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Edible insects as a source of biopeptides and their role in immunonutrition

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Many edible insect species are attracting the attention of the food industry and consumers in Western societies due to their high content and quality of protein, and consequently, the potential to be used as a more environmentally friendly dietary source could be beneficial for humans. On the other hand, prevention of inflammatory diseases using nutritional interventions is currently being proposed as a sustainable and cost-effective strategy to improve people's health. In this regard, finding bioactive compounds such as peptides with anti-inflammatory properties from sustainable sources (*e.g.*, edible insects) is one area of particular interest, which might have a relevant role in immunonutrition. This review aims to summarize the recent literature on the discovery of immunomodulatory peptides through *in vitro* studies from edible insects, as well as to describe cell-based assays aiming to prove their bioactivity. On top of that, *in vivo* studies (*i.e.*, animal and human), although scarce, have been mentioned in relation to the topic. In addition, the challenges and future perspectives related to edible-insect peptides and their role in immunonutrition are discussed. The amount of literature aiming to demonstrate the potential immunomodulatory activity of edible-insect peptides is scarce but promising. Different approaches have been employed, especially cell assays and animal studies employing insect meal as supplementation in the diet. Insects such as *Tenebrio molitor* or *Gryllodes sigillatus* are some of the most studied and have demonstrated to contain bioactive peptides. Further investigations, mostly with humans, are needed in order to clearly state that peptides from edible insects may contribute to the modulation of the immune system.

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1. Introduction

1.1. Edible insects as a source of protein

There is a critical necessity to reconsider the relationship among food, environment and health in the framework of the increase in worldwide population, the climate crisis and the rise in the human population suffering from chronic diseases, usually related to a situation of inflammation, implying a loss of quality of life and an increase of healthcare costs.[1] Nutritional interventions have been proved to exert a biologically relevant effect on an individual's health, promoting healthier ageing, by modifying the dietary patterns, for instance, promoting the consumption of vegetable sources, and avoiding a high intake of salt. In this regard, consumption of high-quality protein and peptides is one of the approaches considered as relevant in the proper development of humans, as it can participate in the modulation of several physiological processes.[2]

The usage of current dietary sources of protein as well as the exploration of new ones that might contribute to the safe and secure provision of potentially health-promoting components is a key factor in the current food system. The exploration and exploitation of edible insects as a source of protein and peptides has gained the interest of researchers in recent years, and it is a promising topic that can imply a paradigm shift in the coming years. With regard to consumer acceptance, the most relevant strategies proposed recently to convince consumers to eat insects have been described.[3]

In their dried forms, most of the species contain high quantities of proteins, unsaturated fatty acids, vitamins, minerals, and fibre, which makes them a suitable component for human consumption.[4–6] These edible insects can be treated to yield ingredients like protein isolates or concentrates. Following that, enzymatic hydrolysis and fermentation can be employed to manufacture protein hydrolysates, which can include peptides with potential physiologically relevant effects, and would be considered bioactive peptides.[7] Enzymatic hydrolysis is generally carried out with food-grade proteases such as subtilisin (Alcalase), digestive enzymes (trypsin, pepsin, chymotrypsin), or Flavourzyme, among others.

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Several regulatory-oriented revisions of insects as food and feed, and the relevance of assessing their safety prior to authorisation have been recently published.[8–10] For instance, in Europe, different insects have received a positive opinion from the European Food Safety Authority (EFSA), implying their authorisation and the possibility to use them as food in the European market, including yellow mealworm (*Tenebrio molitor*, 57% of protein), the migratory locust (*Locusta migratoria*, 48.5% of protein) and house cricket (*Acheta domesticus*, 60.3% of protein).[9] On the other hand, human studies investigating the effects on human health are increasing recently, although they are still very scarce.[11] For instance, the similarity in the postprandial state of protein from smaller amounts of mealworm compared to milk protein on protein digestion, amino acid absorption, and muscle synthesis has been reported.[12]

