting cells (Nayak, Roth, & McGavern, 2014). In this context, they can also be defined as M1 or M2 microglia. A study conducted in the LPS-stimulated BV2 microglia cell line shows the anti-inflammatory effects of LPH1 treatment by decreasing IL-1β, IL-6, and TNF-α mRNA gene expression and increasing IL-10 gene expression. LPH1 also reduced the gene expression of the M1 phenotype markers CCR7 and iNOS, and increased the expression of the M2 phenotype markers, Arg, and chitinase-3-like 3 (Ym-1) (Lemus-Conejo, Millan-Linares, et al., 2020). Thus, LPH1 promotes M2 polarization, preventing prolonged activation of microglia, which can contribute to the development of neurodegenerative diseases. LPHs showed similar effects in an animal model fed with a WD (Lemus-Conejo, Millan-Linares, et al., 2020). LPH1 treatment (1 mg/mL) overcame the WD effects by decreasing total microglia, as well as the gene expression of the M1 markers CCR7, CD80, TNF-α, IL-1β, and IL-6. In addition, an increase in the expression of the Arg, Ym-1, CD200R, and IL-10 gene was found in LPH1-treated mice, confirming the polarization towards the protective M2-microglia phenotype (Lemus-Conejo, Millan-Linares, et al., 2020).

Similar results were obtained in monocyte-differentiated osteoclasts, in which LPH1 promoted an anti-inflammatory phenotype by increasing the IL-4 and IL-10 and reducing IL-1β, IL-6, and TNF-α gene expression (Millan-Linares et al., 2018).

In addition to the effects on the CNS, WD feeding might contribute to the generation of liver inflammation. In this regard, LPH1 treatment (1 mg/kg/day) for eight weeks decreases pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and increases the expression of anti-inflammatory cytokine genes (IL-10) in the liver of WD-fed mice (Lemus-Conejo, Grao-Cruces et al., 2020).

A similar scenario has been observed in the ARPE-19 cell line (Millan-Linares et al., 2019). RPE is located between the retina and the choroid, creating a bridge between the vascular system and the light-sensitive layer of the eye. RPE regulates the volume, chemical composition, and transport of nutrients between these two structures (R Sparrrow, Hicks, & P Hamel, 2010). Therefore, RPE is an important

indicator of the health status of the eye. Vascular endothelial growth factor (VEGF) overproduces the growth of blood vessels in the retina, leading to microcirculatory problems and, finally, to possible retinal ischemia. LPH1 has been shown to decrease VEGF, in addition, it reduces pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α) (Millan-Linares et al., 2019).

All these findings show the anti-inflammatory potential of LPH_s and peptides, mainly from the *L. angustifolius* specie.

5.3. Antihypertensive effect

Hypertension (HT) is an important risk factor implicated in CVDs and one of the main causes of premature death worldwide, affecting 1.13 billion people (Zhou et al., 2017).

Endocrine system regulates blood pressure with the help of renin and angiotensinogen proteins. When blood pressure decreases, the juxtaglomerular cells (JGs), specific cells located within the afferent arterioles of the kidney, release the active form of renin into blood flow. This protein can act on angiotensinogen, another protein produced by the liver and constitutively present in plasma. Thus, renin cleaves angiotensinogen into angiotensin (A)-I, an inactive form and precursor of A-II (the biologically active form). The presence of an ACE in the vascular endothelium of the lungs and kidneys allows the cleavage of A-I into A-II. A-II acts on different targets, in particular, increasing vasoconstriction, inflammation, and atherosclerosis (Fig. 2C).

Dietary strategies for the prevention of HT include reducing salt and alcohol intake, as well as increasing fiber, vegetables and fruits ingestion. Furthermore, physical activities have been widely associated with the prevention of high blood pressure. However, when the diet and lifestyle are not enough to prevent HT, drug treatments are necessary. In this context, different drugs have been shown to prevent HT, especially acting on the ACE activity (Stewart, Lavie, & Ventura, 2019).

Several studies have shown the beneficial effects of LPHs on HT. Both lupin proteins and LPHs exert protective effects on blood pressure (Nowicka, Klosiewicz-Latoszek, Sirtori, Arnoldi, & Naruszewicz, 2006). In particular, 21 different hydrolysates of *L. albus*, *L. angustifolius*, and *L. luteus*, obtained by the use of different enzymes (including specific endopeptidases such as trypsin, pepsin, and chymotrypsin, and different enzymatic complexes such as Corolase PP, Umamizyme, and Flavourzyme), showed to inhibit the ACE activity in a cell-free system (Boschin, Scigliuolo, Resta, & Arnoldi, 2014a; 2014b). Specially, hydrolysates generated by chymotrypsin (LPH_{chy}) with molecular weight (MW) < 3 kDa from *L. luteus* showed the highest inhibitory activity of ACE. This is of special interest because small-sized peptides are better absorbed by the intestine (Karami & Akbari-Adergani, 2019). The effectiveness range of *L. luteus* hydrolysis with several enzymes was: chymotrypsin > pepsin > pepsin + trypsin > trypsin > Corolase PP > Umamizyme > Flavourzyme.

Chymotrypsin preferentially cleaves protein chains on the carboxyl side of aromatic amino acids, whereas pepsin breaks the peptide bond between hydrophobic and aromatic amino acids. Released peptides with hydrophobic or aromatic terminal amino acids have been shown to interact with the catalytic site of ACE (Vermeirssen, Van Camp, & Verstraete, 2004). Consequently, the ACE activity inhibition by pepsin-generated peptides (MW < 3 kDa) from several species of lupine has also been described. In fact, hydrolysates from *L. angustifolius* have shown the highest inhibition rate compared to those of *L. albus*. Moreover, α and β conglutins mixture (the principal lupin storage proteins) show a greater inhibitory effect of ACE than γ and δ conglutins mixture (the minor lupin proteins) or the total proteins (Boschin, Scigliuolo, Resta, & Arnoldi, 2014b). In this line, the hydrolysis by enzymes such as Alcalase, pepsin, and trypsin significantly improves the inhibition of ACE activity of the hydrolysates, reaching a range between 50 and 80% with respect to the non-hydrolyzed lupin proteins. The small size (MW 3–10 kDa) fraction of Alcalase-obtained LPHs shows the best inhibition compared to non-fractionate LPHs (Guo et al., 2018). Focusing on a specific sequence, the nonapeptide LTFPGSAED (LPH4), a biopeptide obtained by hydrolysis of *L. albus* with pepsin, encapsulated into the RADA 16 peptide, a self-assembling peptide hydrogels used as a drug delivery system, inhibits the ACE activity in Caco-2 cells by 80% at 100 μM (Pugliese et al., 2022).

All of these findings support that the hydrolysis of lupin proteins could be an effective strategy to develop nutritional strategies aimed at preventing the hypertensive process, high blood pressure, vasoconstriction, atherosclerosis, and subsequent inflammation.

5.4. Hypoglycemic effect

The association between altered glucose metabolism and HT is well known (Sasaki, Ozono, Higashi, Maeda, & Kihara, 2020). During the physiological process of digestion, the intestine releases glucagon-like peptide-1 (GLP-1) into the blood circulation, which induces insulin secretion from pancreatic islets and inhibits glucagon release leading to a decrease in blood glucose levels. GLP-1 is rapidly degraded by DPP-IV enzyme (Jones, Bloom, Buenaventura, Tomas, & Rutter, 2018). Thus, DPP-IV inhibitors have been described as a new class of antidiabetic drugs (Sneha & Doss, 2016).

Regarding lupin, the nonapeptide LPH4 has been shown to inhibit the DPP-IV activity by 35% (Lammi, Zanoni, Arnoldi, & Vistoli, 2016) (Fig. 2C). Authors described that this peptide complies with four key characteristics (Boots, 2012): (i) LPH4 contains hydrophobic amino acids, such as leucine, phenylalanine, proline, glycine, and alanine; (ii) it is a small peptide (9 amino acids); (iii) it contains at least one proline, and (iv) proline is bound to phenylalanine/glycine.

The DPP-IV protein is detectable both in serum and in the luminal surface of enterocytes (Nargis & Chakrabarti, 2018). Therefore, Caco-2 cells have been used to demonstrate the inhibitory activity of DPP-IV by this nonapeptide (the concentration needed to observe a 50% inhibition –IC_{50}- was 207 μM) (Lammi, Bollati, et al., 2018). Although this intestinal cell line express surface and intracellular proteases, which could compromise the integrity of peptides, the same authors previously showed that LPHs can be absorbed by the Caco-2 cells without any modification (Lammi, Aiello, et al., 2016). Furthermore, LPH4 also inhibits DPP-IV activity in human serum from healthy volunteers (Lammi, Bollati, et al., 2018). This dual effect, which can be found in intestinal cells and in systemic circulation, confirms the great potential of this nonapeptide to control glycemia. Encapsulation of LPH4 into the self-assembling peptide RADA 16 improved its metabolic stability; consequently, its inhibiting power for the DPP-IV activity (RADA16+LPH4 IC_{50} = 28 μM) increased by seven times with respect to LPH4 in plain solution (IC_{50} = 207 μM) (Pugliese, Bollati, Gelain, Arnoldi, & Lammi, 2019; Pugliese et al., 2022). Furthermore, RADA16/LPH4 also decreases circulating DPP-IV activity by 29%. Thus, RADA16 encapsulation is a very interesting strategy to improve both the LPH4 bioactivity and intestinal metabolic stability.

