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Unlocking the potential of EU-approved edible insects: A review of protein hydrolysates and their technological and biological properties

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ABSTRACT

The practice of eating insects, known as entomophagy, is common in many parts of the world, and the nutritional benefits of edible insects are well established. In the European Union (EU), four insect species have been authorised for human consumption under novel food regulations: house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), lesser mealworm (larval form of *Alphitobius diaperinus*), and yellow mealworm (larval form of *Tenebrio molitor*). Their significant protein content makes them excellent substrates for enzymatic hydrolysis, a process that produces amino acids and bioactive peptides which can improve the technological and functional characteristics of food products. The use of specific enzymes during hydrolysis can optimise the nutritional profile, the technological and biological properties of the resulting hydrolysates, making them suitable for a wide range of food applications. This review focuses on the potential of protein hydrolysates from EU-approved edible insects. It assesses the effects of using different enzymes and details how these treatments modify technological and biological properties of the final protein hydrolysates. The variability of insect protein composition highlights the critical importance of selecting the appropriate enzyme or enzyme combination and carefully controlling operational parameters such as pH, time, and temperature. The presence of interferents such as lipids, as well as the application of emerging technologies such as high hydrostatic pressure, also significantly influences the final properties. The use of edible insects in food is not without challenges, particularly regarding safety and allergens.

1. Introduction to entomophagy: A global traditional practice with a modern application

The global population increases, so does the demand for protein, place significant strain on conventional livestock production, which is known for its high environmental impact, including high feed conversion ratios and substantial greenhouse gas and methane emissions (Van Huis, 2013). Sustainable and novel protein production systems are therefore essential to address these challenges (Pastrana-Pastrana, Rodríguez-Herrera, Solanilla-Duque, & Flores-Gallegos, 2025; Van Huis, 2013). Edible insects have emerged as a highly promising alternative protein source, with an average protein content of around 60% (w/w), although this can vary depending on species, sex, developmental stage, environmental conditions and diet (Garofalo et al., 2019; Gkinali, Matsakidou, Vasileiou, & Paraskevopoulou, 2022; Ruschioni et al., 2020). Furthermore, insect proteins are well documented as being highly digestible and having high nutritional value (Sharma et al., 2024). The practice of consuming insects, known as entomophagy, is followed by approximately two billion people worldwide, with strong traditions in Asia, Latin America, and Africa. The scientific literature identifies over 2,000 edible insect species, including beetles, lepidopterans (caterpillars and moths), and hymenopterans (ants, bees, and

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wasps), which can be consumed at all life stages, from eggs to adults (FAO, 2021; Gonçalves, Chavez, & Jorge, 2022; Van Huis, 2013). In Europe, following safety assessments by the European Food Safety Authority (EFSA) and approval from the European Commission, four insect species are now authorised for the market under novel food regulations (European Commision, 2017): house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), lesser mealworm (larval form of *Alphitobius diaperinus*), and yellow mealworm (larval form of *Tenebrio molitor*). Freezing, drying or heat treatments are commonly used for product preservation (Cacchiarelli et al., 2022; Queiroz et al., 2023) and to enhance consumer acceptability (Belleggia et al., 2019; Meyer-Rochow, Gahukar, Ghosh, & Jung, 2021). On the market, they are available as whole processed insects or larvae, or as powders, with the latter widely used to fortify a variety of food products, including bread, crackers and flatbreads (Osimani et al., 2018; Roncolini et al., 2020, 2019), as well as meat analogues (Krawczyk, Fernández-López, & Zimoch-Korzycka, 2024; Nakagawa, Chantanuson, Boonarsa, Seephua, & Siriamornpun, 2024), and other food preparations (Kozlu, Ngasakul, Klojdová, & Baigts-Allende, 2024).

More recently, enzymatic interventions have been proposed to steer technological, functional, and sensory properties of insect proteins (Leni, Soetemans, Jacobs, et al., 2020; Osimani et al., 2018; Purschke, Meinlschmidt, Horn, Rieder, & Jäger, 2018; Roncolini et al., 2020; Zhang et al., 2017). This strategy can improve protein quality by producing amino acids and peptides (Zhao et al., 2012). Specifically, enzymatic hydrolysis of insect flour proteins, such as those from *L. migratoria*, has been shown to improve their technological properties (Purschke et al., 2018) and increase the digestibility of the protein fraction, thereby enhancing the nutritional value of food products (Purschke et al., 2018; Zielińska, Baraniak, Karaś, Rybczyńska, & Jakubczyk, 2015). In addition, proteases have demonstrated strong efficacy in separating proteins from interfering compounds such as lipids and other insoluble components (Leni, Soetemans, Jacobs, et al., 2020).

Beyond improving technological properties, enzymatic hydrolysis can generate bioactive peptides with beneficial effects on human health (Apostolopoulos et al., 2022; Chai, Ee, Kumar, Manan, & Wong, 2021). Depending on their specific amino acid composition, these peptides play significant roles in regulating the nervous, digestive, endocrine, and cardiovascular systems. Moreover, they act as bioactive regulators and exhibit a broad spectrum of biological activities, including antioxidant, anticancer, antithrombotic, antidepressant, antimicrobial, anti-obesity, and anti-inflammatory properties (Akbarian, Khani, Eghbalpour, & Uversky, 2022; de Carvalho Oliveira et al., 2024; Korhonen & Pihlanto, 2006; Liu et al., 2024; Sánchez & Vázquez, 2017; Shahidi & Zhong, 2008; Zaky, Simal-Gandara, Eun, Shim, & Abd El-Aty, 2022).

The objective of this review is to explore the potential of protein hydrolysates derived from edible insects approved for human consumption under European regulations. It also aims to assess the impact of using different enzymes and highlights how the technological and health effects are affected.