The composition of some edible insects and how different parameters (feed, developmental stage, or growth conditions) affect the quantity of these have been recently reviewed.[13–16] Protein content in the dry matter of insects differs among diverse species, although it is usually considered to be around 40%–60%.[17] However, the presence of chitin (*i.e.*, non-protein nitrogen) has led to an overestimation of this value, which should be reviewed employing a lower nitrogen-to-protein factor, aiming to correct the limitation of the protein content measurement.[18,19] In fact, the content of chitin has been correlated with lower protein digestibility, potentially affecting the protein quality of the products if intended to be used as food and/or feed.[20] Regarding the amino acid content, in general terms, it has been highlighted that the contents of methionine, cysteine, and histidine are lower compared to other commodities.[5,14]

1.2. Immunonutrition

The immune system's reaction to damaging stimuli including tissue damage, infection, or toxic substances is inflammation, which has the dual goals of removing irritants or pathogenic bacteria and promoting tissue healing. Chronic inflammatory disorders are often treated with anti-inflammatory drugs (*e.g.*, aspirin, naproxen, ibuprofen, diclofenac, celecoxib and meloxicam), but the majority of them are insufficient to manage chronic reactions and are also linked to negative side effects (affecting the gastric mucosa, renal system, cardiovascular system, hepatic system, *etc.*).[21] Uncontrolled long-lasting acute inflammation can progressively proceed to chronic inflammation, leading to a range of chronic inflammatory diseases. As a result, several attempts are being made to provide alternative and more focused anti-inflammatory treatments using natural ingredients.[21,22] The immune response system is classified into innate and adaptive immune systems. One of the most relevant immune cells are the macrophages, which upon activation because of an external stimulus produce mediators such as nitric oxide or cytokines including proinflammatory (tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-17) and anti-inflammatory (IL-10, IL-4, and IL-13) ones.[23]

The inhibition of inflammatory enzymes is one of the simplest approaches to evaluate the potential of bioactive peptides, including cyclooxygenase-2 (COX-2), lipoxygenases (LOX), and phospholipase A_2 (PLA2), due to their relationship with prostaglandin and leukotriene metabolism.[24] According to Rivera-Jiménez *et al.*,[21] in the study of peptides and protein hydrolysates as anti-inflammatory agents, most of the researchers employ the cell lines RAW 264.7, THP-1 and Caco-2. The induction of the inflammation is usually achieved by the action of lipopolysaccharide (LPS) or TNF-α. In these studies, the main purpose is usually to evaluate the gene expression and protein release of different cytokines (anti- and pro-inflammatory components), generally with a dose–response relation.

This review aims to summarise the currently available evidence on the production and identification of peptides from edible insect species which have been correlated with immunomodulatory activities and, consequently, might have a relevant role in immunonutrition. Furthermore, a critical revision of the gaps and perspectives in the current state of the research has been described, highlighting and proposing the direction in which scientists should go in order to increase the knowledge of this topic.

2. The role of insect peptides in immunonutrition

2.1. Background

Overall, for some insects such as arthropods, the presence of anti-inflammatory peptides (*e.g.*, adolapin or melittin) has been described as they play a key role in the defense mechanisms against pathogens, which might be related to toxicity.[22] In this regard, in this review, the focus will be on insects that are meant to be used as a food/feed source, that is, edible insects. Dietary consumption of insect protein could deliver peptides that, based on their analogy with regulatory compounds endogenously present in humans, might be associated with health-promoting effects. Among the described bioactivities reported in the literature (antidiabetic, antioxidant, antithrombotic, *etc.*),[25,26] the focus of this review will be on the immunomodulatory properties, generally associated with an anti-inflammatory character.

