Trends in Food Science & Technology 127 (2022) 303–318

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Hempseed (*Cannabis sativa*) protein hydrolysates: A valuable source of bioactive peptides with pleiotropic health-promoting effects

Guillermo Santos-Sánchez [a,b,c], Ana Isabel Álvarez-López [a,b], Eduardo Ponce-España [a,b], Antonio Carrillo-Vico [a,b], Carlotta Bollati [c], Martina Bartolomei [c], Carmen Lammi [c,**], Ivan Cruz-Chamorro [a,b,c,*]

[a] Istituto de Biomedicina de Seville, IBiS (Universidad de Sevilla, HUVR, Junta de Andalucía, CSIC), Seville, 41013, Spain
[b] Departamento de Bioquímica Médica y Biología Molecular e Inmunología, Universidad de Sevilla, 41009, Seville, Spain
[c] Department of Pharmaceutical Sciences, University of Milan, 20133, Milan, Italy

ARTICLE INFO

Keywords:
Bioactive peptide
Antioxidant
Inflammation
Hypertension
Hyperglycemia
Hypercholesterolemia

ABSTRACT

Background: Recently, the study of hydrolysates from food proteins has been increasingly due to their wide range of biological activities. Hydrolysates contain peptides of 2–20 amino acids that are inactive within the sequence of the parent protein, but, once released after proteolytic processes, they exert numerous beneficial health effects. Hemp, the non-drug variety of *Cannabis sativa*, is known as an important source of bioactive peptides due to the high quality of hempseeds protein (20–25%) and well-balanced amino acid profile. For this reason, during the last decade, numerous investigations have searched to elucidate the beneficial effects on the health of these hempseed protein hydrolysates.

Scope and approach: The aim of this review was to collect all the scientific evidence on the demonstrated beneficial effects of hempseed protein hydrolysates (HHs).

Key findings and conclusions: HHs have showed to possess antioxidant, immunomodulatory, hypotensive, hypoglycemic, and lipid-lowering capacities in *vitro* systems. All these effects have pointed out HHs as future ingredient for the development of functional foods or dietary supplements useful for the prevention of chronic diseases such as metabolic syndrome, diabetes or hypertension. However, few studies have evaluated the *in vivo* effects of HHs. For this reason, further studies carried out in animal models or human are necessary to better exploit the use of HHs for the development of new dietary supplements.

1. Introduction

Cannabis sativa is an annual, dioecious herbaceous plant from family *Cannabinaceae* that has been documented as a source of food and medicine throughout history (Hartsel, Eades, Hickory, & Makriyannis, 2016). Although it is originated of central Asia and Europe, currently is widely cultivated in other zones such as Canada, United States of America, and Africa (Farinon, Molinari, Costantini, & Merendino, 2020). Its cultivation has been prohibited for many years in numerous countries since some of their varieties have high levels of the euphoria-inducing chemical named Δ9-tetrahydrocannabinol (THC) (Cannizzaro & Diana, 2016). Nonetheless, the hemp crop is currently legalized worldwide due to many countries have differentiated the varieties of *C. sativa* with low-THC levels (i.e., <0.3 or 0.2%) from the rest of drug varieties (i.e., marijuana) (Valizadehderakhshan et al., 2021). In this line, the crop of non-drug varieties of *C. sativa*, collectively known as "hemp" or "industrial hemp", is even expanding for its multipurpose and environmental benefits (not requiring fertilizers nor pesticides) (Karche, 2019). Data show that hemp production was from 48,590 tons in 2009 to 174,027 in 2019 (FAOSTAT, 2019). Each part of the plant is used in different industrial field; flowers are used for cosmetic or pharmaceuticals purposes, *shives* for animal bedding and construction, while fiber is used for paper and textile elaboration (Farinon et al., 2020). Whole hempseed is used for feed and food (i.e. functional foods, dehulled seed, hempseed oil, and hempseed meal) due to its high nutritional value and potential functionality (Farinon et al., 2020). Hempseed contain over 35.5% oil (rich in polyunsaturated fatty acids), 25% protein, 20–30% carbohydrates, 28% total fiber (5.4% digestible and 22.2% no digestible) and 5.6% ash. In addition, hempseed is rich in vitamins and

* Corresponding author. Istituto de Biomedicina, IBiS (Universidad de Sevilla, HUVR, Junta de Andalucía, CSIC), Seville, 41013, Spain.
** Corresponding author.
E-mail addresses: carmen.lammi@unimi.it (C. Lammi), icruz-ibis@us.es (I. Cruz-Chamorro).

https://doi.org/10.1016/j.tifs.2022.06.005
Received 4 January 2022; Received in revised form 5 June 2022; Accepted 7 June 2022
Available online 9 June 2022

Abbreviation

ACh	acetylcholine
ACE	angiotensin I converting enzyme
AChE	acetylcholinesterase
Caco-2 cells	human colorectal adenocarcinoma cell
CAT	catalase
ChAT	choline acetyltransferase
CV	cardiovascular
DPP-IV	dipeptidyl peptidase-IV
FRAP	ferric reducing antioxidant power
GPx	glutathione peroxidase
GR	glutathione reductase
HHs	hempseed protein hydrolysates
HepG2 cells	human hepatoma cells
HH1	NHAV
HH2	HVRETALV
HH3	WVSPLAGRT
HH4	IGFLIIWV
HMG-CoAR	3-hydroxy-3-methylglutaryl coenzyme A reductase
Ho	hydrophobicity
HT	Hypertension
IL	interleukin
iNOS	inducible nitric oxide synthase
LDL	low-density lipoprotein
LDL-R	LDL receptor
LPS	lipopolysaccharide
Mφ	macrophage
NF-κB	nuclear factor kappa B
Nrf2	nuclear factor erythroid 2-related factor 2
NO	nitric oxide
ORAC	oxygen radical absorbance capacity
pAMPK	phosphate-activated protein kinase
PCSK9	proprotein convertase subtilisin/kexin type 9
pHMG-CoAR	phosphorylated HMG-CoAR
RNS	reactive nitrogen species
ROS	reactive oxigen species
SBP	systolic blood pressure
SGD	simulated gastrointestinal digestion
SREBP	sterol-responsive element-binding protein
SOD	superoxide dismutase
THC	Δ9-tetrahydrocannabinol
TNF	tumor necrosis factor

minerals and contains lower concentration of antinutritional factors (phytic acid, tannins, and trypsin inhibitor) in comparison to soybean or rapeseed (Leonard, Zhang, Ying, & Fang, 2020). Due to its high protein content, the interest in the study of the beneficial properties of hempseed proteins and peptides has increased over the last ten years. This interest has come with the rise in the study of the beneficial properties of protein hydrolysates and peptides from vegetable sources.

In the field of plant foods studied as source of bioactive peptides, hempseed stands out together with soybean, oat, wheat, canola, flaxseed, and pulses. Hence, in light with these observations, this review takes into consideration all studies reporting the biological activities of hempseed protein hydrolysates (HHs) with special references on their production strategies and molecular mechanisms of action, highlighting their exploitation as ingredients for the development of dietary supplements and or functional foods (Fig. 1).

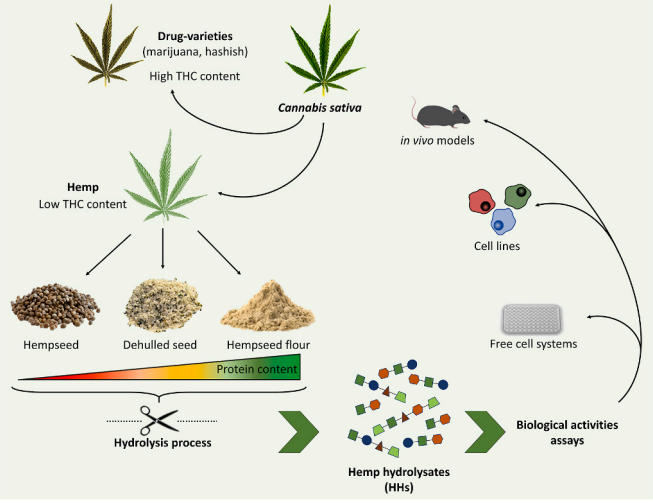

Fig. 1. Schematic representation of the hempseed protein hydrolysate obtaining and their *in vitro* and *in vivo* applications. THC, Δ9-tetrahydrocannabinol.

2. Protein content and hydrolysates production

As above mentioned, hempseeds contain 20–25% protein depending to environmental factors. Furthermore, the protein account can be increased in some hempseed products such as hempseed cake (35–50%) or dehulled seed (30–40%) (House, Neufeld, & Leson, 2010). Its protein content is higher than chia (13%), quinoa (16%), rice (8%), or wheat (14%) seeds, but lower than other vegetable as soybean seed (38–42%) (Farinon et al., 2020). Hempseed protein mainly consist in globulins and albumins, being the former the most predominant (60–80%). Among the globulins, edestin, a hexamer of about 300 kDa, is the most abundant (X. Sun, Sun, Li, Wu, & Wang, 2021). Focus on the quality of hempseeds protein, it contains all of the essential amino acids in nutritionally sufficient amounts. In fact, it meets the suggested requirements of the Food and Agriculture Organization/World Health Organization (FAO/WHO) for infants and children (House et al., 2010). On the other hand, hempseed proteins can be easily digested, showing a higher digestibility (61%) when compared to lentil (52%) or whole wheat (40%) (House et al., 2010). In addition, hempseed protein has higher digestibility when compared to soybean protein after hydrolysis with pepsin and trypsin enzymes (Girgih et al., 2014). All these characteristics increased the interest in the study of the beneficial properties of hemp seed proteins (Farinon et al., 2020; Q.; Wang & Xiong, 2019).

Although the nutritional and functional properties of hempseed proteins are widely known, the study of the bioactive properties of peptides derived from their hydrolysis is an emerging area with numerous potential applications, as new ingredients for the development of nutritional products (functional foods, infant formulas, and sports beverages) and cosmetics.

Peptides are sequences of 2–20 amino acids residues that are inactive in the parent protein, but after a hydrolysis process, they have demonstrated to exert numerous beneficial effects for health (antioxidant, antihypertensive, hypoglycemic, lipid lowering, etc.) (Amigo & Hernández-Ledesma, 2020). The process of hydrolysis can carry out by chemical or enzymatic methods. Specifically in enzymatic-aided hydrolysis, the proteolytic enzymes pepsin, pancreatin, trypsin, papain, and alcalase are the most used in the generation of bioactive peptides (Mazorra-Manzano, Ramírez-Suarez, & Yada, 2018). In this context, protein substrate, enzymes used, and the hydrolysis conditions (pH, temperature, and time) result in protein hydrolysate with different

Table 1
Antioxidant effects exerted by hemp hydrolysates.

Enzyme	Time	T (°C)	pH	Antioxidant effects	Reference
Cells-free system					
Alcalase	1 h	50	9.4	↑ Hydroxyl radical scavenging activity ↑ Superoxide radical scavenging activity ↑ DPPH radical scavenging activity	Lu et al. (2010)
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↑ Hydroxyl radical scavenging activity ↑ DPPH radical scavenging activity ↑ Metal chelating activity ↑ FRAP ↑ORAC ↓ Lipid peroxidation	(Girgih et al., 2014; Girgih et al., 2013; Girgih et al., 2014; Girgih, Udenigwe, & Aluko, 2011)
Alcalase	1 h	50	8	↑ DPPH radical scavenging activity ↑FRAP	Rodriguez-Martin et al. (2019)
Alcalase (A) + flavourzyme (F)	1 h (A) + 1 h (F)	50	7		
Protamex	2 h	55	7	↑ Hydroxyl radical and ABTS scavenging activity ↑ Metal chelating activity	Gao et al. (2021)
AFP 4000	4 h	45	6.5	↑ DPPH radical scavenging activity ↑ ORAC	Teh et al. (2016)
HT Proteolytic Concentrate	4 h	45	6.5		
Protease G	4 h	45	8		
Actinidin	4 h	45	5.5		
Zingibain	4 h	65	6		
Alcalase	2 h	50	8	↑ DPPH and ABTS radical scavenging activity ↑ Metal chelating activity ↑ FRAP	Samaei et al. (2021)
Alcalase	n.a.	n.a.	n.a.	↑ORAC	(Logarušić et al., 2019)
Neutrase	n.a.	n.a.	n.a.		
Protamex	n.a.	n.a.	n.a.		
Alcalase	4 h	55	8.5	↑ DPPH radical scavenging activity ↑ Metal chelating activity ↑ Reducing power	Tang et al. (2009)
Flavourzyme	4 h	50	7		
Neutrase	4 h	55	7		
Protamex	4 h	50	7		
Pepsin	4 h	37	1.5		
Trypsin	4 h	37	7		
Neutrase	4 h	55	7	↑ DPPH radical scavenging activity ↑ Metal chelating activity ↑ Reducing power	(X.-S. Wang et al., 2009)
WVSPLAGRT and IGFLIIWV (from pepsin)	16 h	37	2	↑ DPPH and ABTS radical scavenging activity ↑ ORAC ↑ FRAP	Bollati et al. (2021)
PSLPA, WVYY, SVYT, IPAGV, WYT [from pepsin (P) + pancreatin (Pa)]	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	↑ DPPH radical scavenging activity ↑ Metal chelating activity	Girgih et al. (2014)
HepG2 cell line					

(*continued on next page*)

Table 1 (*continued*)

Enzyme	Time	T (°C)	pH	Antioxidant effects	Reference
Protamex	2h	55	7	↓ ROS ↑ SOD ↑ CAT ↑ GPx	Gao et al. (2021)
WVSPLAGRT and IGFLIIWV (from pepsin)	16 h	37	2	↓ ROS ↓ Lipid peroxidation ↓ NO ↓ iNOS ↑ Nrf-2	Bollati et al. (2021)
HaCaT and HeLa cells lines					
Neutrase	n.a.	n.a.	n.a.	↓ ROS	(Logarušić et al., 2019)
HUVEC cell line					
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↓ ROS	Mahbub et al. (2022)

ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CAT, catalase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GPx, glutathione peroxidase; iNOS, inducible nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; ROS, reactive oxigen species; SOD, superoxide dismutase; n.a., not available.

profiles and a wide range of bio-functional activities. Thus, HHs from different enzymatic processes have demonstrated numerous potential food applications, gained the attention of the food, nutraceutical, and pharmaceutical industries.