2. Methodology

2.1. Literature search

The literature search was conducted using the Scopus database (www.scopus.com). Only papers published in English were included and only results within the following subject areas were considered: “agricultural and biological science”, “food chemistry”, “food technology” and “food science”. The following search words were entered into the Scopus database, selecting the search option “title, abstract, and keywords”: “protein*, hydrolysate*, insect*”. The time range was set from 2010 to 2025, as there is no significant research history on the topic before 2010. The results were further refined using the keywords “Acheta domesticus”, “Locusta migratoria”, “Alphitobius diaperinus”, and “Tenebrio molitor”. Document types different from articles, such as conference contributions, were exluded. The abstracts were analysed to select papers and reviews specifically focused on the technological and biological properties of protein hydrolysates mainly on insects approved in EU. As a result, 102 articles were finally selected.

2.2. Preparation of raw insect material

The quality of the final protein hydrolysate depends greatly on the initial preparation of the raw insect material. The main aims are to maximise protein extraction by removing interfering substances such as water and lipids, and to increase the surface area for enzymatic reactions. Various strategies have been reported in the literature to achieve these objectives (Table 1).

2.2.1. Insect manipulation and moisture removal

Insect preparation involves pre-treatments such as cleaning, enzyme inactivation, and disinfection steps to minimize nutrient losses and thus improve the nutritional and sensorial value of dried insects (Hernández-Álvarez, Mondor, Piña-Domínguez, Sánchez-Velázquez, & Melgar Lalanne, 2021). Dehydration of insects removes moisture and extends their shelf life by increasing the microbiological quality, reduces the rancidity, and improves the color and texture of the final products. This step is considered a prerequisite and/or pretreatment for some extraction technologies for ingredient production. Different drying technologies such as sun drying, smoke drying, roasting, freeze drying and oven drying have been used to reduce water content of insects, both at a laboratory and industrial level (Hernández-Álvarez et al., 2021). Many studies on protein extraction use commercially available insect powders that have been pre-dried or freeze-dried (Dion-Poulin, Laroche, Doyen, & Turgeon, 2020; Grossmann, Merz, Appel, De Araujo, & Fischer, 2021; Messina et al., 2019; Purschke et al., 2018; Song et al., 2020; Sousa, Borges, & Pintado, 2020; Yu, Oh, & Kim, 2021). Other approaches involve the drying of whole insects directly by hot air oven drying, as for *T. molitor* larvae (Chen, Jiang, Gan, Chen, & Huang, 2019) or freeze-drying (Hernández-Álvarez et al., 2021; Kim, Setyabrata, Lee, Jones, & Kim, 2016; Leni, Soetemans, Caligiani, Sforza, & Bastiaens, 2020; Leni, Soetemans, Jacobs, et al., 2020; Tan et al., 2022) followed by grinding and sieving (typically through 20, 100, or 800 mesh sieves) to create a stable and homogeneous starting material. Innovative approaches include microwave and mid-infrared light that takes less time to dry the insects than freeze drying and oven drying (Kröncke et al., 2019; Tang et al., 2018).

2.2.2. Defatting

The removal of fats is a crucial preliminary step, as it increases protein yield and improves the technological properties of the final product (Bußler et al., 2016; Choi, Wong, & Auh, 2017; Purschke et al., 2018). While traditional methods use organic solvents, such as hexane, ethanol, isopropanol, and 2-methyl tetrahydrofuran, more recently “green” solvents have been proposed to ensure process sustainability and consumer requirements. The specifics of the defatting process, including the solvent-to-substrate ratio and extraction time, are optimised to maximise both fat removal and protein recovery (Table 1). In particular, Chen et al. (2019) defatted dried and ground yellow mealworms using isopropyl alcohol at 50°C for 1 h to optimise hydrolytic conditions for generating antithrombotic peptides from larvae proteins. Song et al. (2020) used 95.5% (v/v) ethanol at a 1:20 ratio, combined with ultrasonic extraction (20 kHz, 4 h, 70°C), to treat the powder and obtain functional peptides exhibiting anti-obesity activity from *T. molitor*. Other researchers have used apolar solvents such as petroleum ether to defat yellow mealworm powder to produce protein-derived peptides with dipeptidyl peptidase-4 (DPP-4) inhibitory activity relevant to blood glucose control in diabetic patients (Tan et al., 2022). Hexane has also been applied in a comparative study of *T. molitor* larvae, adult crickets, and silkworm pupae (Yoon, Wong, Chae, & Auh, 2019) or to obtain a hydrolysate of mealworm proteins intended as sausage emulsifier (Kim et al., 2016). Physical methods have rarely been

Table 1
Overview of the raw materials and pre-treatments.

Insect species	Raw material	Pretreatment	Reference
Acheta domesticus	Raw frozen crickets (whole) (Ovipost, LaBelle, FL, USA)	Blending with distilled water, pasteurisation (90°C for 15 min), pH adjustment	Luna et al. (2021)
	Protein powder (68% protein) (Thailand Unique, Udon Thani, Thailand)		Grossmann et al. (2021)
	Powder (Kreca Ento-Food, Harderwijk, Gheldria, The Netherlands)		Messina et al. (2019)
Locusta migratoria	Protein flour (Crawlers, Auckland, New Zealand)		Purschke et al. (2018)
Alphitobius diaperinus	Larvae (whole) (Protifarm, Ermelo, the Netherlands)	Freeze-drying and grinding	Leni, Soetemans, Jacobs, et al. (2020)
	Larvae (whole) (Protifarm, Ermelo, The Netherlands	Freeze-drying and grinding	Leni, Soetemans, Caligiani, et al. (2020)
	Powder (Kreca Ento-Food, The Netherlands)		Sousa et al. (2020)
Tenebrio molitor	Larvae (local market in Hangzhou, China)	Air-drying (50°C until the moisture content reached 5%), grinding, defatting (isopropyl alcohol at 50°C for 1 h)	Chen et al. (2019)
	Larvae (CREA-DC Florence, Italy)	Freeze-drying	Cito et al. (2017)
	Meal (39.6% protein) (Entomo Farms, Norwood, ON, Canada)		Dion-Poulin et al. (2020)
	Protein powder (70% protein) (Ynsect, Evry, France)		Grossmann et al. (2021)
	Powder (Kreca Ento-Food, Harderwijk, Gheldria, The Netherlands)		Messina et al. (2019)
	Freeze-dried larvae (Mealworm Nara, Damyang, JeonBuk, Korea)	Grinding, defatting (ethanol 99.5%; solid/liquid ratio 1:20 w/v; sonication 20 kHz, 4 h, 70°C)	Song et al. (2020)
	Fresh larvae (Cricket Farm, (Hwaseong si, Gyeonggi-do, Korea)	Freeze-drying, defatting (petroleum ether)	Tan et al. (2022)
	Fresh larvae (Cricket Farm, (Hwaseong si, Gyeonggi-do, Korea)	Drying under mid-infrared light, defatting (pressure), grinding	Tang et al. (2018)
	Whole freeze-dried larvae (Chubby Meal Worms, Kennesaw, GA)	Grinding, sieving (20 mesh), defatting (hexane; solid/liquid ratio 1:5 w/v; 30 min)	Kim et al. (2016)
	Dried whole larvae (Insect Vision, Yangju, Korea)	Defatting (hexane; solid/liquid ratio 1:20 w/v; 36 h)	Yoon et al. (2019)
	Larvae powder (Edible-Bug Co., Seoul, Korea) (protein, 48%)	Defatting (ethanol 99.5%; solid/liquid ratio 1:5 w/v; 40°C; 60 min)	Yu et al. (2021)