Recently, sequential hydrolysis of *L. albus* proteins with Alcalase, trypsin, and Flavourzyme has also been demonstrated to generate peptides with DPP-IV inhibitory activity. Low-MW peptides (from 400 to 3000 Da) showed the highest bioactivity. *In silico* analysis identified seven possible peptides with DPP-IV inhibitor activity within the *L. albus* protein hydrolysates (Rivero-Pino, Espejo-Carpio, & Guadix, 2021).

In addition, the hydrolysis of seed proteins from *L. mutabilis* (pepsin 1 h, 37 °C, pH 2 + pancreatin 1 h, 37 °C, pH 7.5) generates peptides that can inhibit DPP-IV activity by 70% (Muñoz, Luna-Vital, Fornasini, Baldeón, & de Mejia, 2018). Also, the same hydrolysates have been demonstrated to increase glucose consumption and the translocation of its main membrane transporter (GLUT4), as well as to reduce gluconeogenesis by 50% in a dual-layered enterocyte/adipocyte culture system (Muñoz et al., 2018) (Supplementary Table S2).

Our group has also described the capacity to inhibit the DPP-IV activity of *L. angustifolius* protein hydrolysates obtained with Alcalase both in *in vitro* and *in vivo* models (Submitted manuscript). LPH_{alc} exerts a

dose-dependent inhibitory effect in a cell-free system with a highest inhibition rate (up to 80%) at 5 mg/mL. In addition, LPH_{alc}-treated Caco-2 cells show a decrease in DPP-IV activity of 40 and 65% at 1 and 5 mg/mL of LPH_{alc}, respectively. We have also shown the inhibition of the DPP-IV activity (by 80%) in plasma of WD-fed $ApoE^{-/-}$ mice treated with LPH_{alc} for 12 weeks. Accordingly, LPH_{alc} also decreases the plasma glucose concentration of treated animals. In addition, the daily ingestion of a beverage containing 1 g of LPH_{alc} for 4 weeks reduced DPP-IV activity in healthy subjects. In line with this fact, the reduced DPP-IV activity was correlated with a decrease in glucose concentration.

The subsequent peptidomic analysis of LPH_{alc} identified 141 sequences containing DPP-IV activity inhibition motives that were absorbed by Caco-2 cells cultured in a transwell system. The most representative were valine-arginine, glutamic acid-glycine, and glutamine-aspartic acid, among others.

Although the ingestion for 30 days of LPHs, obtained with Alcalase and Flavourzyme digestion (LPH_{a+f}), decreases plasmatic and renal glucose in hypercholesterolemic rats (Kapravelou et al., 2013), the study of Cruz-Chamorro et al. is the first showing the LPH_{alc} hypoglycemic effect in humans through direct DPP-IV inhibition (Submitted manuscript).

5.5. Hypolipidemic effects

Hyperlipidemia is a serious problem in modern society, both at health and economic levels. An increase in the consumption of ultra-processed food and a fast and stressed lifestyle leads to an over-eating habit in terms of food quantity, and a poor and unhealthy diet in terms of quality. Generally, these foods are rich in carbohydrates (mainly sucrose) and lipids (mainly saturated fatty acids), and generate an increase in LDL cholesterol (LDL-C) in the serum. LDL-C concentrations below 100 mg/dL are considered normal values which define an optimal physical situation. An increase in LDL-C leads to hyperlipidemia, i.e. an elevated lipid concentration in the blood (Karr, 2017). This excessive concentration of circulating cholesterol can lead to vessel wall obstruction, generating a CVD, or to excessive storage in the liver, leading to hepatic steatosis (defined as an abnormal retention of more than 5% fats in the liver) (Stefan, Häring, & Cusi, 2019). These two pathologies affect more than 1.5 billion people in the world, and are the first global cause of mortality (World Health Organization, 2020). For these reasons, many approaches try to find the best strategy to reduce the circulating LDL-C concentration. In this sense, LPHs have been shown to be a very interesting natural product capable of modulating

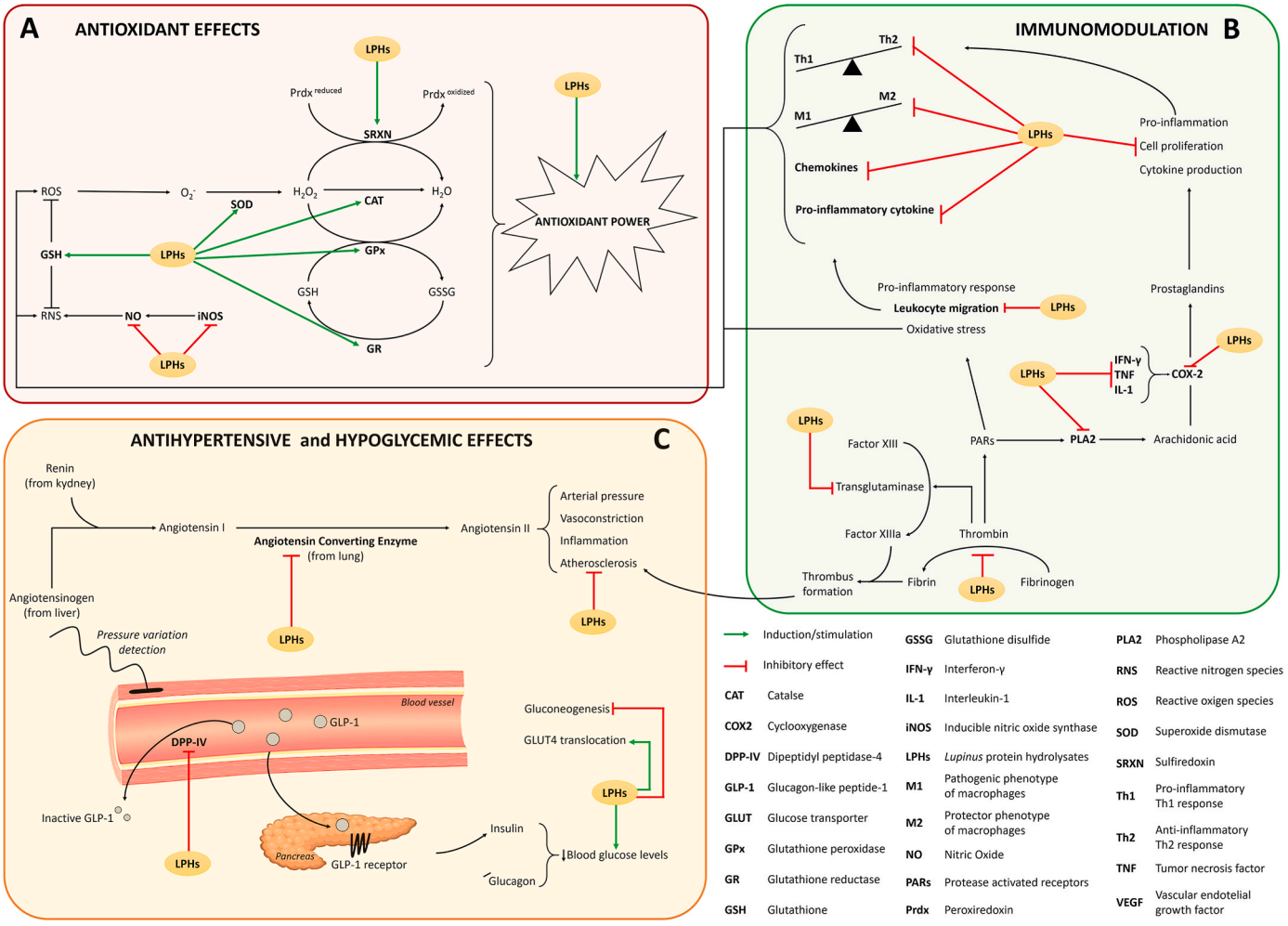

Fig. 2. Schematic representation of the different antioxidant (A), immunomodulatory (B), and antihypertensive and hypoglycemic (C) effects exerted by lupin protein hydrolysates (LPHs).

lipid metabolism. The molecular mechanism of LPHs on lipid metabolism has been validated in both *in vitro* and *in vivo* samples and clinical studies (Fig. 3A).