Although recently the hempseed protein functions have been described and revised (Farinon et al., 2020; Leonard et al., 2020; Shen, Gao, Fang, Rao, & Chen, 2021; Q.; Wang & Xiong, 2019; Xu et al., 2021), in this review, we detail additional key concepts to understand how these hydrolysates exert their biological effects (hydrolysis process, hydrophobicity, physical-chemical characteristics, docking, absorption, etc.). Moreover, we have exclusively focused our attention on HHs and peptides, without considering protein extract function or physico-chemical techniques used to obtain them. Thus, in this review, we described in detail each paper that investigates the HHs (total hydrolysate or single peptide), providing information about their generation (the enzyme used, time, temperature, etc.) and their biological activity. In this context, HHs have been generated starting from different hemp matrices (hemp meal/cake, hemp protein powder or hemp bran), from which total proteins were extracted using dedicated protocols and subsequently hydrolyzed using different enzymes/conditions. In this context, the residual presence of other compounds (fiber, oligosaccharides, fat or polyphenols) within the HHs, that may contribute to the health promoting activity of HHs, cannot be excluded, however, it is also reasonable considering that they are not the mainly responsible for the observed biological activities.

In particular, a detailed analysis of each biological activity (antioxidant, hypocholesterolemic, anti-inflammatory, etc.), hydrolysis conditions (temperature, enzyme, and time), physicochemical characteristics (molecular weight, hydrophobicity, aromatic rings contents, etc.), experimental models (*in vitro* and *in vivo* systems), bioavailability (studies with Caco-2 cells), docking studies (interaction with enzymes), as well as a critical analysis of future studies that will be necessary to gain knowledge in this area (absorption, metabolism, etc.) have been discussed.

3. Biological activities of hempseed protein hydrolysates

Nowadays, numerous HHs and specific hempseed peptides have been identified and have shown to exert numerous bioactive effects including antioxidants (Table 1), immunomodulatory (Table 2), hypocholesterolemic (Table 3), antihypertensive (Table 4), and hypoglycemic effects (Table 5). The starting matrices from which proteins have been extracted and then hydrolysates are detailed summarized and highlighted in **Supplementary Table S1. Moreover, the *in vivo* effects exerted by HHs were reported in Table 6.**

3.1. Antioxidant effects

Oxidative stress is a biological process involved in cardiovascular diseases (CV), cancers, and other chronic diseases that are the leading causes of mortality worldwide (Senoner & Dichtl, 2019). Oxidative stress is defined as an imbalance between free radicals and the antioxidant system defense. Part of this defense are antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR), which are regulated by several transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) (Fig. 2A). Numerous articles have shown that the activity of these enzymes is reduced in certain diseases such as non-alcoholic fatty liver disease (Casas-Grajales & Muriel, 2017). On the other hand, it is well known that antioxidant-rich compounds, such as polyphenols, play a key role in the prevention of this disease (Jelena et al., 2018). There are numerous experimental tests to measure the oxidative status, although the frequently used are: (I) ferric reducing antioxidant power (FRAP) for the measurement of the total antioxidant status; (II) oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxyl, and superoxide radical scavenging activities for the measurement of the radical scavenging potentials; (III) the metal chelating activity for evaluating the ability to chelate metal ions thereby inhibiting the induction and formation of free radicals; (IV) the lipid peroxidation quantification to measure the level of lipid oxidation products; and (V) the direct measurement of the CAT, SOD, GPx, and GR enzyme activities.

Tang et al. were pioneers in the study of the antioxidant activity of HHs in cell-free systems (Tang, Wang, & Yang, 2009). They obtained several HHs, using different enzymes (alcalase, flavourzyme, neutrase, pepsin, protamex, and trypsin) and hydrolysis times (2 and 4h), that exhibited radical scavenging and iron chelating activities, as well as increased the total antioxidant status, being that obtained by neutrase the most powerful (Tang et al., 2009). Moreover, they evaluated the antioxidant power of HHs obtained by neutrase at different hydrolysis times and correlated these effects with their hydrophobicity (Ho). Thus, all studied HHs had a potent antioxidant power, although the results varied in each test depending on the characteristics of their peptides content. These results confirmed a positive correlation between the Ho and the trichloroacetic acid (TCA)-soluble peptides yield (Ysp) of peptides and their antioxidant power (X.-S. Wang, Tang, Chen, & Yang, 2009).

In this sense, Lu et al. demonstrated that the most hydrophobic fraction of alcalase-hydrolyzed HHs possesses better radical scavenging activity than the complete hydrolysate (Lu et al., 2010). The authors identified two peptides, NHAV (HH1) and HVRETALV (HH2), with the

typical characteristics of the already described antioxidant peptides: 4–16 amino acid residues, presence of histidine, and good proportion of hydrophobic amino acids (Lu et al., 2010).

Girgih et al. produced and analyzed eight fractions of a hydrolysate from pepsin + pancreatin with different Ho using reversed-phase high-performance liquid chromatography (Girgih, Udenigwe, & Aluko, 2013). Total antioxidant capacity, the chelation of metal ion chelation, radical scavenging activities, and lipid peroxidation were measured in the total hydrolysate and in each fraction. Although both total HHs and their fractions showed strong antioxidant properties, the antioxidant activity of the peptides were strongly correlated to the composition of amino acids (hydrophobic amino acids) and their Ho (Girgih et al., 2013). Thus, they identified 23 peptides from the better antioxidant fraction and showed that the peptides PSLPA and WVYY have the highest antioxidant power (radical activity scavenging and metal chelation activity) and calculated hydrophobicity equal to +7.89 and + 3.93 kcal/mol, respectively. With these results, it was possible to determine that tetrapeptides and pentapeptides have better antioxidant properties than tripeptides, and these properties are enhanced by the presence of hydrophobic amino acids within their sequences (Girgih et al., 2014).

Other authors have shown the antioxidant properties of HHs. In this sense, HHs obtained from alcalase, alcalase + flavourzyme, and pepsin + pancreatin, show radical scavenging activity (Girgih, Udenigwe, & Aluko, 2011; Logarušić et al., 2019; Rodriguez-Martin et al., 2019; Teh, Bekhit, Carne, & Birch, 2016) and increase the total antioxidant status (Rodriguez-Martin et al., 2019). On the contrary, HHs obtained by neutrase, and proteases preparation [AFP 4000 (AFP), HT Proteolytic Concentrate (HT), Protease G (Pro-G), actinidin, protamex, and zingibain] only show radical scavenging activity (Girgih, Udenigwe, & Aluko, 2011; Logarušić et al., 2019; Rodriguez-Martin et al., 2019; Teh et al., 2016). Furthermore, pepsin + pancreatin hydrolysate has also shown metal chelating activity (Girgih, Udenigwe, & Aluko, 2011).

Regarding studies in cell models, a hydrolysate obtained by the same condition of the Girgih et al., using the pepsin and pancreatin combination, shows to reduce the reactive oxygen species (ROS) in human umbilical vein endothelial cells (HUVEC) cell culture stimulated with 200 mM hydrogen peroxide (H_2O_2) (Mahbub et al., 2022). The effective dose of the treatment was 50 μg/mL of this hydrolysate. This ROS reduction shows the ability of HHs to scavenge free radicals leading to less endothelial dysfunction, a key marker in the physiopathology of CV disease.

On the other hand, Logarusic et al. evaluated the antioxidant effect of HHs obtained with neutrase on normal human keratinocyte cell lines (HaCaT) and cervical cancer cells (HeLa cells) stimulated with H_2O_2. In addition to not showing cytotoxic effects, the HHs decrease the ROS production in both cell lines (Logarušić et al., 2019). In addition, Gao et al. demonstrated that HHs obtained from hydrolysis with protamex reduce ROS production and increase CAT, SOD, and GPx activities in H_2O_2-stimulated human hepatoma cells (HepG2 cells) and identified the peptides with the highest antioxidant potential: YGRDEISV and LDLVKPQ (Gao, Li, Chen, Gu, & Mao, 2021) and calculated hydrophobicity of +16.30 and + 12.29 kcal/mol. These results confirmed that the molecular weight, the presence of aromatic and hydrophobic amino acids, and the presence of valine and tyrosine at the N-terminal and C-terminal ends are responsible to the antioxidant activity. In addition, by molecular docking analysis, both peptides could spontaneously interact with myeloperoxidase (MPO), an endogenous enzyme that induces the production of ROS and reactive nitrogen species (RNS), inactivating it (Gao et al., 2021). Moreover, HH1 and HH2 show protective effects against cell death and oxidative apoptosis in H_2O_2-stimulated rat Pheochromocytoma 12 (PC12) cells (Lu et al., 2010).

Recently, Lammi et al. showed that five peptides identified in HHs obtained with pepsin, DVFSPQAGRL, WVSPLAGRT (HH3), IGFLIIWV (HH4), DVFTPQAGRIST, and IRALPEAV, are able to cross the human colorectal adenocarcinoma (Caco-2) cells barrier and to exert antioxidant activity (Bollati et al., 2021). Furthermore, the most active peptides, HH3 and HH4, reduce H_2O_2-induced oxidative stress, ROS, lipid peroxidation, and nitric oxide (NO) levels in HepG2 cells, by the modulating Nrf-2 and inducible nitric oxide synthase (iNOS) signal pathways (Bollati et al., 2021).

Finally, Girgih et al. evaluated the antioxidant power of HHs in *vivo* model (Girgih et al., 2014; Girgih et al., 2014). Thus, a diet containing 20% casein supplemented with 1% HHs obtained from pepsin + pancreatin increase the SOD and CAT activity in plasma of spontaneously hypertensive rats (SHRs). Furthermore, the diet supplemented with HHs reduce the lipid peroxidation in SHRs as well as in normotensive rats (Girgih et al., 2014).

All these findings clearly demonstrate the strong antioxidant effects of HHs. However, additional *in vivo* studies are needed to confirm its possible therapeutic effects. The antioxidant activity of HHs is in line with that of other plant derived hydrolysates. Interestingly, legumes hydrolysates such as soybean, lupin, pea, and lentils peptide mixtures showed antioxidant activity both *in vitro* and in cellular systems (Cruz-Chamorro et al., 2019; Lammi, Arnoldi, & Aiello, 2019).

The *in vitro* and *in vivo* antioxidant effects exerted by HHs are summarized in Table 1 and in Table 6, respectively.

3.2. Immunomodulatory effects

Chronic inflammation is deeply involved in the development and progression of many chronic diseases (including, diabetes, cancer, obesity, depression, metabolic syndrome, and neurodegenerative diseases), that generate the sixty percent of all deaths around the world (Cote et al., 2022).

The *in vitro* immunomodulatory effects exerted by HHs are summarized in Table 2.

3.2.1. Anti-inflammatory effects

The anti-inflammatory effect of hemp seed hydrolysates and specific hemp seed peptides has been widely studied. Rodriguez et al. showed that HHs, obtained from alcalase and alcalase + flavourzyme, reduce the mRNA expression of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL) −6, and IL-1β and increase the mRNA expression of anti-inflammatory cytokine, such as IL-10, in lipopolysaccharide (LPS)-stimulated BV-2 microglia cells. Since oxidative stress has been associated with the chronic inflammation present in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, the authors attributed the neuroprotective effects of HHs to their potent antioxidant activity previously demonstrated in *vitro* assays (Rodriguez-Martin et al., 2019).