applied for defatting insect powders. Tang et al. (2018) pressed dried *T. molitor* larvae to remove the oil fraction before extraction.

3. Enzymatic hydrolysis: A Key to unlocking functionality

3.1. The role and selection of enzymes

The enzymatic hydrolysis of insect proteins is a common and effective method for generating amino acids and peptides that significantly modify their technological, functional, and sensory properties. This process enhances the technological properties of insect proteins, such as solubility, and can also improve its nutritional value by increasing digestibility and preserving essential amino acids. The enzymes used include endo- and exo-peptidases from fungal, bacterial, or plant sources (Table 2). The choice of enzyme is critical, as different enzymes have optimal operating conditions (pH, temperature), which are typically provided by the manufacturer, and are often combined to maximise the hydrolytic effect and achieve the desired functional properties in the resulting protein hydrolysates. Proteases are also effective at separating proteins from interfering substances such as lipids.

Chemical hydrolysis has been exploited to a much lesser extent. Kim et al. (2016) showed a decline of extraction yields after defatting from 48.64% to 35.84%, and further decreased to 30.29% following acid hydrolysis.

3.2. Hydrolysis parameters for EU-approved insect species

3.2.1. Tenebrio molitor

A variety of commercial enzymes have been studied for hydrolysing *T. molitor* proteins, including Alcalase®, Flavourzyme®, Protamex®, Neutrase®, papain, and trypsin (Table 3). Alcalase® and Flavourzyme® have been found to yield high degree of hydrolysis (DH), significantly reducing protein band sizes (Song et al. 2020). Tan et al. (2022) reported that Flavourzyme® produced the highest DH (23.25% after 4 h) when creating DPP-4 inhibitory peptides as compared to Alcalase®, papain, and trypsin. In a related study, Tang et al. (2018) assessed the free radical scavenging activity of *T. molitor* larvae hydrolysed with Alcalase® and Flavourzyme® that yielded the highest DH (36.4%). Yoon et al. (2019) characterised *T. molitor* protein hydrolysates produced with a combination of Flavourzyme® (12%) and Alcalase® (3%) at 55°C for 8 h, which resulted in a marked reduction in protein band sizes from 75 kDa to below 15 kDa. Optimisation of hydrolysis conditions was further explored using Response Surface Methodology (RSM) by Chen et al. (2019), who applied pepsin and trypsin. Researchers have also investigated the effect of pre-treatments, such as High Hydrostatic Pressure (HHP), on enzymatic reaction. Dion-Poulin et al. (2020) examined the functional properties of *T. molitor* hydrolysates produced with Alcalase®, through both conventional processing and after pasteurisation pre-treatment by HHP. The HHP pre-treatment reduced the DH in the pasteurised sample from 33.8 to 25.6% due to protein denaturation and aggregation, which in turn lowered the efficiency of enzymatic hydrolysis.

Some studies have simulated the gastrointestinal digestion of *T. molitor* proteins with pepsin, trypsin, and α-chymotrypsin to assess the bioactivity of the resulting peptides. Cito et al. (2017) observed an increased ability of the protein hydrolysate to inhibit angiotensin-converting enzyme (ACE). Overall, *T. molitor* proteins can be effectively modulated through the targeted selection of hydrolytic enzymes and processing conditions. Broad-specificity enzymes such as Alcalase® promote extensive hydrolysis and the generation of low–molecular weight peptides, whereas more complex enzymatic systems like Flavourzyme® allow greater control over the resulting peptide profile.

3.2.2. Acheta domesticus

Acheta domesticus is commonly hydrolysed by Alcalase® and Flavourzyme®, achieving high DH in relatively short times. Luna et al.

Table 2
Enzymes and experimental conditions involved in the hydrolysis of insect proteins.