On top of the evaluation of peptides as immunomodulatory agents, the particularity of the insects containing a high amount of chitin is of relevance in this framework. Chitin is an insoluble polysaccharide acting as fibre in humans, and it is not digested. For this reason, chitin is likely to have an impact on the gut microbiota,[27] while at the same time, it has been correlated with lower *in vitro* crude protein digestibility.[28]

Recent reviews have been published aiming to encompass general information on the health potential of edible insects, including modulation of oxidative stress in different kinds of assays,[29] or the effect on microbiota and resistance against diseases in several animal species, including fish, crustaceans, poultry, pigs, and rabbits,[30] and even the use in agriculture.[31]

Table 1 Immunomodulatory peptides identified from edible insects and evaluated by means of *in vitro* studies with synthetic peptides

Insect spp.	Developmental stage	Source of the identified peptides	Bioactivities	Sequences	Ref.
Bombyx mori	Pupa	Alcalase hydrolysate of ultramicro-pretreated protein	Splenocyte proliferation	PNPNTN	35
Tenebrio molitor	Larva	Simulated gastrointestinal digested (pepsin, pancreatin, bile extract) hydrolysate	LOX inhibitory activity, COX inhibitory activity	NYVADGLG AAAPVAVAK YDDGSYKPH AGDDAPR	36 and 37
Gryllodes sigillatus	Adult			IIAPPER KVEGDLK LAPSTIK VAPEEHPV	
Schistocerca gregaria	Adult			FDPFPK AIGVGAIER GKDAVIV YETGNGIK	

LOX: lipoxygenase; COX: cyclooxygenase.

In the following sections, studies from *in vitro* assays, *in silico* analyses, and *in vivo* studies with animals and humans are described, providing a critical revision of the literature, and describing the relevance and limitations of the evidence.

2.2. *In vitro* and cell-culture assays

The first approach to screen the potential bioactivity of candidates is *in vitro* assays by spectrophotometric or cell-based assays. These strategies aim to evaluate the potential of several candidates in a cost-effective and fast manner, thus, allowing comparison among compounds. In Table 1, a list of bioactive peptides evaluated *in vitro* to potentially exhibit immunomodulatory properties is reported, indicating the methodology employed by the authors. Teixeira *et al.*[26] recently carried out a systematic review aiming to identify research articles reporting bioactive peptides identified from edible insects, as demonstrated by *in silico*, *in vitro*, and/or *in vivo* assays.

Yoon *et al.*[32] described the anti-inflammatory properties of hydrolysates obtained from *T. molitor* (mealworm) larvae, *Gryllus bimaculatus* (cricket), and *Bombyx mori* (silkworm) pupae, *via* LPS-induced nitric oxide (NO) production from macrophages (RAW 264.7 cells). According to the authors, *B. mori* pupa samples displayed significant activity, with 0.5 mg mL^{-1} test item used. Recently, Lee *et al.*[33] reported the structural, physicochemical, and immune-promoting activity of protein isolates from *Protaetia brevitarsis* larvae. According to the authors, the samples exposed to macrophages lead to their activation, implying secretion of pro-inflammatory markers such as NO, TNF-α, and IL-1β. In addition to that, it was observed that these events were occurring through the mitogen-activated protein kinase signaling pathway (MAPK) and NF-κB pathways. In the same line, the anti-inflammatory ability of protein hydrolysates derived from *Antheraea assama* and *Philosomia ricinii* was evaluated by Sarkar *et al.*[34] In this regard, the peptides were released, assisted by enzymatic hydrolysis with Alcalase and papain, and the bioactivity was evaluated in LPS-treated HUVEC cells. Several findings were obtained, including the prevention of p65 nuclear translocation and the inhibition of p38 MAPK phosphorylation in the cells, together with the upregulation of COX-2 expression and IL-1β secretion.