5.5.1. *de novo synthesis of cholesterol*

There are many studies on the efficacy of LPHs in modulating lipid metabolism. In all of these studies, the effects of LPHs on key enzymes involved in the synthesis and catabolism of cholesterol, as well as on LDL-C uptake, were investigated.

Cholesterol can be i) generated from hepatocyte cells by the *de novo* synthesis or ii) assumed from the diet (Berg, Tymoczko, & Stryer, 2002). Both ways are tight regulated in order to guarantee the intracellular cholesterol homeostasis (Berg et al., 2002). In the first case (*de novo* synthesis), the key role in the reaction of cholesterol synthesis is exerted by 3-hydroxymethylglutaryl (HMG)-Coenzyme A (CoA) reductase (HMG-CoAR). This enzyme reduces a molecule of HMG-CoA to mevalonate, using two molecules of NADPH and releasing one molecule of CoA, being the first and the rate-controlling enzyme implicated in cholesterol synthesis (Berg et al., 2002). Thus, the synthesis of mevalonate is the main step involved in the production of cholesterol. For this reason, many pharmaceutical strategies are focused on inhibition of HMG-CoAR activity in order to block cholesterol synthesis. Statins are the best drugs for this purpose at the moment (Hu, Cheung, & Tomlinson, 2012).

The enzymatic hydrolysis of *L. albus* protein with pepsin (LPH_{pep}) or trypsin (LPH_{try}) for 18 h generates peptide mixtures capable of inhibiting the *in vitro* HMG-CoAR activity by 17 and 61%, respectively, at 2.5 mg/mL (Lammi, Zanoni, Scigliuolo, D'Amato, & Arnoldi, 2014), suggesting that LPH_{pep} is more effective than LPH_{try}. A multidisciplinary study has been carried out in order to characterize the ability of LPH_{pep} and LPH_{try} to be transported across differentiated human intestinal Caco-2 cells. The results indicated that, eleven and nine peptides, respectively, from tryptic and peptic hydrolysates, are found to linearly permeate the intestinal epithelium (Lammi, Aiello, et al., 2016). Notably, among the absorbed ones, four peptides, LPH4 (Zanoni, Aiello, Arnoldi, & Lammi, 2017), GQEQSHQDEGVIVR (LPH5) (Lammi, Bollati, Lecca, Abbracchio, & Arnoldi, 2019), LILPKHSDAD (LPH6) (Lammi et al., 2022; Zanoni et al., 2017), and YDFYPSSTKDQQS (LPH7) (Lammi, Zanoni, Arnoldi, & Aiello, 2018) successfully drop the HMG-CoAR activity *in vitro* with the dose-response trend and IC_{50} value equals to 68.4 μM, 99.5 μM, 147.2 μM, and 75–100 μM, respectively.

Both LPH_{pep} and LPH_{try} were also used to treat HepG2 cells, as well as Caco-2 cells, for 24 h and the HMG-CoAR protein levels were measured by western blotting experiments. Therefore, an increase in the enzyme protein levels is observed after treatment with these LPHs (Lammi et al., 2014; Lammi et al., 2016). This increase was also measured when HepG2 cells were treated with LPH4 and LPH6 peptides (Lammi et al., 2021; Zanoni et al., 2017). In addition, it is important to note that intracellular HMG-CoAR is present in an unphosphorylated active form (30%) and a phosphorylated inactive form (70%) (Pallottini et al., 2005). An increase in phosphorylated HMG-CoAR (pHMG-CoAR) leads to an inactivation of its function and, therefore, to a decrease in cholesterol *de novo* synthesis. This inhibitory effect of the HMG-CoAR enzyme is detected with LPHs (Lammi et al., 2016). In particular, an increase in the active form phosphorylated of adenosine monophosphate-activated protein kinase (pAMPK) is observed by LPH4 and LPH6 peptide (Zanoni et al., 2017). In this sense, AMPK is responsible for the phosphorylation of HMG-CoAR on Ser872, and, consequently, for its inactivation. Furthermore, LPH_{alc} (from *L. angustifolius*)-treated mice showed a decrease in HMG-CoAR protein levels, as well as an increase in its inactive phosphorylated form in the liver tissue. In addition, inactivation of HMG-CoAR by its phosphorylation is accompanied by an increase in its inhibitor AMPK (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022), which confirms the mechanism observed *in vitro* studies (Zanoni et al., 2017).

5.5.2. *LDL-R pathway*

LDL receptor (LDL-R), which is expressed mainly under the control of the sterol regulatory element-binding protein (SREBP)-2, is involved in the uptake of circulating LDL-C into hepatocytes (Hui-xian et al., 2021). In fact, this receptor is located on the surface of hepatocytes, and it is a therapeutical target in the treatment of hypercholesterolemia, since the improvement of the uptaking through the LDL-R lowers circulating LDL-C, reducing free total cholesterol (TC) in the blood. Thus, both LPH_{pep} and LPH_{try}, from *L. albus*, increase the levels of SREBP-2 protein *in vitro*, and, therefore, LDL-R, both in HepG2 and Caco-2 cells (Lammi et al., 2016).

Moreover, the isolated peptides LPH4, LPH5, and LPH6 can increase the SREBP-2 and the LDL-R protein levels in HepG2 cells (Lammi et al., 2019; Lammi et al., 2021; Lammi et al., 2022; Zanoni et al., 2017).

The SREBP-2 augmentation leads to an increase in LDL-R but also in HMG-CoAR. For this reason, as mentioned above, an increase in HMG-CoAR is observed in LPHs-treated cells. LPHs also induced an increase in the inactive pHMG-CoAR form. Interestingly, LPH7 increases LDL-R protein levels through upregulating SREBP-1, but not SREBP-2(Lammi, Zanoni, et al., 2018). In this way, SREBP-1, unlike SREBP-2, is involved only in increasing LDL-R gene expression but not in HMG-CoAR, indicating the clinical interest in LPH7 as a future candidate to prevent hypercholesterolemia by controlling of LDL-R levels.

All these results confirmed that LPHs not only reduce the *de novo* synthesis of cholesterol but also increase the number of LDL-R molecules in the membrane of the hepatocytes, which accelerates the uptake of serum LDL-C, leading to a hypocholesterolemic effect.

In contrast, *L. angustifolius* protein hydrolysates show the ability to reduce the transcription factor SREBP-2 in liver tissue from LPH_{alc}-treated mice fed with WD (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022). Thus, non-treated hypercholesterolemic mice (control group) show an increase in SREBP-2 compared to normolipidemic mice to remove the high levels of serum LDL-C, as well as an increase in LDL-R. LPH_{alc} restore normal levels as in normolipidemic mice, reducing SREBP-2 and LDL-R proteins (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022).

Glycogen synthase kinase-3β (GSK3β) is a serine/threonine-protein kinase involved in the inactivation of SREBP by its phosphorylation and, consequently, by its degradation (Punga, Bengoechea-Alonso, & Ericsson, 2006). Therefore, the presence of an unphosphorylated active form of GSK3β increases the degradation of SREBP, and, consequently, the decrease of LDL-R. Of great interest is that LPHs act on the phosphorylation of the same GSK3β. LPHs have been shown to induce protein kinase B (Akt) production, which is involved in GSK3β phosphorylation, and, therefore, its inactivation (Manning & Toker, 2017). This interesting Akt/GSK3β kinases pathway is shown to be affected by LPHs in HepG2 cells. LPHs increase the active phosphorylated form of Akt and the inactive phosphorylated form of GSK3β (Lammi et al., 2014), demonstrating that LPHs also act at another level in cholesterol modulation.

The molecular modulation of the main target in the SREBP-2-LDLR pathway by LPH_{pep} and LPH_{try} (Lammi et al., 2014), as well as by single LPH4 (Zanoni et al., 2017), LPH5 (Lammi et al., 2016), LPH6 (Lammi et al., 2019; Lammi et al., 2021; Lammi et al., 2016) and its analogs (Lammi et al., 2022), and LPH7 (Lammi, Zanoni, et al., 2018), is translated by a functional point of view in an improved ability of HepG2 cells to uptake extracellular LDL-C with a final hypocholesterolemic effect.

5.5.3. *PCSK9 pathway*

Although the use of statins is widely accepted in clinical intervention, an uninterrupted intake does not lead to normal lipid values recovery. In addition, some people are intolerant to these substances and can manifest some side effects, such as myopathy (Fitchett, Hegele, & Verma, 2015). Thus, a new very important target has been found to treat hypercholesterolemia: convertase subtilisin/kexin type 9 (PCSK9)

protein. It has been very recently described and, in a few years, new strategies to inhibit or block its effect were developed (Chen, Shi, Cui, Hou, & Zhao, 2019). PCSK9 is implicated in LDL-R degradation, after internalization of LDL-R bound to LDL-C, and in the release of LDL-C in the cytosol, it also addresses LDL-R to its lysosome-mediated degradation. Therefore, a reduction of the PCSK9 protein, the inhibition of its gene expression or its ability to bind to LDL-R are efficacy strategies to reduce its effects (Momtazi, Banach, Pirro, Katsiki, & Sahebkar, 2017).