Insect species	Enzyme	Specifications	Company	Hydrolysis conditions	Degree of hydrolysis (DH %)	Enzyme inactivation	Ref.
Acheta domesticus	Alcalase®	Serine endopeptidase from *Bacillus licheniformis,* > 2.4 U/g	Sigma Aldrich (St. Louis, MO, USA)	pH 8; T = 63°C; E/S = 0.1-0.3; t = 5-30 min	8.11-13.98 (max DH% after 10 min-E/S=0.3)	Heating 90°C for 15 min	Luna et al. (2021)
	Flavourzyme®	Endoprotease and exopeptidase from *A. oryzae,* > 500 U/g	Sigma Aldrich (St. Louis, MO, USA)	pH 7; T = 55°C; E/S = 0.5-3.0; t = 10-30 min	8.21-14.54 (max DH% after 30 min-E/S=3)	Heating 90°C for 15 min	Luna et al. (2021)
	Protamex®	n.a.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min	20.1 (max DH% after 1180 min)	Heating 90°C for 5 min	Messina et al. (2019)
	Flavourzyme®	Endoprotease and exopeptidase from *A. oryzae*	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min		Heating 90°C for 5 min	Messina et al. (2019)
	Alcalase®	n.a.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min	25.8 (max DH% after 1180 min)	Heating 90°C for 5 min	Messina et al. (2019)
	Flavourzyme®	From *A. oryzae;* aminopeptidase content 17% (w/w)	Novozymes (Bagsværd, Denmark)	pH 7.3; T = 50°C; E/S = 5; t = 120 min	33 (max DH% after 120 min)	Heating 90°C for 15 min	Grossmann et al. (2021)
	Protease A	From *A. oryzae;* protease content of 65%	Amano Enzyme Inc. (Nagoya, Japan)	pH 7.3; T = 50°C; E/S = 5; t = 120 min	46 (max DH% after 120 min)	Heating 90°C for 15 min	Grossmann et al. (2021)
Locusta migratoria	Alcalase® 2.4 L FG	Endoprotease from *B. licheniformis*	Novozymes A/S (Bagsvaerd, Denmark	pH 8; T = 50°C; E/S = 0.05-1; t=24 h	31.1 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
	Flavourzyme® 1000 L (Fla)	Endoprotease and exopeptidase from *A. oryzae*	Novozymes A/S (Bagsvaerd, Denmark	pH 8; T = 50°C; E/S = 0.05-1; t=24 h	24.1 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
	Neutrase® 0.8 L (Neu)	Endoprotease from *B. amyloliquefaciens*	Novozymes A/S (Bagsvaerd, Denmark)	pH 8; T = 50°C; E/S = 0.05-1; t=24 h	26.2 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
	Papain	Cysteine-protease from papaya latex; ≥ 10 U/mg protein	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 50°C; E/S = 0.05-1; t = 24 h	20.6 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
	Neu + Fla			pH 8; T = 50°C; E/S = 0.5 (Neu); E/S = 1.0 (Fla)	28.5 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
	Neu + Fla			pH 8; T = 50°C; E/S = 0.5 (Neu); E/S = 1.0 (Fla)	28.0 (max DH% after 24h)	Heating 90°C for 20 min	Purschke et al. (2018)
Alphitobius diaperinus	EC 3.4.21.62	Protease from *Bacillus licheniformis*; ≥ 2.4 U/g	Sigma-Aldrich (St. Louis, MO, USA)	pH 7.5; T = 60°C; E/S = 1; t = 18 h	21.8 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Dispase I (EC 255-914-4)	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 7.3; T = 37°C; E/S = 1; t = 18 h	15.9 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Pepsin (EC 3.4.23.1)	From porcine gastric mucosa (≥ 250 U/mg)	Sigma-Aldrich (St. Louis, MO, USA)	pH 3; T = 37°C; E/S = 1; t = 18 h	15.8 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Pancreatin (EC 232-468-9)	From porcine pancreas	Sigma-Aldrich (St. Louis, MO, USA)	pH 7.8; T = 37°C; E/S = 1; t = 18 h	17.6 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Trypsin (EC 3.4.21.4)	From porcine pancreas (1.000-2.000 BAEE units/mg)	Sigma-Aldrich (St. Louis, MO, USA)	pH 7.8; T = 37°C; E/S = 1; t = 18 h	21.9 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Bromelain (EC 232-572- 4)	From pineapples (2 mAnson U/mg)	Sigma-Aldrich (St. Louis, MO, USA)	pH 7; T = 50°C; E/S = 1; t= 18 h	23.1 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Papain (EC 3.4.22.2)	From papaya latex (1.5-10 U/mg)	Sigma-Aldrich (St. Louis, MO, USA)	pH 6.5; T = 60°C; E/S = 1; t = 18 h	7 (max DH% after 18 h)	Heating 90°C for 5 min	Leni, Soetemans, Jacobs, et al. (2020)
	Protease (EC 3.4.21.62)	From *Bacillus licheniformis* (≥ 2.4 U/g)	Sigma-Aldrich (St. Louis, MO, USA)	pH 7.5; T = 60°C; E/S = 0.25; t = 300 min	14.9 (max DH% after 300 min)	Heating 90°C for 5 min	Leni, Soetemans, Caligiani, et al. (2020)
	Alcalase®	n.a.	Aquitex (Portugal)	pH 8; T = 50°C; E/S = 0.5-3; t = 24 h	15 (max DH% after 4h E/S=3)	Heating 100°C for 15 min	Sousa et al. (2020)
	Corolase®	n.a.	AB Enzymes GmbH (Germany)	pH 8; T = 50°C; E/S = 0.5-3; t= 24 h	25 (max DH% after 4h E/S=3)	Heating 100°C for 15 min	Sousa et al. (2020)
Tenebrio molitor	Pepsin (Pep)	3000 U/mg	Local chemical company	pH 2.0; T = 37°C; t = 1-2.5 h		Heating 95°C for 15 min	Chen et al. (2019)
	Trypsin (Try)	250 U/mg	Local chemical company	pH 8; T = 37°C; t = 5 h		Heating 95°C for 15 min	Chen et al. (2019)
	Pep + Try (2 steps)				0.14-28.85		Chen et al. (2019)

(*continued on next page*)

Table 2 (*continued*)