The same authors showed that HHs can shift macrophage (Mφ) polarization from phenotype M1 (M1) to phenotype M2 (M2) in LPS-activated primary human monocytes. Mφ are phagocytic mononuclear cells that may play a major role in immunity (Parihar, Eubank, & Doseff, 2010). They are cells whose polarization (classically clustered in M1 or M2) is different depending on the several infectious disease conditions, stimuli, or cellular substrate. M1-like phenotype is typical in inflammatory disease, and it is characterized by classically activated macrophages that can produce pro-inflammatory cytokines such as IL-1β, IL-6, TNF, and iNOS, and chemokines such as (C–C motif) ligand (CCL) 2 (CCL2) and chemokine (C–C motif) receptor (CCR) (CCR2 or MCP-1). CCR7 is a transcriptional marker of M1-phenotype that is used for differentiating M1 to M2. On the other hand, M2 phenotype is characterized by IL-10 and IL-4 anti-inflammatory cytokines production. In addition, they are characterized by the expression of high levels of cluster of differentiation 200 receptor (CD200R) and mannose receptor C-type 1 (MRC-1) (Atri, Guerfali, & Laouini, 2018) (Fig. 2B). In this context, HHs obtained from alcalase and alcalase + flavourzyme reduced the pro-inflammatory cytokines TNF, IL-6, IL-1β production, as well as increased the IL-10 and IL-4 anti-inflammatory cytokines production in LPS-stimulated monocytes (Rodriguez-Martin et al., 2020). In

Table 2
Immunomodulatory effects exerted by hemp hydrolysates.

Enzyme	Time	T (°C)	pH	Immunomodulatory effects	Reference
Cells-free system					
Alcalase	4 h	50	8	↑ AChE inhibition	Malomo and Aluko (2016)
Papain	4 h	65	6		
Pepsin	2 h	37	2		
Thermoase	4 h	50	8		
Flavourzyme	4 h	50	8		
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)		
Pepsin	2 h	37	2	↑ AChE inhibition	Malomo and Aluko (2019)
BV-2 microglia cell line					
Alcalase	20 min	50	8	↓IL-1β	Rodriguez-Martin et al. (2019)
Alcalase (A) + flavourzyme (F)	1h (A) + 15 min (F)	50	7	↓IL-6 ↓TNF ↑ IL-10	
HepG2 cell line					
WVSPLAGRT and IGFLIIWV (from pepsin)	16 h	37	2	↓ NF-κB ↓ pNF-κB ↓ pNF-κB/NF-κB ↓ IFN-γ ↓ TNF ↓ IL-6 ↓ NO ↓ iNOS ↑ IL-10	Cruz-Chamorro et al. (2022)
PC12 cell line					
Alcalase	1 h	50	9.4	↑ Cell viability	Lu et al. (2010)
HUVEC cell line					
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↓IL-1β ↓IL-8 ↓IL-12 ↓VCAM	Mahbub et al. (2022)
Ex vivo					
Alcalase	20 min	50	8	↓IL-1β	Rodriguez-Martin et al. (2020)
Alcalase (A) + flavourzyme (F)	1 h (A) + 15 min (F)	50	7	↓IL-6 ↓TNF ↓CCR7 ↓iNOS ↓CCR2/CCL2 ↑ IL-10 ↑ IL-4 ↑ CD200R ↑MRC1	
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↓CD62P	Mahbub et al. (2022)

AChE, acetylcholinesterase; CCL, (C–C motif) ligand; CCR, (C–C motif) receptor; CD200R, cluster of differentiation 200 receptor; CD62P, platelet P-selectin; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; MRC1, mannose receptor C-type 1; NF-κB, nuclear factor kappa B; NO, nitric oxide; pNF-κB, phosphorylated NF-κB; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

Table 3
Hypocholesterolemic effects exerted by hemp hydrolysates.

Enzyme	Time	T (°C)	pH	Hypocholesterolemic effects	Reference
Cells-free system					
Pepsin	16 h	37	2	↓ HMG-CoAR activity	Aiello et al. (2017)
Trypsin	16 h	37	2		
Pancreatin	16 h	37	2		
Pepsin (P) + Trypsin and Pancreatin (TP)	2 h (P) + 4 h (TP)	37	2 (P) + 8.5 (TP)		
HepG2 cell line					
Pepsin	16 h	37	2	↓ HMG-CoAR activity ↑SREBP ↑ LDL-R ↑ pHMG-CoAR ↑pAMPK ↑ LDL uptake ↑ PCSK9	Zanoni et al. (2017)

HMG-CoAR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low-density lipoprotein; LDL-R, LDL receptor; pAMPK, phosphate-activated protein kinase; PCSK9, proprotein convertase subtilisin/kexin type 9; pHMG-CoAR, phosphorylated HMG-CoAR; SREBP, sterol-responsive element-binding protein.

addition, typical markers of M1 phenotypes macrophages such as CCR7 and iNOS were reduced with HHs, while markers characteristics from M2 phenotypes macrophages such as CD200R and MRC1 were increased. Moreover, HHs-treated macrophages showed a reduction in the CCR2 and CCL2 mRNA expression, that could clarify their polarization toward M2, with a lower migratory ability (Rodriguez-Martin et al., 2020).

Recently, we have shown that bioavailable hempseed peptides HH3 and HH4 exert anti-inflammatory activity in LPS-stimulated HepG2 cells (Cruz-Chamorro et al., 2022). Specifically, HHs reduce the pro-inflammatory cytokines production (IFN-y, TNF, and IL-6) and increase the production of anti-inflammatory cytokine IL-10. These results agree with the reduction of the protein levels of nuclear factor kappa B (NF-κB), as well as its more functionally active form (phospho-NF-κB, pNF-κB) with the HH3 and HH4 treatments. Additionally, a reduction in iNOS protein levels and NO production was observed in LPS-stimulated HepG2 cells treated with both peptides (Cruz-Chamorro et al., 2022).

On the other hand, since an increase in vascular cell adhesion molecule (VCAM) is a consequence of a higher endothelial permeability and this increase leads to a vascular inflammatory response, Mahbub et al. assayed a hemp protein hydrolysate, obtained with pepsin and pancreatin, in H_2O_2-stimulated HUVEC cell culture (Mahbub et al., 2022). Thus, this hydrolysate showed to reduce the VCAM protein levels and decrease the inflammatory markers (IL-1β, IL-8, and IL-12) in the same cell culture. Moreover, the hydrolysate obtained by Mahbub et al. can reduce the platelet P-selectin (CD62P), an important marker involved in platelet activation and therefore in the thrombogenesis, in blood isolated from healthy volunteers. These results pointing out to HHs as a possible ingredient in functional food to protect against endothelial dysfunction.

3.2.2. Neuroprotective effects

In the pathogenesis of neurodegenerative diseases such as dementia or Alzheimer's the enzyme acetylcholinesterase (AChE) plays an essential role (Colovic, Krstic, Lazarevic-Pasti, Bondzic, & Vasic, 2013). This enzyme is responsible for the clearance of acetylcholine (ACh), that is generated during the neurotransmission from choline and an acetyl-CoA molecule by the action of the enzyme choline acetyltransferase (ChAT). ACh is released from the nerve to the synaptic cleft, then binding to postsynaptic membrane receptors and subsequently transmitting the signal to the brain. Specifically, AChE inactivates ACh transforming it into the metabolites choline and acetic acid, being the former used again for the generation of ACh (Malomo & Aluko, 2016). For this reason, the function of AChE is to maintain the homeostatic recycling of ACh. However, different factor such as aging, unhealthy diets, stress, or other diseases can reduce the amount of ACh synthesized, while enhancing the activity of enzyme AChE (Malomo & Aluko, 2016). These processes lead to a ACh reduction, generating an inadequate signal transmission to the brain, which cause the memory impairment (Fig. 2C).

In this regard, there are numerous drugs used for dementia or Alzheimer's whose mechanism of action is the inhibition of the enzyme AChE (e.g. memantine). However, the consumption of these drugs can lead to numerous side effects (headaches, nausea, anorexia, etc.) (Colovic et al., 2013). For this reason, the search for natural AChE inhibitors is an interesting field of research. Malomo et al. in 2015 demonstrated that HHs obtained from six different enzymes (alcalase, papain, pepsin, thermoase, flavourzyme, and pepsin + pancreatin)

Table 4
Antihypertensive effects exerted by hemp hydrolysates.

Enzyme	Time	T (°C)	pH	Antihypertensive effects	Reference
Cells-free system					
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↑ ACE activity inhibition ↑ Renin inhibition	(Girgih et al., 2014; Girgih, Udenigwe, Li, et al., 2011)
WVYY, WYT, SVYT, and IPAGV [from Pepsin (P) + Pancreatin (Pa)]	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)	↑ ACE activity inhibition ↑ Renin inhibition	Girgih et al. (2014)
Alcalase	4 h	50	9	↑ ACE activity inhibition ↑ Renin inhibition	Malomo et al. (2015)
Papain	4 h	65	6		
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4h (Pa)	37	2 (P) + 7.5 (Pa)		
Chemical hydrolysis (6M HCl)	6 h	110	n.a.	↑ ACE activity inhibition	Orio et al. (2017)
GVLY, IEE, LGV, and RVR (from chemical hydrolysis)	6 h	110	n.a.	↑ ACE activity inhibition	Orio et al. (2017)
AFP 4000	4 h	45	6.5	↑ ACE activity inhibition	Teh et al. (2016)
HT Proteolytic Concentrate	4 h	45	6.5		
Protease G	4 h	45	8		
Actinidin	4 h	45	5.5		
Zingibain	4 h	65	6		
Alcalase	2 h	50	8	↑ ACE activity inhibition	Samaei et al. (2021)

ACE, angiotensin I converting enzyme; n.a., not applicable.

Table 5
Hypoglycemic effects exerted by hemp hydrolysates.

Enzyme	Time	T (°C)	pH	Hypoglycemic effects	Reference
Cells-free system					
Corolase	4 h	50	7	↑ DPP-IV activity inhibition	Nongonierma and FitzGerald (2015)
Promod	4 h	50	7		
Protamex	4 h	50	7		
Pepsin (P) + Corolase (C)	90 min (P) + 150 min (C)	37	2 (P) + 7.5 (C)	↑ DPP-IV activity inhibition	Nongonierma and FitzGerald (2015)
Flavourzyme	150 min	50	7	↑ α-glucosidase activity inhibition	Ren et al. (2016)
Protamex	3 h	50	6.5		
Neutrase	150 min	50	7		
Papain	2 h	65	7		
Trypsin	2 h	37	8		
Alcalase	3 h	60	8.5		

DPP-IV, dipeptidyl peptidase-IV.

proved to be an excellent source of AChE inhibitor peptides, being the pepsin HHs the most active (Malomo, Onuh, Girgih, & Aluko, 2015). Furthermore, the authors showed that mainly the peptide chain amino acid sequence and secondarily the length of the peptide are responsible for this effect. In this regard, the authors have demonstrated that peptides with hydrophobic amino acids and with low molecular weight have the highest inhibitory effects (Malomo & Aluko, 2019; Malomo et al., 2015). Furthermore, the authors observed that HHs act as non-competitive enzyme inhibitor (Malomo & Aluko, 2019).

All these findings clearly support the immunomodulatory role of

Table 6
In vivo studies.

Enzyme	Time	T (°C)	pH	Animal model	Administration Method	Dosage	Period	Effects	Reference
Antioxidant effects									
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	SHR	Supplemented diet	0.5% and 1%	8 weeks	↑ SOD ↑ CAT ↓ Lipid peroxidation	Girgih et al. (2014)
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	HR	Supplemented diet	0.5% and 1%	4 weeks	↑ SOD ↑ CAT ↓ Lipid peroxidation	Girgih et al. (2014)
Antihypertensive effects									
PSLPA, WVYY, SVYT, IPAGV, WYT [from pepsin (P) + pancreatin (Pa)]	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	SHR	Oral	30 mg/kg	1 day	↓ Systolic blood pressure	Girgih et al. (2014)
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	SHR	Oral	200 mg/kg	1 day	↓ Systolic blood pressure	Girgih, Udenigwe, Li, et al. (2011)
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	SHR	Supplemented diet	1%	8 weeks	↓ Systolic blood pressure ↓ Plasma ACE activity ↓ Plasma renin concentration	Girgih et al. (2014)
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)	HR	Supplemented diet	1%	4 weeks	↓ Systolic blood pressure ↓ Plasma ACE activity ↓ Plasma renin concentration	Girgih et al. (2014)
Alcalase	4 h	50	9	SHR	Oral	200 mg/kg	1 day	↓ Systolic blood pressure	Malomo et al. (2015)
Papain	4 h	65	6						
Pepsin (P) + Pancreatin (Pa)	2 h (P) + 4 h (Pa)	37	2 (P) + 7.5 (Pa)						

ACE, angiotensin I converting enzyme; CAT, catalase; HR, hypertensive rat; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase.