Hepatocyte nuclear factor 1 (HNF-1) is the principal transcription factor of the premature form of PCSK9 (pre-PCSK9). Cleavage of pre-PCSK9 leads to a mature and functional PCSK9, which is secreted and bound to LDL-R (Momtazi et al., 2017). Many studies describe the effects of LPHs on the PCSK9 pathway, inhibiting its synthesis by reducing the concentration of HNF-1 protein, and impeding its cleavage to a mature form or inhibiting its binding to LDL-R (Lammi et al., 2021; Lammi et al., 2016; Lammi et al., 2016; Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022; Zanoni et al., 2017). Therefore, LPHs of *L. albus* obtained with pepsin and trypsin can decrease the protein concentration of premature and mature PCSK9, the HNF-1 transcription factor, and the circulating PCSK9 in HepG2 cells (Lammi et al., 2022; Lammi et al., 2016). The cholesterol-lowering activity of LPHs was confirmed developing a co-culture system between Caco-2 and HepG2 cells, in order to reproduce the physiological cross-talk between intestine and liver (Lammi et al., 2016). More in detail, LPH_{pep} or LPH_{try} were incubated in the apical side of differentiated Caco-2 cells and the hypocholesterolemic activity of absorbed peptide mixtures (basolateral portion) were assessed directly on HepG2, cultured in the basolateral side of differentiated intestinal Caco-2 cells (Lammi et al., 2016). Thus, although 1 μg/μL LPHs do not affect PCSK9 production in Caco-2 cells, peptides capable of crossing the monolayer and getting in contact with HepG2 cells reduce PCSK9 production and activate SREBP-2/LDLR pathway. Another important effect exerted by these LPHs and their absorbed peptide is the inhibition of PCSK9/LDL-R binding, demonstrating that absorbed peptides can inhibit this binding by 81 (pepsin hydrolysates) and 58% (trypsin hydrolysates) (Lammi et al., 2016).

Two peptides that proceed from β-conglutin (LPH5 and LPH6) were individuated and synthesized by molecular dynamics simulation and peptide binding energy estimation. The IC_{50} of PCSK9/LDL-R binding was 320 μM for LPH5, while 1.6 μM for LPH6. These data are also supported by the increase in LDL-C uptake by 55 and 66% for LPH5 and LPH6, respectively (Lammi et al., 2021; Lammi et al., 2016). Recently, similar effects were observed also in LPH6 analogs (Lammi et al., 2022).

Undoubtedly, PCSK9 is a target for preventing the LDL-C accumulation in serum; however, an important mutation of PCSK9 can improve binding to LDL-R by more than 25% at pH 7.4, hindering its inhibition (Cunningham et al., 2007). Grazioso et al. demonstrated that LPH5 can interfere in the protein-protein interaction between the mutated PCSK9 ($PCSK9^{D374Y}$) and LDL-R. It was the first time it was shown that a peptide from vegetable food has this capacity (Grazioso, Bollati, Sgrignani, Arnoldi, & Lammi, 2018). This gain-of-function mutation increases the PCSK9-LDL-R interaction by 25 times with respect to the wild type. Thus, LPH5 inhibits the protein-protein interaction with an $IC_{50} = 285$ μM and it increases LDL-R on the surface and LDL-C uptake compared to the untreated HepG2 cell.

The non-hot- and hot-spots between the peptide LPH5 and $PCSK9^{D374Y}$ were investigated through computational analysis. Hot-spots are the region where the peptide interacts with $PCSK9^{D374Y}$. Thus, the non-hot-spots were replaced with other amino acids in order to identify the best peptide capable to inhibit the protein-protein interaction. This study led to obtaining a peptide that inhibits the interaction between $PCSK9^{D374Y}$ and LDL-R with 83.8%, to 12.7% more efficacy than the original one (LPH5) (Lammi, Sgrignani, Roda, Arnoldi, & Grazioso, 2018).

Finally, also LPH_{alc} from *L. angustifolius* demonstrate to interfere with the PCSK9 pathway, reducing its protein concentration and HNF-1 in the mouse model (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022). Thereby, mice fed with a WD showed an increase in PCSK9 and HNF-1 compared to control mice. LPH_{alc} reduces these proteins, restoring normal levels as in the control group.

All these data confirm the great potential of LPHs to inhibit PCSK9 at different levels, so it might be a great nutraceutical, capable of controlling serum LDL-C concentration by the modulation of the PCSK9 pathway.

5.5.4. Clinical evidence of the pleotropic activity of LPHs

All molecular mechanisms implicated in the metabolism of cholesterol described above are a description of how these LPHs can be useful to prevent lipid disorders. Some *in vivo* studies demonstrate that LPHs treatment is very effective in the reduction of lipid concentration in the organism. Thus, LPH_{a+f} of *L. albus* reduces hepatic TC and plasmatic and hepatic triglycerides (TG) in rats which followed a LPHs treatment for 30 days (Kapravelou et al., 2013). Furthermore, LPH_{alc} from *L. angustifolius* reduces plasmatic LDL-C, as well as plasmatic and hepatic TC and TG in mice treated with LPH_{alc} for 12 weeks (Santos-Sánchez, Cruz-Chamorro et al., 2021; Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022). In addition, this reduction in lipid content improved the health status of the liver, decreasing hepatic steatosis. Moreover, these LPH_{alc} can reduce the CD36 gene expression, and they are able to increase adiponectin receptor 2 (AdipoR2). CD36 is a scavenger receptor involved in fatty acid uptake, and, therefore, it has a key role in the accumulation of hepatic TG. Consequently, a decrease in this receptor reduces lipid accumulation. On the other hand, AdipoR2 allows the adiponectin uptake, an important protein involved in the inhibition of fatty acid and cholesterol synthesis, and promotes beta-oxidation. An increase in AdipoR2 can allow a greater uptake of adiponectin and, therefore, help to develop its functions. All these data are also supported by the observation of a reduction in adipocyte hypertrophy, showing a smaller lipid droplet in white adipose tissue from LPH_{alc}-treated mice in comparison to the control group (Santos-Sánchez, Cruz-Chamorro et al., 2021).

A derivative of these hydrolysates, the LPH1, showed its ability to decrease TG, body weight, and hepatic steatosis in obese mice treated with LPH1 for 8 weeks (Lemus-Conejo, Grao-Cruces et al., 2020). These results are associated with a decrease in fatty acid synthase gene expression and an increase in uncoupling protein 1 and peroxisome proliferator-activated receptors α, increasing the energy metabolism and avoiding lipid accumulation. The health status of the liver is also confirmed by a decrease in transaminases and the weight of this organ compared to the untreated group. In addition, in this study, a decrease in hepatic leptin resistance is observed in obese mice, and, therefore a restoration of normal serum levels of leptin and its hepatic receptor (Lemus-Conejo, Grao-Cruces et al., 2020).

Finally, the hypocholesterolemic effect produced by LPH_{alc} is observed in a clinical study. The administration of LPH_{alc} in healthy human subjects for 28 days led to a decrease in the LDL-C/HDL-C ratio, an important index used as a risk predictor factor for CVDs (Cruz-Chamorro et al., 2021).

All these findings place LPHs as a relevant vegetable nutraceutical, capable of reducing the concentration of circulating lipids and, therefore, reducing the risk of developing all diseases associated with it.

5.6. Others effect

In addition to the numerous effects described above, LPHs have also been shown to increase osteoblastogenesis versus osteoclastogenesis (Millan-Linares et al., 2018), reduce the atheroma plaque formation (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022), improve renal function (Kapravelou et al., 2013) and exert an anxiolytic effect (Santos-Sánchez, Ponce-España et al., 2022), among others.