Insect species	Enzyme	Specifications	Company	Hydrolysis conditions	Degree of hydrolysis (DH %)	Enzyme inactivation	Ref.
	Pepsin	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 2; T = 37°C; E/S = 1/250; t = 2.5 h	n.a.	n.a.	Cito et al. (2017)
	Trypsin	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 6.5; T = 37°C; E/S = 1/250; t = 2.5 h	n.a.	n.a.	Cito et al. (2017)
	α-chymotrypsin	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 6.5; T = 37°C; E/S = 1/250; t = 2.5 h	n.a.	n.a.	Cito et al. (2017)
	Alcalase®	From *Bacillus licheniformis*	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8.5; T = 55°C; E/S = 3; t = 2 h	25.5-33.8	Heating 80°C for 15 min	Dion-Poulin et al. (2020)
	Flavourzyme®	From *A. oryzae*; aminopeptidase content 17% (w/w),	Novozymes (Bagsværd, Denmark)	pH 7.3; T = 50°C; E/S = 5; t = 120 min	51 (max DH% after 120 min)	Heating 90°C for 15 min	Grossmann et al. (2021)
	Protease A	From *A. oryzae*; protease content 65%	Amano Enzyme Inc. (Nagoya, Japan)	pH 7.3; T = 50°C; E/S = 5; t = 120 min	71 (max DH% after 120 min)	Heating 90°C for 15 min	Grossmann et al. (2021)
	Corolase® 7090	n.a.	AB Enzymes (Germany)	pH 7; T = 50°C; E/S = 0.5-; t = 4 h	n.a.	n.a.	Hoffmann et al. (2020)
	Flavourzyme®	n.a.	Novozymes (USA)	pH 7; T = 50°C; E/S = 0.5-1; t = 3 h	n.a.	n.a.	Hoffmann et al., (2020)
	Protamex®	n.a.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min	20.1 (max DH% after 180 min)	Heating 90°C for 5 min	Messina et al. (2019)
	Flavourzyme®	n.a.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min		Heating 90°C for 5 min	Messina et al. (2019)
	Alcalase®	n.a.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 8; T = 60°C; t = 15-195 min	25.8 (max DH% after 180 min)	Heating 90°C for 5 min	Messina et al. (2019)
	Corolase® 7090 (Cor)	Endopeptidase from *Bacillus amyloliquefaciens*	AB Enzymes GmbH (Darmstadt, Germany)	T = 50°C; t = 5 h			Mikołajczak et al. (2020)
	Flavourzyme® (Fla)	Endopeptidase and exopeptidase from *Aspergillus oryzae*	Novozymes A/S, (Denmark)	t = 3 h			Mikołajczak et al., (2020)
	Cor + Fla (2 steps)				n.a.	n.a.	Mikołajczak et al., (2020)
	Alcalase®	Endopeptidase from *Bacillus licheniformis*	Novo Nordisk A/S Korea (Seoul, Korea)	pH 6.4; T = 50°C; E/S = 0.1; t = 48 h	n.a.	n.a.	Song et al. (2020)
	Protamex®	Endo + exopeptidase from *Bacillus* sp.	Sigma Aldrich (St Louis, Missouri, MO, USA)	pH 6.4; T = 50°C; E/S = 0.1; t = 48 h	n.a.	n.a.	Song et al. (2020)
	Neutrase®	Endopeptidase from *Bacillus amyloliquefaciens*	Novo Nordisk A/S Korea (Seoul, Korea)	pH 6.4; T = 50°C; E/S = 0.1; t = 48 h	n.a.	n.a.	Song et al. (2020)
	Flavourzyme®	Endo + exopeptidase from *A. oryzae*	Novo Nordisk A/S Korea (Seoul, Korea)	pH 6.4; T = 50°C; E/S = 0.1; t = 48 h	n.a.	n.a.	Song et al. (2020)
	Trypsin	250 N.F.U/mg	Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China)	pH 8; T = 37°C; E/S = 5; t = 300 min	<6.5 (max DH% after 300 min)	Heating 85°C for 20 min	Tan et al. (2022)
	Flavourzyme®	≥ 30,000 U/g	Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China)	pH 7; T = 50°C; E/S = 5; t = 300 min	23.25 (max DH% after 300 min)	Heating 85°C for 20 min	Tan et al. (2022)
	Papain	≥ 800,000 U/g	Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China)	pH 5; T = 55°C; E/S = 5; t = 300 min	<6.5 (max DH% after 300 min)	Heating 85°C for 20 min	Tan et al. (2022)
	Alcalase®	≥ 200,000 U/g	Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China)	pH 11; T = 50°C; E/S = 5; t = 300 min	<6.5 (max DH% after 300 min)	Heating 85°C for 20 min	Tan et al. (2022)
	Alcalase®	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 8; T = 55°C; E/S = 0.01; t = 8 h	38.7 (max DH% after 8h)	Heating 100°C for 10 min	Tang et al. (2018)
	Flavourzyme®	n.a.	Sigma-Aldrich (St. Louis, MO, USA)	pH 8; T = 55°C; E/S = 0.01; t = 8 h	14 (max DH% after 8h))	Heating 100°C for 10 min	Tang et al. (2018)
	Alcalase® Flavourzyme®			pH 8; T = 55°C; E/S = 0.01; t = 8 h;	36.4 (max DH% after 8h)	Heating 100°C for 10 min	Tang et al. (2018)
	Flavourzyme® 1000 L	n.a.	Novozymes (Bagsvaerd, Denmark)	T = 55°C; t = 8 h	n.a.	n.a.	Yoon et al. (2019)
	Alcalase® 2.4 L	n.a.	Novozymes (Bagsvaerd, Denmark)	T = 55°C; t = 8 h	n.a.	n.a.	Yoon et al. (2019)

(*continued on next page*)

Table 2 (*continued*)

Insect species	Enzyme	Specifications	Company	Hydrolysis conditions	Degree of hydrolysis (DH %)	Enzyme inactivation	Ref.
	Neutrase®	n.a.	Novozymes (Bagsvaerd, Denmark)	T = 55°C; t = 8 h	n.a.	n.a.	Yoon et al. (2019)
	Protamex®	n.a.	Novozymes (Bagsvaerd, Denmark)	T = 55°C; t = 8 h	n.a.	n.a.	Yoon et al. (2019)
	Alcalase® 2.4 L	n.a.	Novozymes (Bagsvaerd, Denmark)	pH 8; T = 60°C; E/S = 2; t = 3 h	n.a	n.a	Yu et al. (2021)

E/S = enzyme substrate ratio, percent (w/w) of enzyme added per g of protein content.

Table 3
Techno-functional properties of insect protein hydrolysates (compared to the native protein isolate).