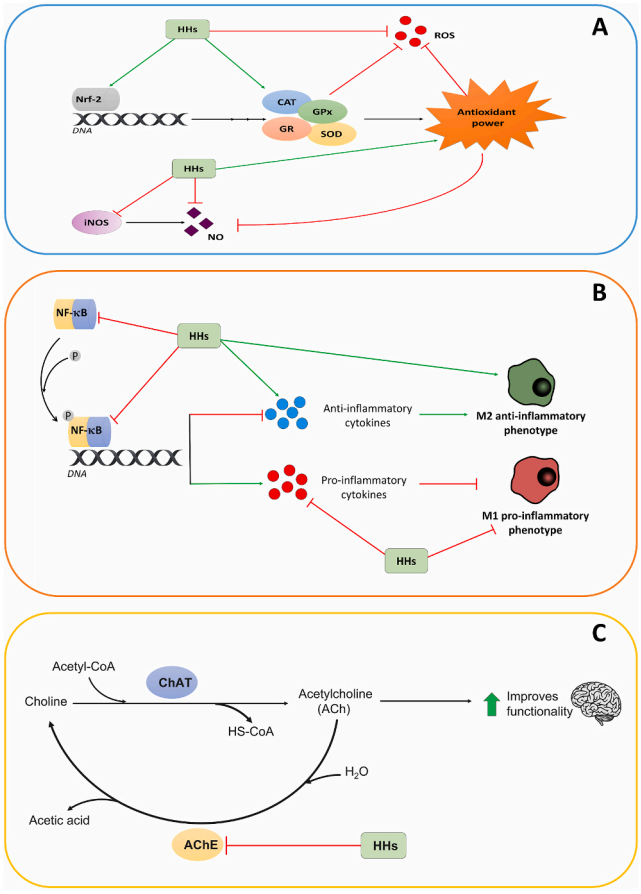

Fig. 2. Summary of the antioxidant (A), anti-inflammatory (B), and neuroprotective (C) effects exerted by hempseed protein hydrolysate. AChE, acetylcholinesterase; CAT, catalase; ChAT, choline acetyltransferase; GPx, glutathione peroxidase; GR, glutathione reductase; HHs, hempseed protein hydrolysates; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; NO, nitric oxide; ROS, reactive oxygen species; SOD, superoxide dismutase. Red line, inhibitory effects; green line, activator effects. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

hemp protein hydrolysates; however, animal, and human tests are urgently needed for confirming their relevance for human health.

3.3. Hypocholesterolemic effects

One of the biggest health problems in modern society is hypercholesterolemia. This pathological condition causes more than 18 million death in the world (World Health Organization, 2020) because it is a key risk factor in CV and fatty liver diseases development (Peters, Singhateh, Mackay, Huxley, & Woodward, 2016; Zhang & Lu, 2015). Although many research lines are focus on the development of drugs able to affect cholesterol metabolism, at the same time, many natural compounds normally present in foods have emerged as potential hypocholesterolemic agents such as extra virgin olive oil phenolic extracts, soybean, and lupin (Cruz-Chamorro et al., 2021; Lammi et al., 2020; Lammi, Zanoni, Arnoldi, & Vistoli, 2015; Lammi, Zanoni, Scigliuolo, D'Amato, & Arnoldi, 2014; Santos-Sánchez et al., 2022).

Besides the positive effects of hempseed enriched diets on arrhythmia (Prociuk, Edel, Gavel, Lukas, & Pierce, 2004), platelet aggregation (Prociuk et al., 2008), and atherogenesis (Gavel et al., 2011), recent investigations have demonstrated the molecular effects of the HHs on the cholesterol pathway (Aiello, Lammi, Boschin, Zanoni, & Arnoldi, 2017; Zanoni, Aiello, Arnoldi, & Lammi, 2017).

Lammi and co-workers evaluated the biological activity of HHs obtained with pepsin on the regulation of the principal limiting enzyme involved in *de novo* cholesterol synthesis, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), as well as the low-density lipoprotein (LDL) receptor (LDL-R) and, their transcription factor, the sterol-responsive element-binding protein 2 (SREBP-2) (Aiello et al., 2017; Zanoni et al., 2017). In addition to the HHs capacity to reduce the HMG-CoAR activity *in vitro*, they increase SREBP-2 protein levels in HHs-treated human hepatic HepG2 cells. As SREBP-2 synchronously activates the gene expression of HMG-CoAR and LDL-R, an increase of the protein levels of both was observed also in the same HHs-treated cells. However, although HMG-CoAR levels protein were augmented, the phosphorylated inactive form (pHMG-CoAR) was predominant. That is in accordance with the results observed in the HHs-treated cells where the increase of the phosphorylated active form of adenosine monophosphate-activated protein kinase (pAMPK) was observed. Thus, pAMPK phosphorylates HMG-CoAR, inactivating it.

On the other hand, the increase of LDL-R protein allows an increase in circulating LDL uptake (Zanoni et al., 2017). This dual effect leads to a decrease in free cholesterol and a diminished *de novo* cholesterol synthesis. The authors also showed that HHs increase the protein levels of the proprotein convertase subtilisin/kexin type 9 (PCSK9), which is a very interesting target to reduce LDL blood concentration. In this line, the statins, which are important drugs used in the control of hypercholesterolemia, have also been shown to increase the levels of LDLR and PCSK9 (Stancu & Sima, 2001). Thus, Lammi and collaborators have showed for the first time, the hypocholesterolemic effect of HHs through a statin-like mechanism (Zanoni et al., 2017).

Interestingly, peptide HH4 was screened and characterized as the first hempseed peptide able to directly drop the HMG-CoAR activity leading to a positively modulation of the LDLR pathway in HepG2. The HH4-induced augmentation of LDLR protein levels on the hepatic cellular surface, determined an improvement of the functional ability of HepG2 cells to uptake extracellular LDL with a final hypocholesterolemic effect. The cholesterol lowering activity is also attributed to the HH4 ability to reduce the PCSK9 protein level and its secretion (Li et al., 2022).

Similar effects were observed when the hempseed protein was hydrolysated with trypsin, pancreatin, or by a simulated gastrointestinal digestion (SGD) (pepsin + trypsin + pancreatin). In fact, these HHs reduce the HMG-CoA activity, being the hydrolysis with trypsin the more effective since 1 mg/mL inhibits the HMG-CoAR activity by 93% (Aiello et al., 2017). The poor effects were shown with pancreatin hydrolysis since a concentration of 2 mg/mL was necessary to reduce the HMG-CoAR activity by 12%. The SGD did not bring an improvement in the activity inhibition of HMG-CoAR compared with the individual hydrolysis with pepsin or trypsin. The cholesterol-lowering activity of hempseed protein hydrolysate is exerted through a mechanism of action which is like that of soybean hydrolysates (Lammi, Arnoldi, & Aiello, 2019). More in details, soybean hydrolysates obtained using pepsin and trypsin modulate the hypocholesterolemic pathways in HepG2 cells, by inhibiting the HMG-CoAR activity and by increasing the LDLR protein levels on cellular membranes.

All these findings point to the HHs as modulators of the cholesterol pathway and the possible integration of these in some commercial products to prevent CV and fatty liver diseases. The hypocholesterolemic effects exerted by HHs are summarized in Fig. 3A and in Table 3.

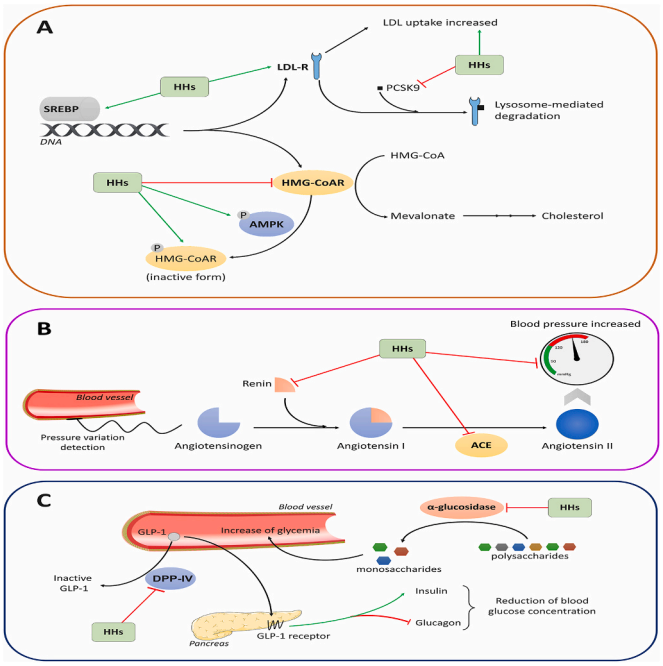

Fig. 3. Summarization of the hypocholesterolemic (A), antihypertensive (B), and hypoglycemic (C) effects exerted by hempseed protein hydrolysate. ACE, angiotensin I converting enzyme; AMPK, activated protein kinase; DPP-IV, dipeptidyl peptidase-IV; GLP-1, glucagon-like peptide 1; HHs, hempseed protein hydrolysates; HMG-CoAR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low-density lipoprotein; LDL-R, LDL receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; SREBP, sterol-responsive element-binding protein. Red line, inhibitory effects; green line, activator effects. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Antihypertensive effects

Hypertension (HT) is defined as systolic blood pressure and diastolic blood pressure values above 140 and 90 mmHg, respectively (Girgih et al., 2014; Singh, Shankar, & Singh, 2017). It is a global health problem that affect to 20–45% of the population (reaching 50% in the elderly population), being responsible of 7.1 million of deaths yearly (Hong et al., 2008; Organization, 2013). HT is the principal controllable risk factor associated to the development of several CV diseases such as coronary heart disease, stroke, peripheral arterial disease, and renal failure (Guessous, Bochud, Theler, Gaspoz, & Pechère-Bertschi, 2012). Although the etiology of hypertension is unknown, it is known that both genetics and lifestyle factors (diet principally) are implicated (Kannel, 1989; O'Shaughnessy, 2006). Currently, the most effective treatment approach is the inactivation of the renin and/or angiotensin I converting enzyme (ACE), key enzymes in the renin-angiotensin-aldosterone system (RAAS) that is in charge of regulating hypertension (Ames, Atkins, & Pitt, 2019; Girgih et al., 2014; Zhou et al., 2010). In the RAAS, kidney-secreted renin converts angiotensinogen to angiotensin I, which, in turn, is converted by the action of the enzyme ACE to angiotensin II, a potent vasoconstrictor (Bernstein, Giani, Shen, & Gonzalez-Villalobos, 2014; Girgih, Udenigwe, Li, Adebiyi, & Aluko, 2011). In condition of disease or due to different genetic/environmental factors, the ACE enzyme may be up regulated, generating an increase in angiotensin II and therefore a high blood pressure (Michel et al., 1993) (Fig. 3B). ACE also is able to inactivate bradykinin, a potent vasodilator (Pirahanchi & Sharma, 2019). In this regard, the most used drugs for hypertension, such as Captopril, Ramipril, Enalapril, or Alacepril, act as ACE-inhibitors (Atkinson & Robertson, 1979; W.; Sun et al., 2016). However, all of these drugs have been linked to numerous side effects such as headache, diarrhea, and tachycardia, among others (Abassi, Winaver, & Feuerstein, 2009). In addition, although all these drugs are reasonably effective, the mortality rate due to HT continues to be elevated (World Health Organization, 2021). For these reasons, the identification of new natural treatments for hypertension is a growing field. Numerous plant bioactive peptides have been shown to have renin and ACE inhibitory properties (Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017). Hempseed, as a high peptide source, has been one of the most studied. Several pieces of evidence have showed numerous hempseed protein hydrolysates with ACE-inhibitory activity. Specifically, hempseed protein hydrolysates obtained from pepsin, pepsin + alcalase, alcalase, papain, and protease preparations [AFP 4000 (AFP), HT Proteolytic Concentrate (HT), Protease G (Pro-G), actinidin, and zingibain] have showed to reduce the activity of ACE in *vitro* experiments (Girgih, Udenigwe, Li, et al., 2011; Malomo et al., 2015; Samaei, Martini, Tagliazucchi, Gianotti, & Babini, 2021; Teh et al., 2016). Some of the cited hydrolysates, specifically those from hydrolysis with pepsin + pancreatin, pepsin, pancreatin and alcalase, have also been shown to be capable of inhibiting the renin activity (Girgih, Udenigwe, Li, et al., 2011; Malomo et al., 2015). These studies concluded that small peptides (2–20 amino acids) that contain residues with aromatic or cyclic rings such as tyrosine, phenylalanine, tryptophan and proline and those that present of aliphatic chains such as glycine, isoleucine, leucine and valine in the N-terminal are the most effective as ACE inhibitors (Girgih, Udenigwe, Li, et al., 2011; Malomo et al., 2015; Samaei et al., 2021; Teh et al., 2016). Since the hempseed hydrolysates are characterized by a very heterogeneous composition where more than one peptides within the mixtures are responsa bile for the biological activity, Malomo et al. concluded that the synergistic interaction between the peptides itself may be also responsible for the hypotensive activity (Malomo et al., 2015). In addition, kinetic studies showed that the inhibition pattern was of mixed type and non-competitive mode (Girgih, Udenigwe, Li, et al., 2011). Girgih and colleagues were the first to study the *in vivo* ACE-inhibitory effect of hempseed protein hydrolysate. Specifically, they measured the systolic blood pressure (SBP) of spontaneously hypertensive rats (SHRs) after short term (24h) oral administration of 200 mg/kg body weight of hempseed protein hydrolysate from pepsin + pancreatin. The hydrolysate showed an antihypertensive effect on SBP (−30 mmHg after 8h) comparable to the effect obtained with the antihypertensive drug captopril (3 mg/kg body weight) (Girgih, Udenigwe, Li, et al., 2011). Others hydrolysate obtained from pepsin, alcalase, and papain hydrolysis have demonstrated the same *in vivo* effects after oral administration (200 mg/kg weight) (Malomo et al., 2015). The results showed a higher ACE inhibitory activity of HHs than others hydrolysate such as pistachio, almond, or sweet potato (Malomo et al., 2015), confirming the importance of food proteins, hydrolytic enzymes, and conditions for producing peptide mixtures with a more effective biological activity. Having previously established the short-term ability of a HHs to reduce SBP, Girgih et al. evaluated the hypotensive effect of 8 weeks treatment with the hydrolysate in a spontaneous hypertension model in young rats (Girgih et al., 2014). In addition, the researchers evaluate the antihypertensive activity of four weeks-HHs treatment in adults' hypertensive rats and normotensive rats. The results showed that the hydrolysate was able to reduce the SBP in both young and adults hypertensive rats, while no effect in SBP was showed in the normotensive rats (Girgih et al., 2014). Nonetheless, a reduction in plasma ACE activity and renin levels were observed in treated mice in the three experimental groups (young and adult hypertensive rats and normotensive ones) (Girgih et al., 2014). The next objective of the investigations was to identify and test the efficacy of antihypertensive peptides present in hempseed protein hydrolysate. Samaei et al. identified 35 peptides in the HHs that had already been described as ACE inhibitory activity, which confirmed the effects observed in the hydrolysate (Samaei et al., 2021). Previously, different *in vitro* investigations had shown a potent ACE-inhibitory activity of the low molecular weight peptides WVYY, PSLPA, GVLY, LGV, RVR, WYT, SVYT, and IPAGV (Girgih et al., 2014; Orio et al., 2017). In addition, the last three peptides