Nonetheless, no inferences regarding bioactivity in humans can be drawn from these data due to the limitations of *in vitro* investigations (*i.e.*, the absence of consideration of physiological circumstances). Additionally, the outcomes of the diverse research are not necessarily comparable as a result of the various approaches used. The matrix in which peptides will be held if intended to be used as a good ingredient, as well as the treatment applied as part of the manufacturing process, also affects their bioactivity and bioavailability because its elements, such as lipids and proteins, may interact with the peptides.[38] In this regard, for instance, the peptide PNPNTN (Table 1) was also reported to be stable after being subjected to a simulated gastrointestinal digestion (pepsin and trypsin) and after being subjected to 37, 80, 100, and 121 °C for 30 min.[35] However, this result is still very preliminary to demonstrate the stability of the peptide as in a commercial product, and further investigations including the impact of other matrices, storage and processing techniques are needed.

In the case of Hall *et al.*,[39] a hydrolysate obtained by Alcalase-assisted hydrolysis of tropical banded crickets (*Gryllodes sigillatus*) was assessed as anti-inflammatory. The cationic peptide fractions contained in the hydrolysate were able to inhibit LPS-induced inflammation in RAW 264.7 cells, although not exhibiting a dose-dependent behaviour in the range of the concentrations evaluated (0.5 to 3 μg mL^{-1}). Similar to this, Chen *et al.*[40] evaluated the potential of a bee pupa-derived peptide in RAW 264.7 cells, and according to the authors, this implied a promotion of IL-2, TNF-α and interferon gamma (IFN-γ) secretion, while also leading to the production of NO and increasing the phosphorylation of extracellular signal-regulated kinase (ERK) and p38, and modulating the expression of intranuclear transcription in the MAPK signalling pathway.

In the context of the prevention of non-alcoholic fatty liver disease (NAFLD), the potential of *Gryllus bimaculatus* protein hydrolysates (obtained with Neutrase or Flavourzyme) in modulating hepatic lipid accumulation, inflammation, and endoplasmic reticulum stress in Hep G2 cells treated with palmitic acid was assessed. In terms of inflammation, the hydrolysates improved the proinflammatory cytokine mRNA expression, such as TNF-α and IL-1β.[41] In this case, no identification of peptides was carried out by the authors, although it shows promising results on the mechanisms by which the peptides might exert some sort of bioactivity.

Regarding the potential of chitin as an immunomodulatory agent, Elieh Ali Komi *et al.*[42] revised by what means chitin might activate the innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes). In this regard, it has been considered that both fractions from edible insects might have an impact on different parameters of the immune system, and consequently, should be evaluated solely and in combination, in order to unravel the underlying mechanisms and understanding the role of these components in immunonutrition.

As yet, it cannot be concluded that a cause–effect relationship between the consumption of proteins from insects and a health status improvement in relation to the immune system of humans exists. Specifically for insect peptides, considering the very few reports available, it is difficult to pinpoint precise physicochemical or structural characteristics that contribute to their bioactivity. However, considering generally the anti-inflammatory peptides from different sources, the abundance of hydrophobic (Val, Ile, Pro) and positively charged (His, Arg, Lys) amino acids, among other traits, does appear to be an essential factor in their response to inflammation.[21] Although promising, *in vitro* and cell-based analyses cannot be used as evidence to support a health promoting effect. In the same line, animal studies, which will be described in the following section, should be exclusively employed to unravel potential mechanisms and modes of action.[43–46]

2.3. *In silico* evaluations

The use of *in silico* tools to evaluate or predict the bioactivity of peptides, by means of prediction tools and/or molecular docking techniques, among others, has gained the interest of researchers due to their versatility, while at the same time being a fast and economical evaluation. However, it must be noted that several limitations have also been identified for these tools, as they are usually not aligned with *in vitro* results, and consequently, their relevance should not be considered as evidence to state the bioactivity of a peptide.[21,47]

The use of *in silico* tools in the discovery of bioactive peptides from insects has been recently reported for other activities, such as antidiabetic or antihypertensive peptides from *A. domesticus*[48] or antithrombotic peptides from *T. molitor.*[49] A tool aiming to predict, design and scan insect neuropeptides was developed, as these sequences, due to their activity towards specific receptors, are proposed as target for pest control, as previously described.[50] Some other *in silico* prediction tools related to the immunomodulatory activity of peptides have been recently developed,[51,52] trying to describe the peptides as anti-inflammatory in a general way or in some cases with a specific target such as IL-6 or IL-2. However, to the authors' knowledge, no reports have been published employing these tools specifically aiming to demonstrate or support the evidence of the bioactivity of peptide sequences.