Insect species	Enzyme	Solubility	Emulsifying properties	Foaming properties	Water holding capacity	Oil holding capacity	Gelling properties	Ref.
Locusta migratoria	Flavourzyme® 1000 L (Fla)	Increased PSI (t = 30, 60, 120 min; pH 5, 7)	Increased EA at pH 5 and/or pH 7	Higher OR at pH 3 but lower at pH 5-9 Sharp FS increase at pH 7 and limited improvement at pH 9	Not improved (WBC)	Increased OBC (30-120 min)		Purschke et al. (2018)
	Neutrase® 0.8 L (Neu)	Increased PSI (t = 30, 60, 120 min; pH 3, 5, 7, 9)	No improvement of EA in the tested pH range	Higher OR at pH 3 but lower at pH 5-9 Sharp FS increase at pH 7 and limited improvement at pH 9	Not improved (WBC)	Increased OBC (30-120 min)		Purschke et al. (2018)
	Fla + Neu	Increased PSI (t = 30, 60, 120 min; pH 3, 5, 7, 9)	Increased EA at pH 5 and/or pH 7	Higher OR under weak acidic conditions (pH 3-5 Sharp FS increase at pH 7 and limited improvement at pH 9	Not improved (WBC)	Increased OBC (30-120 min)		Purschke et al. (2018)
Alphitobius diaperinus	Protease from *Bacillus licheniformis* (EC 3.4.21.62)	Increased (t = 60-180 min; pH 3, 5, 7)	Lower EA (t = 30-180 min)	Foaming capacity appeared in the hydrolysates collected after 60-180 min of hydrolysis. Foams did not display any stability.		Increased OHC (t = 120-180 min)		Leni, Soetemans, Caligiani, et al. (2020)
Tenebrio molitor	Alcalase®	Increased (HHP further increase)	Lower EAI ESI not affected	No foaming properties		Increased OBC (HHP further increase)	Not improved (regardless the treatment)	Dion-Poulin et al. (2020)
	Chemical hydrolysis (HCl)	Decreased	Unaffected				No gel formation	Kim et al. (2016)
	Flavourzyme® 1000 L (Fla)	Increased	Increased EAI Increased ESI					Yoon et al. (2019)
	Alcalase® 2.4 L (Alc)	Increased	Decreased EAI Increased ESI					Yoon et al. (2019)
	Fla + Alc	Increased	Unaffected					Yoon et al. (2019)
	Alcalase® 2.4 L	Increased	Lower EA Higher ES	Higher FEC Higher FS		Higher FAC	Hydrolysate did not form gels	Yu et al. (2021)

EA = Emulsifying Activity; EAI = Emulsion Activity Index; ES = Emulsion Stability; ESI = Emulsion Stability Index; FAC = Fat Absorption Capacity; FEC = Foam Expansion Capacity; FC = Foam Capacity; FS = Foam Stability; OBC = Oil Binding Capacity; OHC = Oil Holding Capacity; OR = Overrun; PSI = Protein solubility index; WBC = Water Binding Capacity.

(2021) used these enzymes to hydrolyse house cricket proteins for use in tortillas. The highest DH achieved was 14.54% with Flavourzyme® at an enzyme-to-substrate ratio (E/S) of 3% after 30 min, and 13.98% with Alcalase® at an E/S ratio of 0.3% after 10 min. Similarly, Messina et al. (2019) tested Protamex®, Flavourzyme® and Alcalase® on *T. molitor* and *A. domesticus* flours and found that Alcalase® produced the highest hydrolytic effect on *A. domesticus* flour, reaching a maximum DH of 24.6% after 195 min.

Protease A has also been shown to be particularly effective. Grossmann et al. (2021) investigated flavour potential using Flavourzyme® and Protease A, finding that Protease A consistently produced a higher DH, reaching 46% for house cricket protein compared to 33% of Flavourzyme®.

Overall, Alcalase® enables rapid and efficient protein breakdown, while Flavourzyme® allows more gradual modification, potentially benefiting flavour and peptide complexity. Protease A shows particularly high efficiency, highlighting the strong responsiveness of house cricket proteins to specific protease systems and their suitability for tailored food applications.

3.2.3. *Alphitobius diaperinus*

A wide range of enzymes, including protease from *B. licheniformis*, Dispase I, papain, bromelain, pancreatin, pepsin, and trypsin, have been tested on *A. diaperinus*. Leni et al. (2020) assessed extraction yield and DH across a pH range of 3-7.8 and temperatures between 37 and 60°C, over an 18-h reaction period, and found that trypsin, *B. licheniformis* protease, and bromelain showed the highest DH (21.9%, 21.8%, and 21.8%, respectively). Further studies aiming to generate bioactive

peptides identified optimal conditions for Alcalase® and Corolase® at a substrate concentration of 3.0% with reaction times of 4 and 6 h. Under these conditions, the highest DH values were 25% and 30% for Alcalase® and Corolase®, respectively (Sousa et al., 2020). *Alphitobius diaperinus* proteins can be effectively tailored through enzymatic hydrolysis by selecting appropriate proteases and reaction conditions. Both digestive and industrial enzymes enable substantial and controllable protein breakdown, allowing the production of peptide-rich ingredients suited to specific functional or bioactive applications.

3.2.4. *Locusta migratoria*

Research on *L. migratoria* has focused on enzymes such as Alcalase®, Neutrase®, Flavourzyme®, and papain, used both individually and in combination. Purschke et al. (2018) conducted hydrolysis at 50°C and pH 8.0 for reaction times ranging from 30 min to 24 h. DH varied among the enzymes, with Alcalase® (31.1%), Neutrase® (26.2%), Flavourzyme® (24.1%), and papain (20.6%) showing progressively lower values. Overall, broad-specificity proteases such as Alcalase® enable extensive protein breakdown, while Neutrase®, Flavourzyme®, and papain provide more moderate and controlled hydrolysis. This flexibility allows tuning of peptide size distribution and functional properties according to the desired application.