(WYT, SVYT, and IPAGV) also inhibited the renin activity (Girgih et al., 2014). Regarding *in vivo* studies, Girgih et al. evaluated the antihypertensive effect of the five peptides (WYT, SVYT, IPAGV, WVYY, and PSLPA) in young hypertensive rats after a short-term oral administration (30 mg/kg body weight). All the animals showed a reduction in the SBP after administration, being PSLPA and IPAGV the most effective. In fact, the antihypertensive effect of these peptides were similar than in the captopril-treated mice (Girgih et al., 2014). Molecular docking of these peptides (WVYY and SVYT) indicated that their higher inhibitory activities of ACE were due to their ability to interact with the enzyme by the formation of higher numbers of hydrogen bonds with the amino acids residues located on the active site of the enzyme (Girgih et al., 2014). Although there is much evidence on the antihypertensive role of HHs, a greater number of studies in animals and humans will be needed to clarify the use of them in future therapeutic therapies. In this line, a randomized, double-blind, crossover clinical trial will be conducted on 35 hypertensive participants to evaluate the hypotensive potential of consuming HHs (Samsamikor, Mackay, Mollard, & Aluko, 2020).

The *in vitro* and *in vivo* antihypertensive effects exerted by HHs are summarized in Table 4 and in Table 6, respectively.

3.5. Hypoglycemic effects

Nowadays only exist two reports on the hypoglycemic activity of HHs focus on the evaluation of dipeptidyl peptidase-IV (DPP-IV) (Nongonierma & FitzGerald, 2015) and α-glucosidase inhibitory activities (Ren et al., 2016).

DPP-IV is a protease involved in the degradation of the glucagon-like peptide-1 (GLP-1), the principal regulator of insulin production. Thus, the DPP-IV-mediated degradation of GLP1 leads to a decrease in insulin production and an increase in glucagon production, with a final result in the increase of glucose blood concentration (Ahrén, 2019; Andersen, Deacon, & Holst, 2018). The inhibition of the DPP-IV activity is the principal key in the increase of insulin production and the consequent glucose blood regulation (Fig. 3C). Gliptins are the principal drugs used in type 2 diabetes (T2D) because they act as DPP-IV inhibitors (Godinho et al., 2015).

However, recently, some natural compounds able to inhibit the DPP-IV activity have been identified. Several studies had demonstrated that some food-derived products are able to inhibit the DPP-IV activity such as hydrolysates obtained from rice, lupin, amaranth, soybean, etc. (Lammi, Zanoni, Arnoldi, & Vistoli, 2016; Nongonierma & FitzGerald, 2015). In particular, lupin peptide LTFPGSAED inhibits the DPP-IV activity in several model systems: *in vitro* on the DPP-IV enzyme, in Caco-2 cells, and on the human serum circulant DPP-IV form (Lammi, Zanoni, Arnoldi, & Vistoli, 2016).

HHs obtained by an *in vitro* SGD (pepsin + pancreatic enzymes) or by the use of different enzymes preparation (protamex®, corolase®, and promod™) showed a strong DPP-IV inhibitory activity (Nongonierma & FitzGerald, 2015). This study shows that the use of different enzymes preparation generates hydrolysates with higher DPP-IV inhibitor activity than those obtained with an SGD. In fact, hydrolysis generates peptides that contain a high number of the tryptophan amino acid, which possess a high DPP-IV inhibitor activity. However, the further proteolytic degradation by the SGD disrupts the Try-containing peptides, losing their function. Thus, this is the first report that shows the DPP-IV inhibitor activity of several HHs, showing that the different hydrolysis can lead to obtain peptides more or less bioactive (Nongonierma & FitzGerald, 2015). Lammi and co-workers demonstrated that HHs obtained digesting total proteins with pepsin and trypsin exert anti-diabetic effect through the inhibition of DPP-IV *in vitro* and on the surface of Caco-2 cells (Lammi, Bollati, Gelain, Arnoldi, & Pugliese, 2019).

Another bioactivity showed by HHs is the inhibition of the α-glucosidase activity (Ren et al., 2016) that it is an important enzyme located in the mucosal brush of the small intestine and implicated in the digestion of carbohydrates, which leads to the liberation of glucose (Alssema et al., 2021). Thus, inhibition of this enzyme delays the carbohydrates catabolism, diminishing the glucose release in the blood (Fig. 3C). For this reason, the discovery of new drugs or natural compounds able to inhibit the α-glucosidase activity is the great interest. In this context, HHs obtained by the use of different enzymes, such as flavourzyme, protamex, neutrase, papain, trypsin, and alcalase, showed an α-glucosidase activity inhibition (Ren et al., 2016). Specifically, the HHs with the best inhibitory activity were the hydrolysates obtained with alcalase. In addition, this study showed that the different degrees of hydrolysis affect the inhibition capacity of these hydrolysates on α-glucosidase. Thus, a high degree of hydrolysis is detrimental to this inhibitory activity. The fraction that showed the highest inhibitory capacity was isolated and analyzed to detect the specific sequences that could be generating this biological activity. Finally, two hemp peptides with antidiabetic biological activity: LR and PLMLP have been identified for the first time (Ren et al., 2016).

Thus, the combined actions of the HHs on the inhibition of the DPP-IV and α-glucosidase activity are the start point to generate new commercial HHs-based functional food able to prevent an exacerbated increase of glucose blood concentration and then diabetes.

The *in vitro* hypoglycemic effects exerted by HHs are summarized in Table 5.

4. Hempseed peptides: challenges and opportunities

4.1. Improving hempseed peptides stability and biological activity by innovative nanotechnological strategy

Even though bioactive peptides have become an emerging field of interest in the scientific community as well as the food, pharmaceutical, and cosmetics industries, some factors limit their nutraceutical and commercial exploitation, including easy chemical degradation (e.g., pH, enzymatic), food matrix interaction, low water-solubility, hygroscopicity, and potential bitter taste. Bearing that in mind, the encapsulation of bioactive peptides in different materials can help to overcome these challenges. Studies have demonstrated that encapsulation of bioactive peptides increases their bioactivity, improves their stability, sensory properties, increases solubility, and decreases hygroscopicity (Aguilar-Toalá, Quintanar-Guerrero, Liceaga, & Zambrano-Zaragoza, 2022). Recently, one innovative strategy have dealt with these issues, consists to the use of well-designed and controlled self-assembly supramolecular systems, such as self-assembling peptides 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 fabricated and assembled according to architectural plans, by applying similar principles it is possible to construct protein and peptide based-hydrogels through the programmable molecular self-assembly (Pugliese, Arnoldi, & Lammi, 2021). In light with this consideration, the hempseed protein hydrolysate stability and its anti-diabetic properties (through DPP-IV inhibition) were improved through their encapsulation into ionic self-complementary RADA16 peptide based-hydrogels (Lammi, Bollati, et al., 2019). A recent study demonstrated that hempseed protein hydrolysates obtained using trypsin and pepsin reduce the DPP-IV activity in cell-free, and cell-based assays. The incubation with the Caco-2 cells slightly impaired the inhibitory potencies of the hydrolysates, with a greater effect on the peptic hempseed protein hydrolysate. This may be possibly explained by metabolic effects. Hence, in order to overcome the observed limitations and improve the DPP-IV inhibitory activity, both hempseed protein hydrolysates were incorporated within the nanofibrous structures of RADA16 self-assembling peptides-hydrogel. Atomic force microscope (AFM) morphological analysis indicated that the new nanomaterials were composed of a nanofibril network, whose increased diameter in respect to native RADA16 suggests the presence of transient

non-covalent interactions among the RADA16 supramolecular assemblies and the embedded hempseed peptides. Structural analysis by Fourier-transform infrared spectroscopy indicated typical β-sheet signatures. The RADA16-hempseed protein hydrolysate hydrogel act as a DPP-IV inhibitor, in Caco-2 cells, with gained more activity with respect to their plain solutions (2.5–2.0-folds more) (Lammi, Bollati, et al., 2019). Indeed, when hempseed peptides are non-covalently entrapped into the RADA16 self-assembling peptide, the stability of both peptides is significantly improved, suggesting that this strategy could be a viable platform for targeting metabolic diseases (Pugliese, Gelain, Arnoldi, & Lammi, 2021).

Since self-assembling peptides are amenable to multi-functionalization with bioactive motifs to intimately interact with cells, cytokines, tissues, etc., it is feasible to functionalize the RADA16 self-assembling peptides with the synthetic analogs of hempseed peptides at the N-terminus, in order to create a food bioactive assembling peptide with improved biological properties. Extending the peptide sequence to the C or N-terminus, either during the peptide synthesis or subsequently using click-chemistry, the biofunctionalization of the self-assembling peptides can be obtained. Furthermore, differently functionalized self-assembling peptides, sharing the same self-assembling sequence, can be mixed together, thus displaying multiple functional motifs. This simple strategy, which is feasible to customize, in terms of bioactivity, has already been developed and applied for fostering the new exploitation of lupin, soybean, and rapeseed peptides (Pugliese et al., 2022), suggesting that it can be easily applied also for hempseed peptides. Notably, it would be interesting to design a new RADA16 scaffold functionalized with active hempseed peptides, i.e., LR and PLMLP, or WYT, SVYT, IPAGV, WVYY, and PSLPA to obtain a new hydrogel endowed by hypoglycemic or hypotensive activity.

Currently, even though the investigations in the field of food-derived peptides are focused to valorize their own bioactive or technological properties, it is interesting to observe that peptides within protein hydrolysates might be also endowed with structural properties. This feature might be exploited in order to characterize the self-assembling behavior of natural peptides, which can give origin to new functional/smart materials, such as hydrogels. For example, the peptide LNALEPDNTVQSEAGTIETWNPK (named T13) stands out for its interesting structural properties (Lammi, Zanoni, Aiello, Arnoldi, & Grazioso, 2016). T13, which is composed of 23 amino acids, 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 (MD) simulations, suggesting that T13 was shaped as a β-hairpin (Lammi, Zanoni, Aiello, et al., 2016). Given its peculiarity of spontaneously organizing into ordered β-hairpin structures, it was demonstrated that T13 is an antioxidant peptide capable of creating nanostructures with hierarchical self-assembly propensity that were enhanced by a straightforward N-terminal biotinylated oligoglycine tag-based methodology (Pugliese, Arnoldi, & Lammi, 2021). In light with these evidences, hempseed protein hydrolysates might also represent useful sources of peptides with structural properties that could be used to develop naturally occurring food peptide-based hydrogels, providing new research directions across food chemistry, biochemistry, and bioengineering.

4.2. Hempseed peptides and structure-function relationship

The bioactivity of a single peptide is typically related to its size, physicochemical properties, and amino acid sequence, whereas that of a protein hydrolysate depends strictly on its total composition, including inactive and active species, and possible synergistic or antagonist effects (Aiello et al., 2017; Zanoni et al., 2017). In this context, the possibility to establish a reasonable structure-function relationship is impaired by the fact that little literature evidence exploring this aspect is available. Indeed, among the hempseed peptide bioactivities, the most studied ones are doubtless the antioxidant and ACE inhibitory.