2.4. Animal studies

As previously mentioned, the relevance of animal studies relies on the description of the modes of action and underlying mechanisms by which a component in the diet has a role in the health status of an organism. Many studies have investigated several aspects of insects as health-promoting agents in relation to specific conditions, such as the lipid-lowering effect in the liver and plasma of hyperlipidaemic rats[53] or the antibiotic potential in infected broiler chicks.[54] However, some of these studies concluded that no specific changes with biological relevance related to the consumption of insects could be defined. Table 2 summarizes the *in vivo* studies with animals, reporting the effect of insect protein and products thereof (not including studies with specific synthesized peptides[40,55]) on modulation of the immune system.

In addition to that, the connection between the gut microbiota and immune system has been widely confirmed. In brief, the dynamic interactions between the gut microbiota and the host's innate and adaptive immune systems are critical for maintaining intestinal homeostasis and lowering inflammation. The metabolism of proteins and complex carbohydrates by the gut microbiota suggests a connection between the gut epithelium and immune cells.[56] Some studies have evaluated the effect of ingesting edible insects on the gut microbiota.[57–59] In these cases, the presence of chitin, as previously mentioned, could have had a key role in this modulation. In addition, it should be noted that the phylogenetic differences among these animal species and humans are hindering the possibility to extrapolate these results as evidence of immunomodulation in humans.

Regarding the health status and response to infections in animals, the body's main defense is carried out by neutrophils. Neutrophils respond to the signal and immediately go to the area where pathogenic bacteria have invaded. The pathogens are then rendered inactive by neutrophils by phagocytosis and the generation of reactive oxygen species (ROS). For the host defense, ROS generation is essential. On the other hand, excessive ROS generation by neutrophils could potentially harm cells over time and may result in cellular aging, cancer, lowered immunity, *etc.* Scavenging ROS by diet may assist to lessen oxidative damage to the body and the consequent health issues.[60]

In relation to the role of proteins and peptides derived from insects in immunonutrition, which is defined as how the intake of these might modulate the activity of the immune system, scarce information was found. However, there is information on markers related to the development of diseases which are associated with the immune system, such as dia-

Table 2 *In vivo* studies evaluating immunomodulatory protein-derived products from edible insects

Animal model	Protein source	Study design	Main outcomes	Ref.
Mice	*Gryllodes sigillatus*	Comparison with traditional sources in terms of growth and immune system, for six weeks	No significant differences in the expression of inflammatory genes in the spleen tissue	62
Rats	*Tenebrio molitor*	Potential amelioration of alcoholic liver injury, for 8 weeks	Downregulation of some hepatic-inflammation-associated genes	68
Broiler chickens	*Tenebrio molitor* and *Zophobas morio*	Whether the addition of insect meal in feed affects immunological response, for 35 days	Levels of IgY and IgM were reduced compared to that in the negative control	63
Broiler chicks	*Hermetia illucens*	Whether addition in feed enhances non-specific immune activities against experimental infection of *Salmonella Gallinarum*, for 20 days	Increased frequency of $CD4^+$ T lymphocytes, serum lysozyme activity, and spleen lymphocyte proliferation	66
Weaning pigs	*Tenebrio molitor*	Whether the addition of insect meal in feed affects immunological response, for 35 days	No significant changes	64
Finishing pigs	*Hermetia illucens*	Whether the addition of insect meal in feed affects immunological response, for 46 days	Downregulation of expression of TLR4 and pro-inflammatory cytokines (IFN-γ) and upregulation of IL-10.	65
Growing pigs	*Tenebrio molitor*	Whether the addition of insect meal in feed affects immunological response, for 4 weeks	No significant changes	67

betes,[61] which might be relevant in understanding the potential reduction of risk factors related to autoimmune diseases.