4. Enhancing food products: functional properties of insect protein hydrolysates

A thorough understanding of the technological properties of insect proteins hydrolysates is essential for their application in novel food products or as substitutes for conventional ingredients and the influence of insect diet, drying, defatting, enzymatic hydrolysis, or other processing methods is key to fully exploiting the potential of these novel ingredients. Functional properties, which include solubility, emulsifying capacity, foaming and gelling ability, water-holding capacity, and oil-holding capacity, are heavily influenced by processing methods such as enzymatic hydrolysis (Bußler et al., 2016; Gkinali et al., 2022; F. G. Hall, Jones, O'Haire, & Liceaga, 2017; Purschke et al., 2018; Q. Wu, Jia, Yan, Du, & Gui, 2015). Hydrolysis alters protein characteristics such as molecular weight, hydrophobicity, and surface activity, which in turn affect their behaviour in food systems.

While the scientific literature offers extensive research on traditional protein sources such as cereals, legumes, milk, and meat, comparatively few studies have examined the technological properties of insect proteins and protein hydrolysates currently listed as approved sources (Table 3).

4.1. Protein solubility

As a critical parameter influencing emulsification, foaming, and gelation, protein solubility is paramount, especially for applications such as beverages and powdered ingredients that must dissolve in an aqueous medium. Protein solubility is determined by the distribution of polar and non-polar groups and is strongly affected by amino acid sequence, peptide size, pH, and salt concentration (Bußler et al., 2016; Purschke et al., 2018; Ulloa et al., 2017). Enzymatic hydrolysis generally enhances solubility by reducing the molecular weight of proteins and increasing the proportion of small peptide fragments, resulting in more ionisable amino and carboxyl groups, which interact with water molecules to enhance protein hydration and, therefore, solubility.

Hydrolysis of *T. molitor* proteins with Flavorzyme® and Alcalase® increased solubility from 39.1% to 89.2% (Yoon et al., 2019). A clear pH-dependent behaviour has also been reported. Native or defatted protein isolates showed minimum solubility around pH 5, indicating a pI close to this value, whereas enzymatic hydrolysates exhibited substantially higher solubility across the entire pH range. In particular, Yu et al. (2021) observed a four-fold increase in solubility at pH 5, while near-complete solubilisation (≈99–99.6%) was achieved at neutral and alkaline pH (7–9). These results indicate that hydrolysis effectively mitigates the solubility limitations typically observed near the isoelectric point (pI). By contrast, acid hydrolysis does not appear to be an effective strategy for improving solubility. Kim et al. (2016) reported no significant enhancement in the solubility of *T. molitor* proteins following acid treatment, even when combined with defatting. Similar trends have been reported for other edible insects. Hydrolysates from *A. diaperinus* and *L. migratoria* have shown significantly improved solubility compared to their native proteins. Purschke et al. (2018) investigated changes in the solubility of *L. migratoria* protein hydrolysates obtained with different enzymes (Flavourzyme®, Neutrase®) and found minimum solubility at pH 5 and maximum solubility at pH 9, except with Flavourzyme®. Prolonged hydrolysis time positively impacted solubility with all enzymes, regardless of pH. Enzymatic hydrolysis with multiple enzymes increases the DH and promotes the generation of low molecular weight peptides with improved solubility. Hydrolysates of *A. diaperinus* produced with protease from *Bacillus licheniformis* exhibited higher solubility than native proteins at pH 3 and pH 7 after 180 min (94.9–90.9 %), while showing minimal solubility at pH 5 (69.9 %), close to the isoelectric point (Leni et al., 2020).

Combining enzymatic hydrolysis with high hydrostatic pressure (HHP) can further enhance solubility, although the outcome strongly depends on processing conditions. While HHP may promote aggregation of high-molecular-weight proteins by exposing hydrophobic residues, its combination with enzymatic hydrolysis can induce favourable conformational changes and alter cleavage patterns. In *T. molitor* hydrolysates obtained with Alcalase®, this combined approach resulted in a more hydrophilic peptide profile and a marked increase in solubility compared to conventional hydrolysis (Dion-Poulin et al., 2020).

4.2. Emulsifying properties

Emulsifying capacity is defined as the amount of lipids that, under specified conditions, can be bound to an amphipathic molecule; it therefore reflects the ability of soluble proteins to migrate to the water–oil interface (Bhaskar, Ananthanarayan, & Jamdar, 2019). Consequently, the balance between hydrophobic and hydrophilic amino acids, which determines the protein's composition and primary structure, has a decisive influence on this property. Environmental parameters such as pH, temperature, and ionic strength further modulate protein solubility and conformation and, in turn, their technological characteristics, including emulsifying properties (Bußler et al., 2016; Mena-Casanova & Totosaus, 2011; Sharma et al., 2024). Across edible insect species, enzymatic hydrolysis can improve emulsifying activity, but only within a limited and optimal range of protein degradation. Enzymatic hydrolysis with Alcalase® has been shown to increase the emulsifying activity index (EAI) of several edible insect species. This effect is largely due to the enzyme's specificity for cleaving at aromatic residues, which exposes hydrophobic amino acids and thereby promotes emulsion formation (Jiang et al., 2018; Jung, Murphy, & Johnson, 2005; Pacheco-Aguilar, Mazorra-Manzano, & Ramírez-Suárez, 2008; Zhai et al., 2013). However, little influence on emulsifying activities was observed when defatting was combined with Alcalase® hydrolysis of *T. molitor* (at pH 8 and 60°C for 3 h) (Yu et al., 2021). Similarly, acid hydrolysis and HHP-assisted treatments did not significantly enhance emulsifying performance of *T. molitor* hydrolysates (Dion-Poulin et al., 2020; Kim et al., 2016). These findings were attributed to excessive protein degradation, which diminishes the interfacial activity of proteins. Moreover, while HHP treatment does not alter the DH, it may nonetheless modify peptide hydrophobicity (Gbogouri, Linder, Fanni, & Parmentier, 2004; Meinlschmidt, Sussmann, Schweiggert-Weisz, & Eisner, 2016).

The emulsifying capacity of *L. migratoria* proteins was enhanced through enzymatic hydrolysis using Flavourzyme® alone and in combination with Neutrase® (Purschke et al., 2018) likely attributable to increased surface hydrophobicity, as also reported for hydrolysed

tropical banded crickets (Hall et al., 2017; Wu, Hettiarachchy, & Qi, 1998). Yoon et al. (2019) reported higher emulsifying activity with Flavourzyme® than Alcalase® used to hydrolyse T. *molitor* proteins.