Many physical-chemical factors may influence the ability of peptides to exert antioxidant activity. In fact, although certain aspects of the structure-function relationship of antioxidant peptides are still poorly understood (Harnedy, O'Keeffe, & FitzGerald, 2017), chain length, type, composition, sequence, and the location of specific amino acids in a peptide chain have been suggested to be critical issues for exerting the antioxidant property (Gallego, Mora, & Toldrá, 2018). In this context, short peptides may be often potent antioxidants. Literature indicates that, besides containing hydrophobic amino acids, such as Leu or Val, in their N-terminal regions, peptides containing nucleophilic sulfur-containing amino acid residues (Cys and Met), aromatic amino acid residues (Phe, Trp, and Tyr) and/or the imidazole ring-containing His are generally found to possess strong antioxidant properties (Nwachukwu & Aluko, 2018, 2019). Based on these considerations, HH4 is a short peptide and it stands out for the presence of two aromatic amino acids (Trp and Phe) within its sequence, which certainly contribute to its antioxidant activity (Bollati et al., 2021). In addition, since the repetitive di- or tri- amino acid residues within a peptide have been linked to enhanced antioxidant activity (Jin, Liu, Zheng, Wang, & He, 2016), the HH4 antioxidant behavior may be linked to the repetitive II sequence (biochemical and physicochemical properties are shown in Table 7).

Similar to hempseed peptide WVYY, the antioxidant activity of peptide HH3 is linked to the presence of Trp residue located in the N-terminal portion of the peptide as well as to the presence of an Arg residue in the C-terminal (Bollati et al., 2021). In particular, the Arg residue in C-terminal may be correlated with its high ABTS radical scavenging ability. This evidence is in line with the fact that the C-terminal Arg residue has been linked to the high antioxidant activity of certain peptides, i.e. GLFGPR and GATGPQGPLGPR (Sae-Leaw et al., 2017).

vBasically, behind the hypocholesterolemic activity of peptides, different mechanisms of action may occur. In particular, in order to function as a competitive inhibitor of HMG-CoAR, a peptide should mimic the HMG moiety. To achieve this goal, the conformation and the side chain groups play a more important role than the total hydrophobicity. Moreover, the correlation of the inhibitory activity with the peptide length is still unclear. Notably, the computed putative complex between HH4 and HMG-CoAR, reveals that peptide HH4 is conveniently accommodated at the interface between the two monomers with which it stabilizes a diversification pattern of interactions (Li et al., 2022). The Phe residue in the third position within the peptide is nicely inserted within a narrow sub-pocket mostly lined by hydrophobic residues. Similarly, Trp-7 approaches the Lα1helix of the other monomer where it stabilizes a clear charge-transfer interaction with Arg-568. Although both charged termini are involved (as expected) in key ion-pairs, the hydrophobic contacts in the HH4 binding played a remarkable role. In detail, almost all the central residues are engaged in hydrophobic interactions involving both alkyl side chains and methionine residues, which can also elicit π-sulfur interactions with aromatic residues (i.e., Phe-5) (Li et al., 2022). The comparison with the key interactions stabilizing the resolved complex of HMG-CoAR with statins reveals an interesting agreement with those observed for the HH4 peptide to be concerned with both the basic residues bridging the ligand's carboxylate (K662 and R590) and hydrophobic side chains which also approach the apolar moieties of the statins (V683, L853, L857) (Nwachukwu & Aluko, 2018, 2019).

Malomo et al. suggested that the better ACE-inhibitory activity of the hempseed protein hydrolysate obtained using 2% papain may be due to the slightly higher Pro content when compared to that of the other hempseed protein hydrolysates obtained using pepsin, Alcalase, and pepsin + pancreatin (Girgih et al., 2014; Girgih, Udenigwe, & Aluko, 2011; Malomo et al., 2015). This is because Pro has been suggested as a potency-enhancing factor for ACE-inhibitory peptides. However, Pro content alone is not directly related to ACE-inhibitory activity of all the

Table 7
Hempseed peptides: biochemical and physicochemical properties.

Peptide	aa	MW (Da)	Charge	SVM Score[a]	Ho (kcal/mol)	BioRank[b]	Total rings[c]	Reference
Antioxidant effects								
NHAV	4	439.52	+0.5	−0.83	+11.12	0.09	1	Lu et al. (2010)
HVRETALV	8	924.18	+0.5	−0.89	+14.25	0.07	1	
WVYY	4	629.76	0	−0.85	+3.93	0.71	4	Girgih et al. (2013)
PSLPA	5	483.62	0	−1.04	+7.89	0.52	2	
YGRDEISV	8	938.11	−1	−0.67	+16.30	0.10	1	Gao et al. (2021)
LDLVKPQ	7	812.08	0	−1.01	+12.29	0.12	1	
DVFSPQAGRL	10	1089.35	0	−0.89	+12.95	0.19	2	Bollati et al. (2021)
WVSPLAGRT	9	986.26	+1	−0.90	+8.41	0.65	3	
IGFLIIWV	8	960.36	0	−0.47	+0.18	0.74	3	
DVFTPQAGRIST	12	1291.59	0	−1.18	+13.58	0.66	2	
IRALPEAV	8	868.15	0	−0.93	+11.65	0.14	1	
Immunomodulatory								
WVSPLAGRT	9	986.26	+1	−0.90	+8.41	0.65	3	Cruz-Chamorro et al. (2022)
IGFLIIWV	8	960.36	0	−0.47	+0.18	0.74	3	
Antihypertensive								
WVYY	4	629.76	0	−0.85	+3.93	0.71	4	(Girgih et al., 2014; Orio et al., 2017)
PSLPA	5	483.62	0	−1.04	+7.89	0.52	2	
GVLY	4	450.59	0	−0.85	+6.63	0.34	1	
LGV	3	287.40	0	−0.85	+7.34	0.21	0	
RVR	3	429.55	+2	−0.82	+11.06	0.19	0	
WYT	3	468.54	0	−0.79	+5.35	0.76	5	
SVYT	4	468.55	0	−0.87	+7.44	0.10	1	
IPAGV	5	455.62	0	−0.95	+8.11	0.28	1	
Hypoglycemic								
LR	2	287.38	+1	−0.80	+8.46	0.57	0	Ren et al. (2016)
PLMLP	5	569.83	0	−1.11	+5.01	0.88	2	
Hypocholesterolemicùù								
IGFLIIWV	8	960.36	0	−0.47	+0.18	0.74	3	Li et al. (2022)

[a] Calculated according to (Gupta et al., 2013).
[b] Calculated according to (Mooney, Haslam, Pollastri, & Shields, 2012).
[c] Calculated as number of rings of H + F + P + Y + Wx2.

hempseed protein hydrolysates obtained using pepsin, alcalase, and pepsin + pancreatin, which suggests that other peptide structural factors (e.g., Pro position, presence of aromatic amino acids, or absence of disulfide bonds) may be responsible for the observed results. For example, the hempseed protein hydrolysate obtained using 2% pepsin has very low Cys level while the hydrolysate obtained using 2% pepsin + pancreatin has slightly higher Tyr level, both of which may have contributed to the poor ACE-inhibitory activities (Malomo et al., 2015). In addition, it has been demonstrated that WYT and SVYT show the highest dual inhibition of ACE and renin activities. Both peptides contain bulky aromatic amino acids, which could have contributed to increased potency by enhancing hydrophobic interactions with the enzyme protein. The higher ACE inhibition by SVYT may also be due to the presence of Val, a branched-chain amino acid with a high affinity for ACE active sub-sites. Some of the peptides such as PSLPA, VSYT, FEQL, and YNI have weak or strong ACE-inhibitory activities but lack detectable inhibition of renin (Girgih et al., 2014).

5. Conclusion

HHs and derived peptides have demonstrated to exert antioxidant, immunomodulatory, hypocholesterolemic, antihypertensive, and hypoglycemic effects (summarized in Fig. 4). Furthermore, enzymes and time used during the hydrolysis process is a key point related to the final bioactivity of the HHs. In fact, the bioactivity of these hydrolysates and peptides depends on the structural properties (length and amino acid composition) and physicochemical characteristics of the amino acid chains (hydrophobicity and charge). Thus, HHs, due to their multifunctional effects, can be considered as an excellent component for the generation of new nutraceuticals. The investigation of the absorption, distribution and metabolism of these peptides would contribute to understanding the molecular base of the action mechanisms that underline their biological activities. However, to date, most of the demonstrated effects have been reported in *vitro* and in free-cell systems. For this reason, and with the purpose of the HHs can be incorporated as ingredients in functional food or in nutraceuticals products, further studies in animals and/or in humans are needed to confirm these beneficial biological activities.

On one hand, studies on hypercholesterolemia, hypertension, or hyperglycemic models in mice should be carried out to confirm the demonstrated *in vitro* effects. A very interesting mouse model would be the high-fat diet fed-C57BL/6 mice to simulate the metabolic dysfunction-associated fatty liver disease (MAFLD). This model would allow to evaluate, at the same time, the antioxidant, immunomodulating, and hypocholesterolemic effects of HHs, as peptides from other vegetable sources have previously been reported (Santos-Sánchez et al., 2021; Santos-Sánchez et al., 2022). On the other hand, once effects have been proved in mice, the following step would be to validate the safety of a product based on the HHs in human subjects, as our group have previously reported with another product containing lupin hydrolysate (Cruz-Chamorro et al., 2021). Finally, in order to be marketed as nutraceuticals in several pathological conditions, it will be necessary to test the efficacy of these HHs in subjects that present some disorders or diseases as well as to determine dose-response relation, bioavailability (absorption, distribution), and pharmacokinetics.

Undoubtedly, this review opens the field to investigate the beneficial effects of HHs in *vivo* models and points out them as new promising nutraceuticals that could be used in several pathological condition.

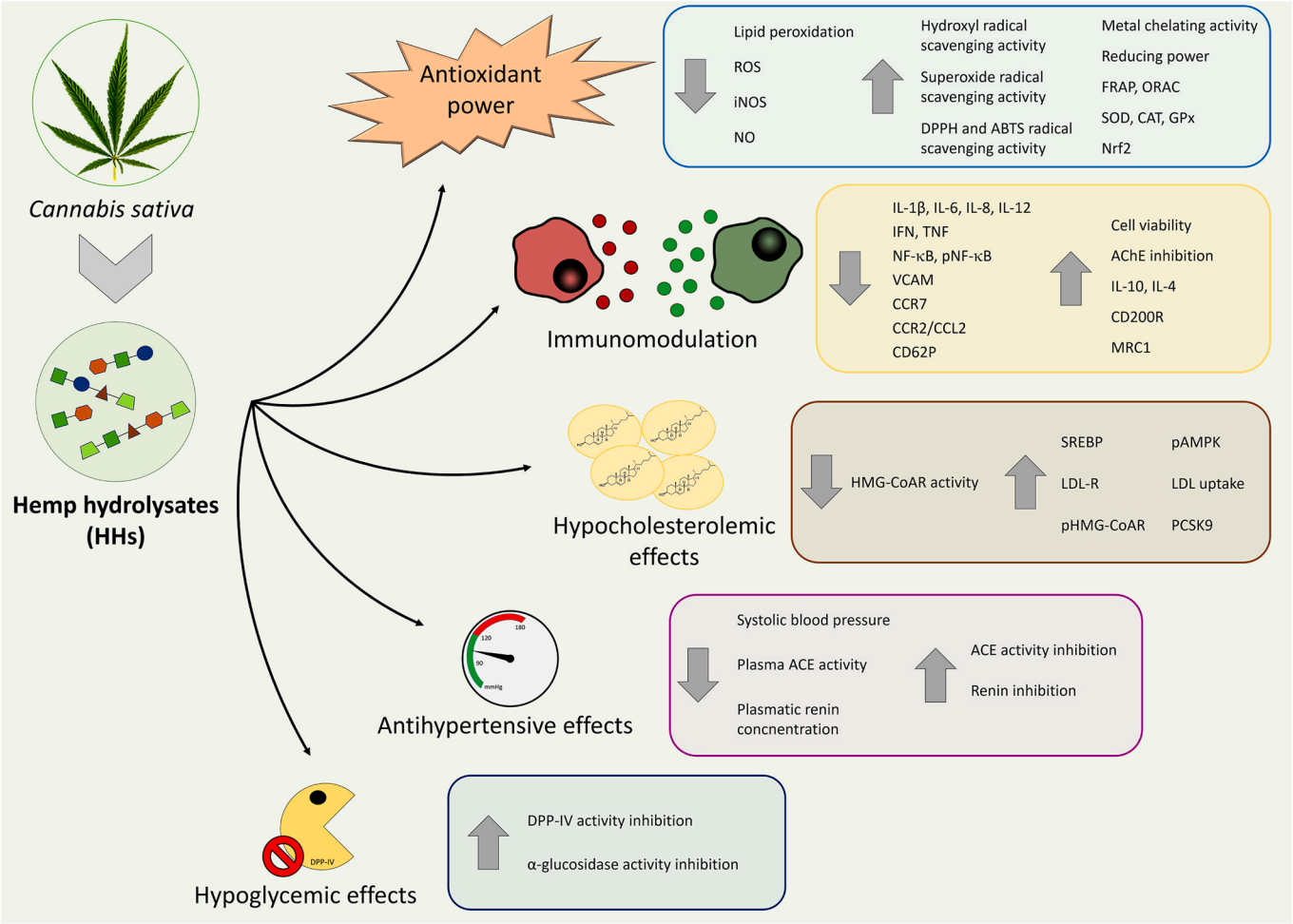

Fig. 4. Visual representation of the biological effects exerted by hempseed protein hydrolysates. ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ACE, angiotensin I converting enzyme; AChE, acetylcholinesterase; CAT, catalase; CCL, (C–C motif) ligand; CCR, (C–C motif) receptor; CD200R, cluster of differentiation 200 receptor; CD62P, platelet P-selectin; DPP-IV, dipeptidyl peptidase-IV; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GPx, glutathione peroxidase; HMG-CoAR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; LDL-R, LDL receptor; MRC1, mannose receptor C-type 1; NF-κB, nuclear factor kappa B; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; pAMPK, phosphate-activated protein kinase; PCSK9, proprotein convertase subtilisin/kexin type 9; pHMG-CoAR, phosphorylated HMG-CoAR; pNF-κB, phosphorylated NF-κB; ROS, reactive oxigen species; SOD, superoxide dismutase; SREBP, sterol-responsive element-binding protein; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