In the framework of malnutrition in early life, Bergsmans *et al.*[62] compared the efficacy of cricket protein (*Gryllodes sigillatus*) with traditional sources (*i.e.*, peanut or milk) in terms of growth and immunological status in mice. However, the authors did not observe significant differences in the expression of select inflammatory genes in spleen tissue, including TLR4, TNF-α, IL-1β, IFN-γ and IL-4. In the same line, Benzertiha *et al.*[63] aimed to evaluate whether the addition of insect meal or substitution of a part of the diet with it (samples being *T. molitor* and *Zophobas morio* larvae, at 0.2 and 0.3%) could imply differences in several parameters, including the immunological response. In the assays, the levels of IgY and IgM were reduced compared to the negative control, and in fact, a negative correlation was suggested between the IgM levels and the body weight gain. Overall, some changes in the immune system traits were observed. In the same line, the effect of dried mealworm (*T. molitor* larvae) was assessed in weaning pigs (supplementation from 0 to 6%). Regarding immunological parameters, after the experimental assay for 35 days, no significant differences in both IgA and IgG were observed.[64]

Yu *et al.*[65] aimed to evaluate whether the ingestion (4 and 8%, compared to a control) of black soldier fly (*H. illucens)* larvae for 46 days would affect the mucosal immune status in finishing pigs. According to the authors, pigs ingesting 4% were reported to have downregulated expression of TLR4 and pro-inflammatory cytokines (IFN-γ) compared with pigs in the control group, and upregulated IL-10 expression. Similarly, Lee *et al.*[66] showed that *H. illucens* larvae (1 to 3% in feed) enhanced non-specific immune activities of broiler chicks against experimental infection of *Salmonella Gallinarum*, reporting an increased frequency of $CD4^+$ T lymphocytes, serum lysozyme activity, and spleen lymphocyte proliferation after an intervention of 20 days.

In contrast, Meyer *et al.*[67] aimed to assess whether the consumption of *T. molitor* larvae (5 or 10%) would have an effect on the physiological and immunological status of growing pigs. According to the authors, based on the absence of modification of the expression of pro-inflammatory mediators in the ileal tissue, it could be concluded that the consumption of this insect did not induce an inflammatory process. Also, in the context of alcoholic liver injury, Choi *et al.*[68] demonstrated that a fermented extract from defatted yellow mealworm (*T. molitor*) in rats (up to 200 mg per kg per day, for 8 weeks) led to the downregulation of some hepatic-inflammation-associated genes, including phosphorylated-inhibitor of nuclear factor-kappa B-alpha and TNF-a among other parameters.

Similarly, Fan *et al.*[55] identified a peptide (AGLQFPVGR) after subjecting *Allomyrina dichotoma* larvae to enzymatic hydrolysis. This sequence was chosen to investigate the effects towards the prevention of hepatic steatosis in the framework of an NAFLD model by feeding a high-fat diet to C57BL/6 mice. The animals ingested 100 mg kg^{-1} sample and 60 mg kg^{-1} orlistat *via* gavage (10 ml kg^{-1}) for 5 weeks. The authors reported, among other variations, the activation of TNF-α, IL-1β and IL-6 in hepatocytes.

Recently, Chen *et al.*[40] hydrolysed bee pupae with alkaline protease and were able to purify two peptides, and one of them was used to assess its immunomodulatory activity *in vivo*. In this regard, cyclophosphamide-treated immunosuppressed mice were exposed to the peptide, and this led to a significant increase in body weight growth rate, organ index, macrophage phagocytosis, delayed-type hypersensitivity reaction, and cytokine levels (IL-2, IFN-γ, IgA, IgG, and IgM).