A relevant mechanism induced by LPH_{alc} is the reduction of osteoclastogenesis, a main step that leads to premature osteoclast differentiation to mature osteoclast, a specialized macrophage involved in bone

resorption (Fig. 3B). Osteoblast cells involved in bone formation present the receptor activator for the nuclear factor-κB (RANK) ligand (RANKL). This ligand can be recognized by two important antagonist receptors: osteoprotegerin (OPG) and RANK. OPG is a soluble decoy receptor that, when it binds to RANKL, impedes binding to RANK, expressed by pre-mature osteoclasts. This inhibition avoids osteoclastogenesis, and, therefore, mature osteoclast formation and, finally, bone resorption. An increase in OPG expression leads to an increase in signaling inhibition mediated by RANKL-RANK. In this sense, differentiated osteoclast-cells treated with 50 and 100 μg/mL LPH1 show an increase in OPG gene expression and a reduction in RANK gene expression compared to the control group. Moreover, LPH1 can reduce the expression and activity of tartrate-resistant acid phosphatase (TRAP), as well as the osteoclast-associated immunoglobulin-like receptor (OSCAR) and cathepsin K (CTSK), all important markers of mature osteoclasts. These findings are very interesting and place LPHs as an excellentopportunity to use them in the prevention of osteoporosis.

Furthermore, LPH_{alc} from *L. angustifolius* shows to reduce important markers of atherosclerosis plaque formation. After 12 weeks of LPH_{alc} treatment, mice showed a reduction in the expression of P-selectin, and CD36 genes in the aorta artery (Santos-Sánchez, Cruz-Chamorro, Álvarez-Ríos et al., 2022). P-selectin is fundamental in the accumulation of monocyte/Mφ and T lymphocytes in the arterial intima, which is an early sign of atherosclerosis plaque formation. Therefore, the accumulation of immune cells in the intima and the CD36 expression in the surface of these cells leads to a massive internalization of oxLDL into the Mφ, transforming it into foam cells, which will participate in the atheroma plaque generation. Thus, the decrease in these markers can prevent the formation of atheroma plaques.

Another important outcome induced by LPHs is the protective effect on renal function, which was demonstrated in rats treated with LPH_{a+f} from *L. albus* for 30 days (Kapravelou et al., 2013). These rats were fed a diet rich in cholesterol and coconut oil in order to alter their renal function. LPH_{a+f} treatment restored all altered values of urinary markers involved in the initial stages of renal injury. In particular, it restored the normal albumin and creatinine values, as well as the urinary citrate concentration.

Another important effect exerted by LPHs is the anxiolytic effect. This is the first important report in which an anxiolytic-like effect of LPH_{alc} is demonstrated in a mouse model (Santos-Sánchez, Ponce-España et al., 2022). In this study, anxiety was induced by WD ingestion for 16 weeks and mice were treated, or not, with LPH_{alc} (from *L. angustifolius*) in the last 14 weeks. By an elevated plus-maze, a well-known established behavior test to study anxiety, it was possible to prove that LPH_{alc}-treated mice showed fewer anxious behaviors. The authors showed that LPH_{alc}-treated mice spent less time in the closed arms and more time in the center and open arms of the maze compared to the control group, which indicates that the mice were calmer. Furthermore, the number of head dips, another important anxiety-related behavior, was evaluated. Also, in this case, LPH_{alc}-treated mice, unlike control anxious mice, showed a number of head dips similar to non-anxious mice. In addition, the authors, after LPH_{alc}

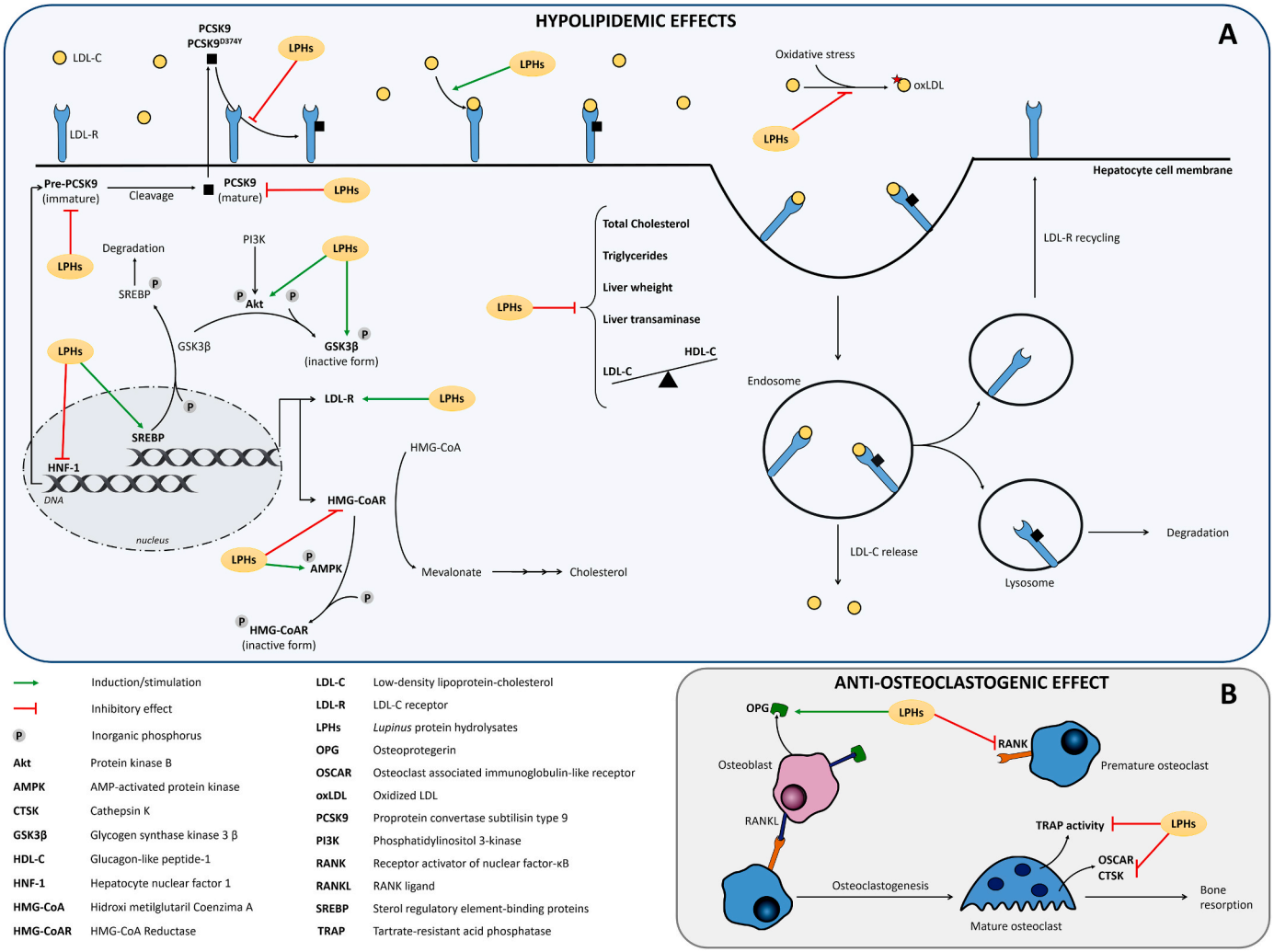

Fig. 3. Schematic representation of the different hypolipidemic (A) and anti-osteoclastogenic (B) effects exerted by lupin protein hydrolysates (LPHs).

sequencing, identified 58 sequences that contained motifs associated with anti-amnesic and anxiolytic effects. This study demonstrated for the first time that LPHs can act on animal behavior and modulate anxiety.

6. Physicochemical characteristics of LPH peptides

As there are controversial opinions about the relationship between bioactivity of the peptides and their length, MW, charge, hydrophobicity (Ho), amino acid composition, etc. (Karami & Akbari-Adergani, 2019; YANG et al., 2021), the LPHs-derived peptides discussed in this review were analyzed *in silico* using the PeptideRanker (Mooney, Haslam, Pollastri, & Shields, 2012) and ToxinPred (Gupta et al., 2013) tools (Table 4).

To predict the possible bioactivity of these peptides, the BioRank score was calculated. This score has a range of punctuation between 0 and 1, being a 1.0 the highest value of bioactivity. A threshold of 0.5 is used to label a peptide as bioactive. As shown in Table 4, only two peptides, LPH1 and LPH2, reached a BioRank score >0.5. Despite this, all of the peptides discussed in this review showed a biological effect. For example, although the BioRank value for LPH4 is 0.13 (low probability of exerting a biological effect), several studies have demonstrated its undisputable biological activity, showing inhibition of DPP-IV, ACE, and HMG-CoAR activities. On the basis of these considerations, we propose that the PeptideRanker database should not be the only source used to categorize a peptide as bioactive, but additional characteristics should be considered.

In addition, all analized peptides possess a SVM score <0 (mean value = −0.96), which predicts a non-toxic effect. In this way, a threshold value of 0.0 is considered the limit to determinate whether peptides released from food proteins might be toxic (positive values) or non-toxic (negative values). This characteristic is of great interest for translating basic studies into food-clinical trials using these peptides.

Within sequences of all LPHs-derived peptides, 116 different motifs with some biological activity were identified. In particular, 1.72%, 3.45%, 35.34%, and 55.17% of these were responsible for the antioxidant, immunomodulating, antihypertensive, and hypoglycemic activities, respectively (Supplementary Table S3).