Acknowledgements

I.C.-C. was supported by the VI Program of Inner Initiative for Research and Transfer of the University of Seville (VIPPIT-2020-II.4) and by a postdoctoral fellowship from the Andalusian Government Ministry of Economy, Knowledge, Business, and University (DOC_00587/2020). G.S.-S. was supported by a FPU grant from the Spanish Ministerio de Educación, Cultura y Deporte (FPU16/02339) and by an Erasmus+ Mobility Programme. A.I.A.-L. was funded by Andalusian Government Ministry of Health (PI-0136-2019). E.P.-E. was supported by the VI Program of Inner Initiative for Research and Transfer of University of Seville.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2022.06.005.

References

Abassi, Z., Winaver, J., & Feuerstein, G. Z. (2009). The biochemical pharmacology of renin inhibitors: Implications for translational medicine in hypertension, diabetic nephropathy and heart failure: Expectations and reality. *Biochemical Pharmacology, 78*, 933–940.

Aguilar-Toalá, J., Quintanar-Guerrero, D., Liceaga, A., & Zambrano-Zaragoza, M. (2022). Encapsulation of bioactive peptides: A strategy to improve the stability, protect the nutraceutical bioactivity and support their food applications. *RSC Advances, 12*, 6449–6458.

Ahrén, B. (2019). DPP-4 inhibition and the path to clinical proof. *Frontiers in Endocrinology, 10*, 376.

Aiello, G., Lammi, C., Boschin, G., Zanoni, C., & Arnoldi, A. (2017). Exploration of potentially bioactive peptides generated from the enzymatic hydrolysis of hempseed proteins. *Journal of Agricultural and Food Chemistry, 65*, 10174–10184.

Alssema, M., Ruijgrok, C., Blaak, E. E., Egli, L., Dussort, P., Vinoy, S., et al. (2021). Effects of alpha-glucosidase-inhibiting drugs on acute postprandial glucose and insulin responses: A systematic review and meta-analysis. *Nutrition & Diabetes, 11*, 1–9.

Ames, M. K., Atkins, C. E., & Pitt, B. (2019). The renin-angiotensin-aldosterone system and its suppression. *Journal of Veterinary Internal Medicine, 33*, 363–382.

Amigo, L., & Hernández-Ledesma, B. (2020). Current evidence on the bioavailability of food bioactive peptides. *Molecules, 25*, 4479.

Andersen, E. S., Deacon, C. F., & Holst, J. J. (2018). Do we know the true mechanism of action of the DPP-4 inhibitors? *Diabetes, Obesity and Metabolism, 20*, 34–41.

Atkinson, A., & Robertson, J. (1979). Captopril in the treatment of clinical hypertension and cardiac failure. *The Lancet, 314*, 836–839.

Atri, C., Guerfali, F. Z., & Laouini, D. (2018). Role of human macrophage polarization in inflammation during infectious diseases. *International Journal of Molecular Sciences, 19*, 1801.

Bernstein, K. E., Giani, J. F., Shen, X. Z., & Gonzalez-Villalobos, R. A. (2014). Renal angiotensin-converting enzyme and blood pressure control. *Current Opinion in Nephrology and Hypertension, 23*, 106.

Bollati, C., Cruz-Chamorro, I., Aiello, G., Li, J., Bartolomei, M., Santos-Sánchez, G., et al. (2021). Investigation of the intestinal trans-epithelial transport and antioxidant activity of two hempseed peptides WVSPLAGRT (H2) and IGFLIIWV (H3). *Food Research International*, Article 110720.

Cannizzaro, C., & Diana, M. (2016). Cannabis and the mesolimbic system. In *Neuropathology of drug addictions and substance misuse* (pp. 795–803). Elsevier.

Casas-Grajales, S., & Muriel, P. (2017). The liver, oxidative stress, and antioxidants. In *Liver pathophysiology* (pp. 583–604). Elsevier.

Colovic, M. B., Krstic, D. Z., Lazarevic-Pasti, T. D., Bondzic, A. M., & Vasic, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Current Neuropharmacology, 11*, 315–335.

Cote, B., Elbarbry, F., Bui, F., Su, J. W., Seo, K., Nguyen, A., et al. (2022). Mechanistic basis for the role of phytochemicals in inflammation-associated chronic diseases. *Molecules, 27*, 781.

Cruz-Chamorro, I., Álvarez-Sánchez, N., del Carmen Millán-Linares, M., del Mar Yust, M., Pedroche, J., Millán, F., et al. (2019). Lupine protein hydrolysates decrease the inflammatory response and improve the oxidative status in human peripheral lymphocytes. *Food Research International, 126*, Article 108585.

Cruz-Chamorro, I., Santos-Sánchez, G., Bollati, C., Bartolomei, M., Li, J., Arnoldi, A., et al. (2022). The hempseed (C. sativa) peptides WVSPLAGRT and IGFLIIWV exert anti-inflammatory activity in LPS stimulated human hepatic cell line. *Journal of Agricultural and Food Chemistry, 70*, 577–583.

Cruz-Chamorro, I., Álvarez-Sánchez, N., Álvarez-Ríos, A. I., Santos-Sánchez, G., Pedroche, J., Millán, F., et al. (2021). Safety and efficacy of a beverage containing lupine protein hydrolysates on the immune, oxidative and lipid status in healthy subjects: An intervention study (the lupine-1 trial). *Molecular Nutrition & Food Research, 65*(2100139).

Daskaya-Dikmen, C., Yucetepe, A., Karbancioglu-Guler, F., Daskaya, H., & Ozcelik, B. (2017). Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from plants. *Nutrients, 9*, 316.

Faostat. (2019). *Food and agriculture organization of the united nations (FAO) statistics reports*. . (Accessed October 2021) Accessed.

Farinon, B., Molinari, R., Costantini, L., & Merendino, N. (2020). The seed of industrial hemp (Cannabis sativa L.): Nutritional quality and potential functionality for human health and nutrition. *Nutrients, 12*, 1935.

Gallego, M., Mora, L., & Toldrá, F. (2018). Characterisation of the antioxidant peptide AEEEYPDL and its quantification in Spanish dry-cured ham. *Food Chemistry, 258*, 8–15.

Gao, J., Li, T., Chen, D., Gu, H., & Mao, X. (2021). Identification and molecular docking of antioxidant peptides from hemp seed protein hydrolysates. *LWT, 147*, Article 111453.

Gavel, N., Edel, A., Bassett, C., Weber, A., Merchant, M., Rodriguez-Leyva, D., et al. (2011). The effect of dietary hempseed on atherogenesis and contractile function in aortae from hypercholesterolemic rabbits. *Acta Physiologica Hungarica, 98*, 273–283.

Girgih, A. T., Alashi, A., He, R., Malomo, S., & Aluko, R. E. (2014). Preventive and treatment effects of a hemp seed (Cannabis sativa L.) meal protein hydrolysate against high blood pressure in spontaneously hypertensive rats. *European Journal of Nutrition, 53*, 1237–1246.

Girgih, A. T., Alashi, A. M., He, R., Malomo, S. A., Raj, P., Netticadan, T., et al. (2014). A novel hemp seed meal protein hydrolysate reduces oxidative stress factors in spontaneously hypertensive rats. *Nutrients, 6*, 5652–5666.

Girgih, A. T., He, R., & Aluko, R. E. (2014). Kinetics and molecular docking studies of the inhibitions of angiotensin converting enzyme and renin activities by hemp seed (Cannabis sativa L.) peptides. *Journal of Agricultural and Food Chemistry, 62*, 4135–4144.

Girgih, A. T., He, R., Malomo, S., Offengenden, M., Wu, J., & Aluko, R. E. (2014). Structural and functional characterization of hemp seed (Cannabis sativa L.) protein-derived antioxidant and antihypertensive peptides. *Journal of Functional Foods, 6*, 384–394.

Girgih, A. T., Udenigwe, C. C., & Aluko, R. E. (2011). In vitro antioxidant properties of hemp seed (Cannabis sativa L.) protein hydrolysate fractions. *Journal of the American Oil Chemists' Society, 88*, 381–389.

Girgih, A. T., Udenigwe, C. C., & Aluko, R. E. (2013). Reverse-phase HPLC separation of hemp seed (Cannabis sativa L.) protein hydrolysate produced peptide fractions with enhanced antioxidant capacity. *Plant Foods for Human Nutrition, 68*, 39–46.

Girgih, A. T., Udenigwe, C. C., Li, H., Adebiyi, A. P., & Aluko, R. E. (2011). Kinetics of enzyme inhibition and antihypertensive effects of hemp seed (Cannabis sativa L.) protein hydrolysates. *Journal of the American Oil Chemists' Society, 88*, 1767–1774.

Godinho, R., Mega, C., Teixeira-de-Lemos, E., Carvalho, E., Teixeira, F., Fernandes, R., et al. (2015). The place of dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapeutics: A "me too" or "the special one" antidiabetic class? *Journal of Diabetes Research, 806979*, 2015.

Guessous, I., Bochud, M., Theler, J.-M., Gaspoz, J.-M., & Pechère-Bertschi, A. (2012). 1999–2009 Trends in prevalence, unawareness, treatment and control of hypertension in Geneva, Switzerland. *PLoS One, 7*, Article e39877.

Harnedy, P. A., O'Keeffe, M. B., & FitzGerald, R. J. (2017). Fractionation and identification of antioxidant peptides from an enzymatically hydrolysed Palmaria palmata protein isolate. *Food Research International, 100*, 416–422.

Hartsel, J. A., Eades, J., Hickory, B., & Makriyannis, A. (2016). Cannabis sativa and hemp. In *Nutraceuticals* (pp. 735–754). Elsevier.

Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: A novel alternative to drugs? *Peptides, 29*, 1062–1071.

House, J. D., Neufeld, J., & Leson, G. (2010). Evaluating the quality of protein from hemp seed (Cannabis sativa L.) products through the use of the protein digestibility-corrected amino acid score method. *Journal of Agricultural and Food Chemistry, 58*, 11801–11807.

Jelena, C. H., Giorgio, R., Justyna, G., Neda, M.-D., Natasa, S., Artur, B., et al. (2018). Beneficial effects of polyphenols on chronic diseases and ageing. In *Polyphenols: Properties, recovery, and applications* (pp. 69–102). Elsevier.

Jin, D.-x., Liu, X.-l., Zheng, X.-q., Wang, X.-j., & He, J.-f. (2016). Preparation of antioxidative corn protein hydrolysates, purification and evaluation of three novel corn antioxidant peptides. *Food Chemistry, 204*, 427–436.

Kannel, W. B. (1989). Risk factors in hypertension. *Journal of Cardiovascular Pharmacology, 13*, S4–S10.

Karche, T. (2019). The application of hemp (Cannabis sativa L.) for a green economy: A review. *Turkish Journal of Botany, 43*, 710–723.

Lammi, C., Arnoldi, A., & Aiello, G. (2019). Soybean peptides exert multifunctional bioactivity modulating 3-hydroxy-3-methylglutaryl-coa reductase and dipeptidyl peptidase-iv targets in vitro. *Journal of Agricultural and Food Chemistry, 67*, 4824–4830.

Lammi, C., Bellumori, M., Cecchi, L., Bartolomei, M., Bollati, C., Clodoveo, M. L., et al. (2020). Extra virgin olive oil phenol extracts exert hypocholesterolemic effects through the modulation of the LDLR pathway: In vitro and cellular mechanism of action elucidation. *Nutrients, 12*, 1723.

Lammi, C., Bollati, C., Gelain, F., Arnoldi, A., & Pugliese, R. (2019). Enhancement of the stability and anti-DPPIV activity of hempseed hydrolysates through self-assembling peptide-based hydrogels. *Frontiers of Chemistry, 6*, 670.