The summarized data may be used as a starting point to examine certain health consequences through human studies, despite the fact that they do not enable drawing definitive conclusions due to the variability of the evidence. Other studies employing water-soluble extracts from different edible insects have been published, also aiming to evaluate the influence on the immune response and are summarized elsewhere.[69] These animal assays are useful in investigating possible biological responses linked to insect consumption, providing mostly indications on causal mechanisms but should not be considered solid evidence towards indicating the immunomodulatory effects of consumption of insects in humans. In the case of the inclusion of insect meal/peptides as feed additives, the purpose of the components in the mixture is to be defined according to the specific needs of the animals and could be useful in a sustainable food system. Further information on the relevance of insect meals in aquaculture, in relation to the immunomodulatory and physiological effects can be found in Mousavi *et al.*[70]

2.5. Human studies

To date, human studies investigating the immunomodulatory effects following the consumption of insects are limited and not directly focused on evaluating these properties and physiological changes. Overall, human studies have mostly focused on evaluating the promotion of growth and modulation of the gut microbiota, among others.[71] In relation to the immunological framework, aging can hasten the effects of free radical damage, which could result in problems with the locomotor and cognitive systems. Similar to this, oxidative stress brought on by an immunological response may harm health.[60] Dietary interventions aiming to promote healthy ageing by tackling immunological traits appear as a promising alternative to improve the quality of life.

In terms of inflammation markers, the most relevant study found among the human studies[69] is the double-blind, randomised crossover trial in healthy adults (n = 20, age: 26.45 years old, 45% males) carried out by Stull *et al.*[72] In this study, a dose of 25 g per day of whole cricket powder (*Gryllodes sigillatus*) for six weeks was ingested by the individuals in order to evaluate the effect on the gut microbiota. Regarding immunomodulatory effects, only a reduction in plasma TNF-α was reported, while the rest of the plasma cytokines were not modified. Apart from this study, no other relevant human studies could be found in which the immune response or immune system traits was evaluated. It must be noted that the test item in this human study was cricket flour; thus it is unknown if the effect observed was due to the protein or the chitin content.

There is growing evidence that eating insects has an impact on the immune system traits of both animals and humans. However, it should be mentioned that the body of research that is now available is insufficient to prove a connection between the consumption of insects and factors that improve the immunological system. To examine the possible positive health consequences of this practice, human trials should be designed and conducted using animal tests using edible insects as testing material. Along with the fate of dietary chitin and the *in vivo* activity of bioactive peptides, the bioavailability of nutrients, mostly micronutrients, from insects and their products is an area that needs more research.

3. Future perspectives and challenges

The consumption of insects is a widespread practice in certain regions of the world such as Asia or Africa, but not in Western societies. In this regard, in the European framework, the safety of certain insect-derived food products has been already established and the consumption is expected to increase in the near future. About 500 tonnes of insect-based products (whole insects and products combined with edible insects) were sold on the European market in 2019. In the next years, the market for edible insect-based food items is anticipated to expand quickly, with production expected to reach roughly 260 000 tonnes by 2030.[73] First, one of the main challenges that this industry faces is the efficient scaling up of insect farming, as it would provide this opportunity for sustainable production of food ingredients.[74]

It must be noted that one of the main problems of insect consumption is supposed to be their allergenicity, since it has been proved that these proteins may cause it *via* sensitisation or cross reactivity in crustaceans, molluscs, and dust mites.[9,75] In this regard, the use of enzymatic hydrolysis is being evaluated as a measure to reduce this problem, as it might change the allergens' conformation by releasing peptides from the native proteins. This enzymatic hydrolysis, or cleaving the proteins, is the basis to release bioactive peptides, with potential immunomodulatory properties. Edible-insect protein composition is influenced by a number of elements, including diet, the developmental stage, and processing.[76] Various nutritional profiles of edible insects indicate varying protein quality levels, which in turn affect the amount of bioactive peptides present. Evaluating their release from native proteins employing different proteases would lead to the release of different sequences. In this sense, the application of non-thermal treatments as pre-treatment, or as an aid during the hydrolysis has not been investigated in the release of immunomodulatory peptides, while it has been for other insect-derived peptides such as antidiabetic[77] or antioxidant.[78] Consequently, this is a research line with a wide range of conditions to be explored, in order to optimize the process of releasing bioactive immunomodulatory peptides. Non-thermal treatments (*e.g.*, ultrasound or high pressures) might lead to modifications of the protein structure, and consequently, different peptidic bonds would be exposed to the action of proteases, potentially, enhancing the release of peptides with higher activity.