Several chemical characteristics have been described to be responsible for the antioxidant activity of peptides, such as length, presence of hydrophobic amino acids in the N-terminal portion, nucleophilic sulfur-containing amino acid residues or the presence of rings in the lateral chain of the amino acid (da Silva et al., 2021). As shown in Table 4, all peptides that showed antioxidant effects, i.e., LPH1, LPH2, LPH3, and LPH8, possess at least 1 ring in their sequences, although their lengths are heterogeneous (4–23 amino acids). This shows that the small size of the peptide is not an essential and unique characteristic in order to exert antioxidant effects. On the other hand, the antioxidant capacity of LPH4, LPH5, LPH6, and LPH7 has not been explored. Although they are large peptides, the aromatic amino acid residues, and pyrrolidine and imidazole rings could give them their antioxidant activities. The evaluation of LPH7, which has 4 rings in the amino acid sequence, could be of great interest.

Some studies have highlighted the importance of a hydrophobic or negative amino acid in the N- or C-terminal residue, respectively, as well as the presence of proline (P) or low MW to interact with the enzyme HMG-CoAR (da Silva et al., 2021). These characteristics have been described to facilitate the peptides interaction with the active sites of the HMG-CoAR enzyme, modulating the enzyme activity and the subsequent biosynthesis of cholesterol. Although the peptides reported here do not contain motifs with demonstrated hypocholesterolemic activity or with capacity to inhibit the HMG-CoAR activity, LPH1 (Lemus-Conejo, Grao-Cruces et al., 2020), LPH4 (Pugliese et al., 2022), LPH5 (Lammi et al., 2019), LPH6 (Lammi et al., 2022; Zanoni et al., 2017), and LPH7 (Lammi, Zanoni, et al., 2018) showed hypolipidemic effects in theHepG2 cell line and in C57BL/6J obese mice. The hydrophobic

Table 4
Physicochemical properties of the lupin peptides discussed in this review.

Peptide sequence	Abbr.	aa	MW (Da)	Charge	pI	BioRank score[a]	SVM Score[b]	Different motifs[c]	Ho (kcal/mol)	Total rings[d]	N-terminal	C-terminal	Biological effect
GPETAFLR	LPH1	8	890.11	0	6.85	0.69	−1.22	8	+12.42	2	G[§]	R[†]	Antioxidant Immunomodulatory Hypolipidemic Others
FVPY	LPH2	4	524.66	0	5.40	0.82	−0.87	2	+5.16	3	F[§]	Y	Antioxidant
IQDKEGIPPDQQR	LPH3	13	1523.86	−1.0	4.27	0.17	−0.91	10	+24.92	2	I[§]	R[†]	Antioxidant Immunomodulatory
LTFPGSAED	LPH4	9	936.10	−2.0	2.82	0.13	−0.85	9	+14.71	2	L[§]	D[‡]	Antihypertensive Hypoglycemic Hypolipidemic
GQEQSHQDEGVIVR	LPH5	14	1581.88	−1.5	4.31	0.15	−1.08	11	+25.97	1	G[§]	R[†]	Hypolipidemic
LILPKHSDAD	LPH6	10	1108.40	−0.5	5.14	0.12	−0.90	9	+17.79	2	L[§]	D[‡]	Hypolipidemic
YDFYPSSTKDQQS	LPH7	13	1565.78	−1.0	3.92	0.11	−1.17	12	+18.16	4	Y[§]	S	Hypolipidemic
LNALEPDNTVQSEAGTIETWNPK	LPH8	23	2528.07	−3.0	3.57	0.20	−0.70	21	+26.02	4	L[§]	K[†]	Antioxidant
Mean		11.75	1332.36	−1.1	4.54	0.30	−0.96	10.25	+18.14	2.50			

Abbr., abbreviation; aa, amino acids; MW, molecular weight; Ho, hydrophobicity; pI, isoelectric point; SVM, support vector machine; [a], calculated according to (Mooney et al., 2012); [b], calculated according to (Gupta et al., 2013); [c], according to BIOPEP-UWM database (Minkiewicz, Dziuba, Iwaniak, Dziuba, & Darewicz, 2008); [d], calculated as the sum of number of H + F + P + Y + W × 2 present in the sequence; [§], hydrophobic aa; [†], aa with positive charge; [‡], aa with negative charge.

amino acid in the N-terminal, present in all of them, and the negative residue (aspartic acid, D) in C-terminal found in LPH4 and LPH6 could explain this effect (Table 4). Similarly, the presence of hydrophobic amino acids at the N-terminus of peptides LPH1, LPH2, LPH3, and LPH8 could point them out as hypolipidemic. However, future studies will be necessary to confirm these physicochemical predictions. In addition, the amino acid sequence and the presence of specific ones in the terminal residue seem to be more related with the hypolipidemic activity of the peptides than with the length of the peptides. There are also contrasting opinions regarding the importance of Ho in this enzyme inhibition (Heres, Mora, & Toldrá, 2021; Pak et al., 2006). Thus, further investigation of the key characteristic/s (length, a number of rings, netload, and/or Ho) by which peptides interfere with the activity of HMG-CoAR by the interaction with L- or S-domains of the enzyme should be very interesting. Other reports have proposed that low MW fractions induce the greatest inhibition of ACE and DPP-IV activity (Lafarga, Wilm, Wynne, & Hayes, 2016). Although the structure-activity relationship of DPP-IV inhibitory peptides has not yet been fully elucidated, the presence of Pro (Malomo, Onuh, Girgih, & Aluko, 2015), aromatic amino acids, and Tyr and Leu at the C-terminal position (Nongonierma & FitzGerald, 2016) has been proposed to influence the capability of peptides to inhibit ACE activity. LPH4 is the only peptide with demonstrated ACE and DPPIV activity inhibition. Although LPH4 is a peptide of 9 amino acids with a MW of 936.10 Da, the presence of Pro, as previously stated by Malomo et al. (Malomo et al., 2015), could be responsible for this effect. LPH1, LPH2, LPH3, LPH6, LPH7, and LPH8 have the amino acid Pro and aromatic amino acids within their peptide sequence. In addition, the peptide LPH2 presents a Tyr at the C-terminal position. Future research could be focus on the study of the ACE-inhibitory capacity of these peptides.

All of these considerations point out that there is a combination of physicochemical characteristics involved in the development of the biological effects. These characteristics are due to the specific hydrolysis conditions that depend on the digestive enzyme, or the combination of different enzymes, temperature, pH, etc.

Therefore, it is difficult to predict the biological action of a peptide only by *in silico* analysis. Although this approach can confirm some information, *in vitro* and subsequent *in vivo* tests are mandatory to demonstrate the effective bioactivity exerted by a certain peptide.

7. LPH bioactivity and other compounds

Unlike some drugs and plant compounds, the different physiological roles of lupin peptides have made them a good choice as therapeutic compounds. Different types of the physiological activity of lupin bioactive peptides have been reported, depending on their species, number, sequence, and properties of the amino acids. Therapeutically, there are many benefits from peptides that make them more useful than traditional medicines. For example, bioactive peptides have more specialized activities on the target tissue and, therefore, have little or no toxic effects, and are also effective even at low concentrations. This feature is very interesting for the treatment of chronic diseases, since normally they require prolonged therapy, which implies the occurrence of secondary side effects. On the other side, synthetic chemical compounds, commonly used as drugs, have a cumulative effect on the body. In addition, these chemicals, once excreted by our organism, may cause environmental problems due to their persistent activity. Conversely, bioactive peptides do not accumulate in the organism, and once excreted they are easily degraded with an eco-green impact.

In line with eco-sustainability, it is important to point out that to obtain products such as polyphenols, more raw material is needed than to obtain peptides. Latter ones, in fact, can be obtained from waste products from several food production chains, which revalorize them. This also offers an economic benefit at the industrial level.

The effects exerted by these hydrolysates and peptides derived from lupin have sometimes been compared to those of a commercial drug (pravastatin as a hypocholesterolemic drug or sitagliptin as a hypoglycemic drug) in *in vitro*, or in free-cells systems (Lammi, Aiello, et al., 2016; Lammi et al., 2016). As an example, it has been observed that a lupin hydrolysate reduced the activity of the HMG-CoAR enzyme by 51.5%, while pravastatin at 1.0 μM reduced it by 90% (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022). To date, no clinical trials have compared the effects of an LPH and a drug in the pathological conditions.

Thus, this review allows knowing the functional effects exerted by lupin biopeptides and hydrolysates as potential future ingredients of nutraceuticals or functional foods for the prevention or aid in the treatment of various diseases.