Lammi, C., Zanoni, C., Aiello, G., Arnoldi, A., & Grazioso, G. (2016). Lupin peptides modulate the protein-protein interaction of PCSK9 with the low density lipoprotein receptor in HepG2 cells. *Scientific Reports, 6*, 1–13.

Lammi, C., Zanoni, C., Arnoldi, A., & Vistoli, G. (2015). Two peptides from soy β-conglycinin induce a hypocholesterolemic effect in HepG2 Cells by a statin-like mechanism: Comparative in vitro and in silico modeling studies. *Journal of Agricultural and Food Chemistry, 63*, 7945–7951.

Lammi, C., Zanoni, C., Arnoldi, A., & Vistoli, G. (2016). Peptides derived from soy and lupin protein as dipeptidyl-peptidase IV inhibitors: In vitro biochemical screening and in silico molecular modeling study. *Journal of Agricultural and Food Chemistry, 64*, 9601–9606.

Lammi, C., Zanoni, C., Scigliuolo, G. M., D'Amato, A., & Arnoldi, A. (2014). Lupin peptides lower low-density lipoprotein (LDL) cholesterol through an up-regulation of the LDL receptor/sterol regulatory element binding protein 2 (SREBP2) pathway at HepG2 cell line. *Journal of Agricultural and Food Chemistry, 62*, 7151–7159.

Leonard, W., Zhang, P., Ying, D., & Fang, Z. (2020). Hempseed in food industry: Nutritional value, health benefits, and industrial applications. *Comprehensive Reviews in Food Science and Food Safety, 19*, 282–308.

Li, J., Bollati, C., Bartolomei, M., Mazzolari, A., Arnoldi, A., Vistoli, G., et al. (2022). Hempseed (Cannabis sativa) peptide H3 (IGFLIIWV) exerts cholesterol-lowering effects in human hepatic cell line. *Nutrients, 14*, 1804.

Logarušić, M., Slivac, I., Radošević, K., Bagović, M., Redovniković, I. R., & Srček, V. G. (2019). Hempseed protein hydrolysates' effects on the proliferation and induced oxidative stress in normal and cancer cell lines. *Molecular Biology Reports, 46*, 6079–6085.

Lu, R.-R., Qian, P., Sun, Z., Zhou, X.-H., Chen, T.-P., He, J.-F., et al. (2010). Hempseed protein derived antioxidative peptides: Purification, identification and protection from hydrogen peroxide-induced apoptosis in PC12 cells. *Food Chemistry, 123*, 1210–1218.

Mahbub, R., Callcott, E., Rao, S., Ansari, O., Waters, D. L., Blanchard, C. L., et al. (2022). The effect of selected hemp seed protein hydrolysates in modulating vascular function. *Food Bioscience, 45*, Article 101504.

Malomo, S. A., & Aluko, R. E. (2016). In vitro acetylcholinesterase-inhibitory properties of enzymatic hemp seed protein hydrolysates. *Journal of the American Oil Chemists' Society, 93*, 411–420.

Malomo, S. A., & Aluko, R. E. (2019). Kinetics of acetylcholinesterase inhibition by hemp seed protein-derived peptides. *Journal of Food Biochemistry, 43*, Article e12897.

Malomo, S. A., Onuh, J. O., Girgih, A. T., & Aluko, R. E. (2015). Structural and antihypertensive properties of enzymatic hemp seed protein hydrolysates. *Nutrients, 7*, 7616–7632.

Mazorra-Manzano, M., Ramírez-Suarez, J., & Yada, R. (2018). Plant proteases for bioactive peptides release: A review. *Critical Reviews in Food Science and Nutrition, 58*, 2147–2163.

Michel, B., Welsch, C., Coquard, C., Grima, M., Barthelmebs, M., & Imbs, J.-L. (1993). Angiotensin converting enzyme variability in hypertensive and normotensive rats. *Hypertension, 21*, 442–445.

Nongonierma, A. B., & FitzGerald, R. J. (2015). Investigation of the potential of hemp, pea, rice and soy protein hydrolysates as a source of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides. *Food Digestion: Research and Current Opinion, 6*, 19–29.

Nwachukwu, I. D., & Aluko, R. E. (2018). Antioxidant properties of flaxseed protein hydrolysates: Influence of hydrolytic enzyme concentration and peptide size. *Journal of the American Oil Chemists' Society, 95*, 1105–1118.

Nwachukwu, I. D., & Aluko, R. E. (2019). Structural and functional properties of food protein-derived antioxidant peptides. *Journal of Food Biochemistry, 43*, Article e12761.

Organization, W. H. (2013). *A global brief on hypertension: Silent killer, global public health crisis: World health day*. World Health Organization, 2013.

Orio, L. P., Boschin, G., Recca, T., Morelli, C. F., Ragona, L., Francescato, P., et al. (2017). New ACE-inhibitory peptides from hemp seed (Cannabis sativa L.) proteins. *Journal of Agricultural and Food Chemistry, 65*, 10482–10488.

O'Shaughnessy, K. M. (2006). Role of diet in hypertension management. *Current Hypertension Reports, 8*, 292–297.

Parihar, A., Eubank, T. D., & Doseff, A. I. (2010). Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death. *Journal of innate immunity, 2*, 204–215.
Peters, S., Singhateh, Y., Mackay, D., Huxley, R. R., & Woodward, M. (2016). Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. *Atherosclerosis, 248*, 123–131.
Pirahanchi, Y., & Sharma, S. (2019). *Physiology, Bradykinin*.
Prociuk, M., Edel, A., Gavel, N., Lukas, A., & Pierce, G. (2004). Influence of dietary hempseed on arrhythmia generation by ischemia/reperfusion. *Journal of Molecular and Cellular Cardiology, 37*, 275. Elsevier, 275.
Prociuk, M., Edel, A., Richard, M., Gavel, N., Ander, B., Dupasquier, C., et al. (2008). Cholesterol-induced stimulation of platelet aggregation is prevented by a hempseed-enriched diet. *Canadian Journal of Physiology and Pharmacology, 86*, 153–159.
Pugliese, R., Arnoldi, A., & Lammi, C. (2021). Nanostructure, self-assembly, mechanical properties, and antioxidant activity of a lupin-derived peptide hydrogel. *Biomedicines, 9*, 294.
Pugliese, R., Bartolomei, M., Bollati, C., Boschin, G., Arnoldi, A., & Lammi, C. (2022). Gel-forming of self-assembling peptides functionalized with food bioactive motifs modulate DPP-IV and ACE inhibitory activity in human intestinal caco-2 cells. *Biomedicines, 10*, 330.
Pugliese, R., & Gelain, F. (2017). Peptidic biomaterials: From self-assembling to regenerative medicine. *Trends in Biotechnology, 35*, 145–158.
Pugliese, R., Gelain, F., Arnoldi, A., & Lammi, C. (2021). Chemistry and functional roles of food protein hydrogels. In *Food proteins and peptides* (pp. 157–172).
Ren, Y., Liang, K., Jin, Y., Zhang, M., Chen, Y., Wu, H., et al. (2016). Identification and characterization of two novel α-glucosidase inhibitory oligopeptides from hemp (Cannabis sativa L.) seed protein. *Journal of Functional Foods, 26*, 439–450.
Rodriguez-Martin, N. M., Montserrat-de la Paz, S., Toscano, R., Grao-Cruces, E., Villanueva, A., Pedroche, J., et al. (2020). Hemp (Cannabis sativa L.) protein hydrolysates promote anti-inflammatory response in primary human monocytes. *Biomolecules, 10*, 803.
Rodriguez-Martin, N. M., Toscano, R., Villanueva, A., Pedroche, J., Millan, F., Montserrat-de la Paz, S., et al. (2019). Neuroprotective protein hydrolysates from hemp (Cannabis sativa L.) seeds. *Food & Function, 10*, 6732–6739.
Sae-Leaw, T., Karnjanapratum, S., O Callaghan, Y. C., O Keeffe, M. B., FitzGerald, R. J., Brien, O., Benjakul, S., et al. (2017). Purification and identification of antioxidant peptides from gelatin hydrolysate of seabass skin. *Journal of Food Biochemistry, 41*, 1–11.
Samaei, S. P., Martini, S., Tagliazucchi, D., Gianotti, A., & Babini, E. (2021). Antioxidant and angiotensin I-converting enzyme (ACE) inhibitory peptides obtained from alcalase protein hydrolysate fractions of hemp (Cannabis sativa L.) bran. *Journal of Agricultural and Food Chemistry, 69*(32), 9220–9228.
Samsamikor, M., Mackay, D., Mollard, R. C., & Aluko, R. E. (2020). A double-blind, randomized, crossover trial protocol of whole hemp seed protein and hemp seed protein hydrolysate consumption for hypertension. *Trials, 21*, 1–13.
Santos-Sánchez, G., Cruz-Chamorro, I., Álvarez-Ríos, A. I., Fernández-Santos, J. M., Vázquez-Román, M. V., Rodríguez-Ortiz, B., et al. (2021). Lupinus angustifolius protein hydrolysates reduce abdominal adiposity and ameliorate metabolic associated fatty liver disease (MAFLD) in western diet fed-ApoE−/− mice. *Antioxidants, 10*, 1222.
Santos-Sánchez, G., Cruz-Chamorro, I., Bollati, C., Bartolomei, M., Pedroche, J., Millán, F., et al. (2022). A Lupinus angustifolius protein hydrolysate exerts hypocholesterolemic effect in western diet-fed-ApoE−/− mice through the modulation of LDLR and PCSK9 pathways. *Food & Function, 13*, 4158–4170.
Senoner, T., & Dichtl, W. (2019). Oxidative stress in cardiovascular diseases: Still a therapeutic target? *Nutrients, 11*, 2090.
Shen, P., Gao, Z., Fang, B., Rao, J., & Chen, B. (2021). Ferreting out the secrets of industrial hemp protein as emerging functional food ingredients. *Trends in Food Science & Technology, 112*, 1–15.
Singh, S., Shankar, R., & Singh, G. P. (2017). Prevalence and associated risk factors of hypertension: A cross-sectional study in urban varanasi. *International Journal of Hypertension, 2017*, 5491838, 2017.
Stancu, C., & Sima, A. (2001). Statins: Mechanism of action and effects. *Journal of Cellular and Molecular Medicine, 5*, 378–387.
Sun, X., Sun, Y., Li, Y., Wu, Q., & Wang, L. (2021). Identification and characterization of the seed storage proteins and related genes of Cannabis sativa L. *Frontiers in Nutrition, 8*, 297.
Sun, W., Zhang, H., Guo, J., Zhang, X., Zhang, L., Li, C., et al. (2016). Comparison of the efficacy and safety of different ACE inhibitors in patients with chronic heart failure: A PRISMA-compliant network meta-analysis. *Medicine, 95*.
Tang, C.-H., Wang, X.-S., & Yang, X.-Q. (2009). Enzymatic hydrolysis of hemp (Cannabis sativa L.) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. *Food Chemistry, 114*, 1484–1490.
Teh, S.-S., Bekhit, A. E.-D. A., Carne, A., & Birch, J. (2016). Antioxidant and ACE-inhibitory activities of hemp (Cannabis sativa L.) protein hydrolysates produced by the proteases AFP, HT, Pro-G, actinidin and zingibain. *Food Chemistry, 203*, 199–206.
Valizadehderakhshan, M., Shahbazi, A., Kazem-Rostami, M., Todd, M. S., Bhowmik, A., & Wang, L. (2021). Extraction of cannabinoids from Cannabis sativa L.(Hemp). *Agriculture, 11*, 384.
Wang, X.-S., Tang, C.-H., Chen, L., & Yang, X.-Q. (2009). Characterization and antioxidant properties of hemp protein hydrolysates obtained with Neutrase. *Food Technology and Biotechnology, 47*, 428–434.
Wang, Q., & Xiong, Y. L. (2019). Processing, nutrition, and functionality of hempseed protein: A review. *Comprehensive Reviews in Food Science and Food Safety, 18*, 936–952.
World Health Organization. (2020). *Global health estimates*.
World Health Organization. (2021). *Hypertension*.
Xu, Y., Li, J., Zhao, J., Wang, W., Griffin, J., Li, Y., et al. (2021). Hempseed as a nutritious and healthy human food or animal feed source: A review. *International Journal of Food Science and Technology, 56*, 530–543.
Zanoni, C., Aiello, G., Arnoldi, A., & Lammi, C. (2017). Hempseed peptides exert hypocholesterolemic effects with a statin-like mechanism. *Journal of Agricultural and Food Chemistry, 65*, 8829–8838.
Zhang, Q.-Q., & Lu, L.-G. (2015). Nonalcoholic fatty liver disease: Dyslipidemia, risk for cardiovascular complications, and treatment strategy. *Journal of Clinical and Translational Hepatology, 3*, 78.
Zhou, A., Carrell, R. W., Murphy, M. P., Wei, Z., Yan, Y., Stanley, P. L., et al. (2010). A redox switch in angiotensinogen modulates angiotensin release. *Nature, 468*, 108–111.