The majority of research looking at the proposed advantageous biological activity related to the immune system are restricted to *in vitro* models or animal studies using it as feed, leaving little room for extrapolation to *in vivo* in humans. More information is anticipated to become accessible in the following years, enabling the investigation of the potential health benefits and/or drawbacks of edible insect consumption on the immune system.

In addition, a proper characterization of the test items is required, since it has been demonstrated that other components beyond peptides and protein-derived components might be responsible for the bioactivity, such as chitin, or other non-peptidic nitrogen components such as alkaloids,[79] for which safety should be addressed as well, or vitamins.[17]

One challenge that the food industry will have to deal with in the coming years is to convince the consumer for including insects in their diet. Instead of eating insects whole, the production of flour (concentrates or isolates) is interesting, and from these ingredients, the fortification of food matrices to manufacture a nutritionally enhanced product. In this regard, for instance, the inclusion of *T. molitor* larvae and its protein derivatives on the antioxidant and anti-inflammatory capacities of tofu has been investigated.[80] These authors produced a soy-based product mixing both sources at 1 : 1, with the edible insect as native or as a hydrolysate. On top of the different compositional data observed, the *in vitro* ileal digestibility of the sample fortified with the hydrolysate was significantly higher than the other samples. Regarding the potential immunomodulatory properties of the product, samples containing the insect led to high anti-inflammatory effects against LPS-induced cytokines, including TNF-α, IL- 1β, and IL-6. Similar results have been obtained with other bioactivities such as antioxidant or antidiabetic, employing edible insects as an ingredient in a food matrix.[81,82] On top of that, the food products available in the market in which insects are considered an ingredient are increasing in recent years.[83] The demonstration of the health benefits of these ingredients to be actually claimed as functional foods is still to be carried out in well-designed studies.

The concept of insects as food and medicine being a sustainable solution for global health and environmental challenges is a trending topic in the current society[84] and, consequently, investments and research are required to fully understand their potential to promote their consumption in a challenging market in which the diversity of food sources is increasing exponentially.

4. Conclusions

The consumption of edible insects is currently being promoted by scientists and authorities, given the sustainability that the inclusion of these components in the diet could imply for the overall food system in the near future, without implying a nutritional disadvantage compared to common foodstuff currently available for consumption. In addition to that, new tendencies and lifestyle patterns are moving towards the consumption of food linked to health benefits for human physiology, promoting healthier ageing and better quality of life. In this sense, the exploration and exploitation of edible insects as a source of bioactive peptides is still in a very early stage, especially in terms of how these peptides can exert an immunomodulatory effect on human health. In this review, a critical and detailed description of the literature available on insect-derived peptides with potential immunomodulatory properties was presented. A summary of studies including *in vitro*, *in vivo* and *in silico* analyses was presented, highlighting the relevance of the results obtained until now, and analysing the gaps and challenges still to be addressed in the future. Overall, the currently available literature on the immunomodulatory potential of these peptides is scarce. Well-designed human studies are still needed, while mechanistic assays in order to completely understand the relationship between the consumption of insects and the potential effects are also missing.

Author contributions

F. R. P.: writing – original draft. T. G.-d. l. R. and S. M.-d. l. P.: writing – review and editing. All authors participated in the production of the final version of the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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