8. Future perspective: nano-nutraceuticals

Although many studies support the biological activity of peptides in human health, there are currently few functional foods on the market that contain them. Calpis™ or Evolus®, for example, contain the milk-derived tripeptides VPP and IPP, which are responsible for reducing blood pressure, while Cholesteblock contains soy-derive biopeptides with hypocholesterolemic effects (Nasri, 2017). Bioactive peptides have become a field of interest in the scientific community, as well as in the food, pharmaceutical, and cosmetic industries, however, some factors limit their nutraceutical and commercial exploitation, such as chemical degradation, modifications during processing, low water solubility, and potential bitter taste (Iwaniak, Hrynkiewicz, Bucholska, Minkiewicz, & Darewicz, 2019; McClements, 2015). It should be noted that the structure of peptides, and therefore their bioactivity, can change during digestion and absorption processes through the intestinal epithelial cells to the blood circulatory system (Jao, Huang, & Hsu, 2012; Xu, Hong, Wu, & Yan, 2019). In addition, numerous studies have shown that peptides can also be modified by enzymes present in the intestinal lumen, such as DPP-IV (Rivero-Pino et al., 2021; Röhrborn, Wronkowitz, & Eckel, 2015; Shao, Xu, Yu, Pan, & Chen, 2020).

Currently, there are numerous useful assays to study the bioavailability of these hydrolysates and bioactive peptides, such as *in vitro* static digestion, the Caco-2 cell intestinal barrier model, the *in situ* intestinal perfusion model, or the theoretical prediction model of the *in silico* digestion. More recently, nanotechnology is an interesting food strategy to counteract several limiting factors. Thus, the application of this technology to generate nano-nutraceuticals can improve the beneficial properties of nutrients administered as nanostructure, such as nanoparticles, nanoemulsions, nanogels, etc., revised in (Paolino et al., 2021).

Some bioactive food peptides may show an intrinsic tendency to spontaneous self-assembly. This unique feature is driven by the propensity to form stable secondary structures (β-sheet) outside the protein environment, or by their peculiar physico-chemical behavior that may give rise to a new generation of bioactive and sustainable self-assembling peptide nanomaterials from food. The forces holding the peptides together within the nanogel structures may enhance their stability and absorption, thus resolving the bioavailability, which is crucial for a successful oral delivery of bioactive peptides. Recently, dedicated efforts have been pursued in order to develop lupin bioactive peptide based nanogels applying innovative nanotechnological strategies.

More in detail, one strategy to deal with these issues might be the use of well-designed and controlled self-assembly supramolecular systems, such as self-assembling peptides (SAPs), to combine them with the food-bioactive peptides, in order to create a hybrid bonding system, in which monomers are bonded non-covalently (Pugliese & Gelain, 2017). Much like the construction of a house, where walls, floors, doors, and windows, can be built according to architectural plans, it is possible, by applying similar principles, to construct protein and peptide based-hydrogels through programmable molecular self-assembly (Zhang, 2003).

Therefore, the first example of a hybrid bonding hydrogel system in

which SAPs and lupin bioactive peptide are polymerized together simultaneously to form a multicomponent supramolecular food peptide hydrogel has been reported. In this system, aqueous solutions of the well-known and characterized self-complementary peptide RADA16 (i.e. Ac-RADARADARADARADA-$CONH_2$, with '+ - + - + - + -' charge orientation) (Yokoi, Kinoshita, & Zhang, 2005) were brought in physical contact with aqueous solutions of LPH4 (derived from β-conglutin, a lupin 7S globulin). By ionic-induced gelation, the simultaneous polymerization of peptide monomers resulted in the formation of a self-supporting polyelectrolytes hydrogel, in which the electrostatic interactions play a pivotal role. The peculiarity of these materials is that they lead to the formation of nanofibrous hydrogels, biomechanically robust (G' > G"), that can be used as a shear-thinning carrier for drug delivery (i.e. Sitagliptin) or as a scaffold for cell growth (such as human intestinal Caco-2 cells) (Dellafiora et al., 2020). Furthermore, since these multicomponent supramolecular food peptide hydrogels contain LPH4 (which can display a multifunctional behavior), it was shown for the first time that they can have useful health benefits as hydrogels with inhibitory activities of DPP-IV (IC_{50} equal to 28.1 μM), one of the main factors implicated in the development of CVDs and diabetes. This nano-technological approach that exploits programmable molecular self-assembly, in which SAPs and food peptides monomers are held together non-covalently (mainly by electrostatic forces, Van der Waals interactions, and hydrogen bonds), could be a valuable strategy for the formulation of renewable, biocompatible, biodegradable, and bioactive food-hydrogels capable of delivering nutraceuticals as well as pharmaceuticals in a controlled and harmless manner. In addition, since the constituent monomers of such supramolecular food peptide hydrogels are designed to polymerize non-covalently, it is possible to include simultaneously various food bioactive peptides, thus providing opportunities to create multicomponent superstructures with multifunctional behavior.

However, the main limitation of this strategy is that we needed to encapsulate more than 100 μM of LPH4 to have a positive effect on DPP-IV, and then that the peptides could be released with different trends over time; for instance, it was shown that LPH4 is regularly delivered by the RADA16 hydrogel during the 6 h that last the controlled release tests.

To bypass these constraints and improve thestability, bioavailability, and propensity of peptides to form bioactive functional hydrogels, the direct incorporation of LPH4 into the RADA16 sequence was carried out (Pugliese et al., 2022). Indeed, by using a click chemistry approach, LPH4 was used as a cue to bio-functionalize the RADA16 SAP, obtaining the peptide Ac-LTFPGSAEDGGGRADARADARADARADA-$CONH_2$. The N-terminus of the peptide was acetylated in order to increase its stability when in solution (Pugliese & Gelain, 2017); instead, a three-glycines spacer was used to avoid interference between the food bioactive motif and the self-assembling backbone. This strategy allowed to improve the nanostructure and biomechanics, as well as to control the density of food biological signals and, therefore, attain enhanced

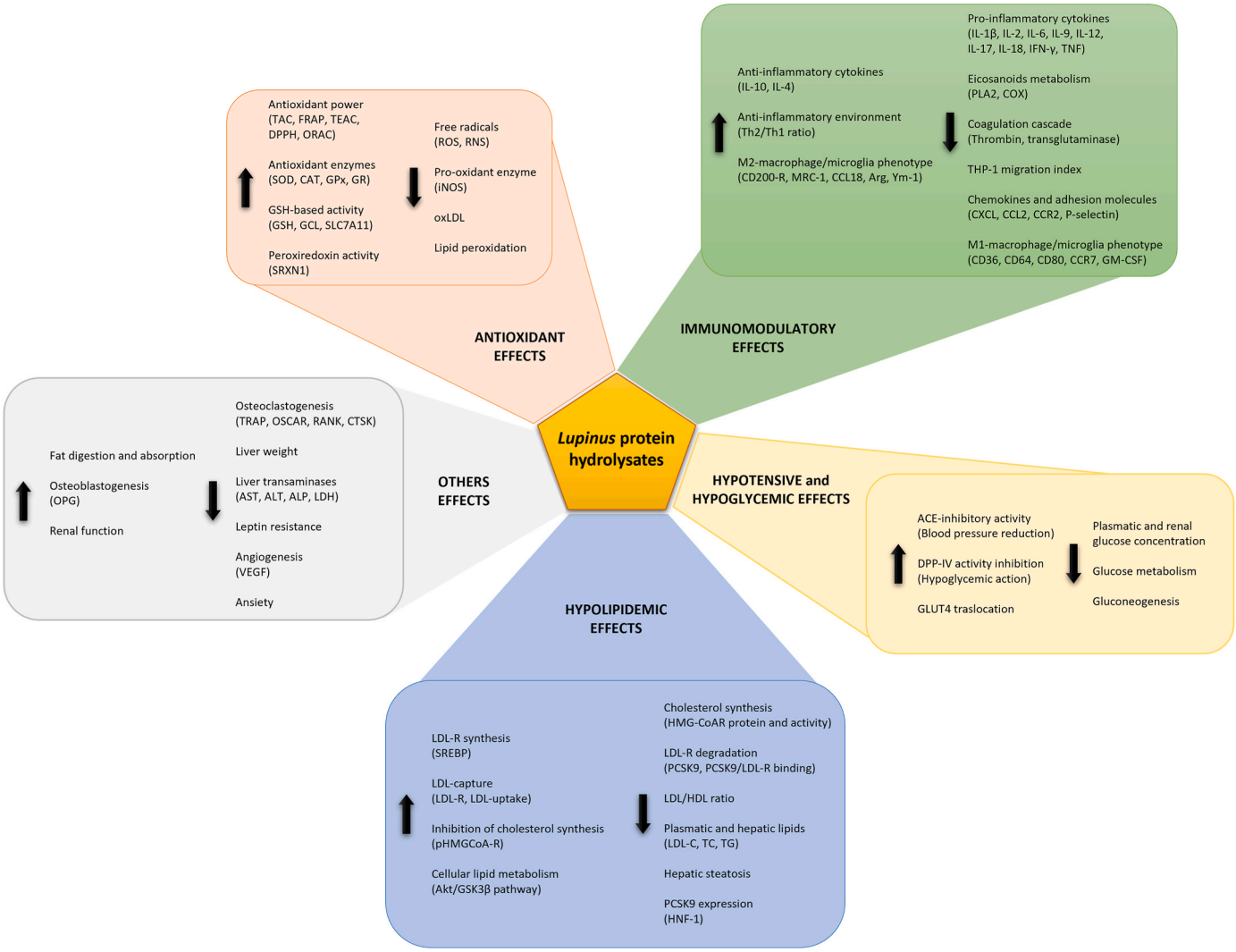

Fig. 4. Schematic representation of the different actions exerted by lupin protein hydrolysates (LPHs) separated by a specific effect.

bioactivity (on both DPP-IV and ACE target, respectively) of LPH4-based nanogel. Interestingly, this is a versatile strategy, indeed, other lupin peptides with different bioactivities can be identified and used as active cues for bio-functionalizing SAP. For instance, since SAP can be also used to encapsulate active compounds (of nutraceutical and pharmaceutical origin) which can be slowly released, they can be functionalized with the lupin peptides contained in the active motive RGD. RGD is the most common peptide motif responsible for cell adhesion to the extracellular matrix. Exploiting its ability to bind integrin, this peptide is widely used in materials science to draw drug delivery systems capable of promoting their interaction with cells and release active compounds and/or for the regenerative tissue proposals. Some peptides containing the active RGD motive have been identified within some LPHs; i.e. QVDRGDKLPL, TRRRHTRGDEGQEEEETTTTT, WKRTTRRRHTRGDEGQEEEE, GGSEWKRTTRRRHTRGDEGQEE from Alcalase hydrolysate (Santos-Sánchez, Cruz-Chamorro, Bollati, et al., 2022) and AGRLRRGDPL, SRGDVDSVSQLFEAF, LPVQVAGKLLRGDVL, VTRPAEALAPGGRGDR from peptic and triptic lupin peptide mixtures (Lammi et al., 2014). This aspect in particular clearly supports the hypothesis that lupin peptides may act as interactive materials which can be used to functionalize SAP surfaces to promote the adhesion and survival of selected cell types involved in the wound healing or tissue regenerative processes (although it should be noted that there are still many clinical applications for which biotolerant materials are optimal). The capacity of a material to support cell adhesion is critical not only for proper tissue development stimulation at implant/tissue interfaces, but it is also necessary for materials that serve as carriers for reparative cells delivery to wound sites.

The lupin peptide LNALEPDNTVQSEAGTIETWNPK (LPH8) stands out for its interesting structural properties (Pugliese et al., 2021). It is made up of 23 amino acids and belongs to the lupin α-conglutin (11S globulin, legumin-like protein). Its computational model was created by homology modeling techniques and the high percentage of sequence homology found with the employed template, the soybean proglycinin (PDB code 1UCX), made it possible to achieve a model of reasonable quality, additionally supported by molecular dynamic simulations, suggesting that LPH8 is shaped as a β-hairpin (Pugliese et al., 2021). Given its peculiarity of spontaneously organizing into ordered β-hairpin structures, it was showed that LPH8 is an antioxidant peptide capable of nanostructure creation with a hierarchical self-assembly propensity that was enhanced by a straightforward N-terminal biotinylated oligoglycine tag-based methodology (Pugliese et al., 2021). Doubtless, these evidences not only provide insights into fine tuned nanostructures, mechanical properties, and antioxidant activities using a naturally occurring food peptide-based hydrogel, but also, in combination with food chemistry, nanotechnology, and synthetic biology approaches, it undoubtedly provides a proof-of-concept strategy to develop functional bioinspired and more sustainable nanonutraceuticals, starting from bioavailable β-sheet peptides obtained by enzymatic hydrolysis of the parent protein.

9. Conclusion

LPHs have demonstrated multifaceted beneficial effects (summarized in Fig. 4). They have antioxidant and immunomodulatory effects, increase antioxidant enzymes and reduce reactive species, and skew the anti-/pro-inflammatory ratio of the immune cells to more protective phenotypes, respectively. These are combined with hypotensive and hypoglycemic effects, mediated principally by the control of ACE and DPP-IV activities, respectively. Furthermore, LPHs can also reduce the lipid concentration in the organism by activating or inhibiting different pathways involved in cholesterol levels control. Given that oxidative stress, inflammation, hiperlipidemia and hiperglycemia are key components of several conditions, LPHs has been shown to modulate atherosclerosis, osteoclastogenesis, renal function and anxiety through *in vitro* and pre-clinical approaches. All of these findings support the possible use of these hydrolysates as functional foods capable of preventing or reducing pathophysiological conditions such as hypertension, liver disease, atherosclerosis, metabolic syndrome, neurodegenerative diseases, etc. However, food-clinical trials are needed to confirm these preliminary *in vitro* and pre-clinical results. In addition, this review points out the digestion conditions during the hydrolysis process as a pivotal point related to the final bioactivity of LPHs. In this line, the physicochemical characteristics of the amino acid chains can explain the bioactivity of these lupin hydrolysates and peptides.

Therefore, this review points out LPHs as promising new nutraceuticals that could be used in several pathological conditions. In addition, it also opens the possibility of evaluating the molecular mechanism, intestinal absorption, bioavailability, stability, and chemical interaction with specific targets of LPHs.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2023.02.011.

Abbreviation

AA	arachidonic acid
ACE	angiotensin-converting enzyme
Akt	protein kinase B
AMPK	adenosine monophosphate-activated protein kinase
Arg	arginase
ARPE-19	human retinal pigment epithelial cell line
CAT	catalase
CCL	chemokine (C–C motif) ligand
CCR	chemokine (C–C motif) receptor
CD	cluster differentiation
CD200R	CD200 receptor
CD36	fatty acid translocase
CNS	central nervous system
COX	cyclooxygenase
CXCL1	chemokine (C-X-C motif) ligand 1
CVDs	cardiovascular diseases
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPP-IV	dipeptidyl peptidase-IV

FRAP ferric reducing antioxidant power
GCL glutamate-cysteine ligase
GM-CSF granulocyte-macrophage colony-stimulating factor
GPx glutathione peroxidase
GR glutathione reductase
GSH glutathione
GSK3β glycogen synthase kinase-3β
H_2O_2 hydrogen peroxide
HepG2 human hepatocyte cell line
HMG-CoAR hydroxymethylglutaryl-Coenzyme A reductase
HNF-1 hepatocyte nuclear factor 1
HT hypertension
IC_{50} half-maximal inhibitory concentration
IFN-γ interferon-γ
IL interleukin
iNOS inducible nitric oxide synthase
LDL low-density lipoprotein
LDL-R low-density lipoprotein receptor
LPHs lupin protein hydrolysates
LPH1 GPETAFLR peptide
LPH2 FVPY peptide
LPH3 IQDKEGIPPDQQR peptide
LPH4 LTFPGSAED peptide
LPH5 GQEQSHQDEGVIVR peptide
LPH6 LILPKHSDAD peptide
LPH7 YDFYPSSTKDQQS peptide
LPH8 LNALEPDNTVQSEAGTIETWNPK peptide
LPS lipopolysaccharide
MAPK mitogen-activated protein kinase
MMP2 matrix metalloproteinase-2
MW molecular weight
Mφ monocytes/macrophages
NO nitric oxide
O_2^- superoxide
ORAC oxygen radical absorbance capacity
OxLDL oxidized LDL
PBMCs peripheral blood mononuclear cells
PCSK9 protein convertase subtilisin/kexin type 9
PG prostaglandins
pHMG-CoAR phosphorylated form of hydroxymethylglutaryl-Coenzyme A reductase
PLA2 phospholipase A2
Prdx peroxiredoxin
P-selectin cell adhesion molecule
RNS reactive nitrogen species
ROS reactive oxygen species
RPE retinal pigment epithelial
RONS reactive oxygen-nitrogen species
SLC7A11 cystine/glutamate transporter
SOD superoxide dismutase
SREBPs sterol regulatory element-binding proteins
SRXN sulfiredoxin
TAC copper reducing assay
TEAC Trolox equivalent antioxidant capacity
TC total cholesterol
TG triglycerides
Th T helper cells
THP-1 human monocytic cell line
Thr thrombin
TNF-α tumor necrosis factor-α
VEGF vascular endothelial growth factor
WD western diet
Ym-1 chitinase-3-like 3

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