While emulsifying activity may increase following hydrolysis, emulsion stability often shows the opposite trend. In general, the emulsifying stability of hydrolysates tends to decline over time. Hydrolysis of *A. diaperinus* proteins with enzymes from *B. licheniformis* also showed a negative correlation with emulsifying capacity (Leni, Soetemans, Jacobs, et al., 2020). These findings suggest that certain hydrolysis conditions may reduce emulsion stability because the excessive degree of protein degradation alter molecular rearrangements at the interface (Hall et al., 2017; Meinlschmidt, Schweiggert-Weisz, Brode, & Eisner, 2016; Meinlschmidt et al., 2016).

Controlled enzymatic hydrolysis can enhance emulsifying activity by increasing surface hydrophobicity and flexibility, whereas excessive hydrolysis diminishes both emulsifying capacity and stability due to the loss of structural integrity at the oil–water interface.

4.3. Water holding capacity

The amount of water that food polymers, including carbohydrates and proteins, can retain is referred to as water-holding capacity (WHC). Concerning proteins, this property is determined by several factors, including amino acid composition, pH, ionic strength, protein conformation, hydrophobicity, temperature, and protein concentration (Verhoeckx et al., 2014). It should also be emphasised that near the isoelectric point (pI), WHC reaches a minimum because protein-protein interactions are favoured over protein-water interactions.

Across edible insect species, protein hydrolysates from *L. migratoria*, produced using various enzymatic treatments (Alcalase®, Flavourzyme®, Neutrase®, papain), showed no significant changes in WHC compared to the untreated control, regardless of hydrolysis time (30, 60, or 120 min) (Purschke et al., 2018). This suggests that, under moderate hydrolysis conditions, the protein network responsible for water retention remains largely intact, allowing bound water to be retained even after extended proteolysis. Similarly, enzymatic treatment with Alcalase® did not significantly affect the WHC of *T. molitor* hydrolysates (Dion-Poulin et al., 2020).

In contrast, lipid removal emerges as a key factor influencing WHC. Under specific hydrolysis conditions (2% Alcalase®, pH 8, 60°C, 3 h), *T. molitor* hydrolysates improve WHC values (Dion-Poulin et al., 2020) while Yu et al. (2021) reported an increase of water adsorption capacity (WAC) of 1.6-fold higher after defatting mealworm.

4.4. Oil binding capacity

Oil holding capacity (OHC) refers to the ability of food polymers, such as proteins and carbohydrates, to retain lipids. When dealing with proteins, this property is influenced by the ratio of polar to non-polar amino acids (Gkinali et al., 2022; Zielińska et al., 2015).

The ability of insect proteins to retain oil can be significantly enhanced through enzymatic hydrolysis promoting the binding of lipids. Enzymatic hydrolysis by Flavourzyme® and Neutrase® has been shown to significantly improve the oil adsorption capacity (OAC) of *A. domesticus* proteins, regardless of hydrolysis time, likely due to the exposure of hydrophobic groups during protein degradation (Purschke et al., 2018). Similarly, proteases derived from *B. licheniformis* and Alcalase® have been shown to increase OAC of hydrolysates obtained from *A. diaperinus* and *T. molitor*, with higher DH having positive effects (Dion-Poulin et al., 2020; Leni et al., 2020).

Lipid removal (defatting) is an even more effective strategy for enhancing OAC, as observed in the case of *T. molitor* meal (Yu et al., 2021).

In addition to hydrolysis and defatting, HHP treatment has also been reported to increase the OAC of *T. molitor* hydrolysates, mainly at pH, 4 while no significant changes were detected at pH 5.5 or 7. The authors suggested that HHP treatment likely modified the protein structures and peptide profile due to different cleavage patterns (Dion-Poulin et al., 2020).

4.5. Foaming properties

A foam is a colloidal system consisting of a dispersed gas phase within an aqueous medium. Foaming is influenced by the ability of proteins to lower surface tension at the gas-water interface, a process involving the migration, unfolding, and rearrangement of molecules. This phenomenon is widely exploited to enhance the texture and visual appeal of food products. Both foaming capacity and stability are affected by various factors, notably surface hydrophobicity and, more broadly, the amphiphilic nature of proteins (Gkinali et al., 2022; Hall et al., 2017).

While hydrolysis can sometimes improve foamability by exposing surface-active residues, it can also cause instability if the protein molecules are excessively degraded. In *L. migratoria* protein hydrolysates, single-enzyme treatment with Neutrase® and Flavourzyme® led to a marked improvement in foamability at pH 3, while the measured overrun (OR) was considerably reduced at all other pH tested (5, 9), suggesting that hydrolysis is pH-dependent. Treatments with enzyme combinations similarly affected the foamability of the hydrolysates, resulting in higher OR under weakly acidic conditions (pH 3–5). Enzymatic hydrolysis enhanced foam stability at pH 7 for both single and combined enzyme treatments, with only limited improvement observed at pH 9 (Purschke et al., 2018). Improvements in foamability can be attributed to the diffusion and stabilisation of the interfacial layer, resulting from enzymatic cleavage that generates small peptides and exposes surface-active residues. However, the loss of foamability of hydrolysates at neutral and alkaline pH may be explained by stronger repulsive forces and increased elasticity of the interfacial layer (Foegeding, Luck, & Davis, 2006; Panyam & Kilara, 1996). Leni et al. (2020) reported that hydrolysates of *A. diaperinus* produced with protease from *B. licheniformis* displayed appreciable foaming capacity but lacked stability at any hydrolysis time, with foam already declining after 1 min. This instability is likely due to the breakdown of large protein molecules essential for improving the viscoelasticity of the air-water interface and stabilising the foam.

Defatting is crucial for the foaming stability as even small amounts of lipids can compromise foam formation and stability. In fact, the lipid content completely compromised the foaming properties of *T. molitor* hydrolysates obtained using Alcalase® (Dion-Poulin et al., 2020). Even low lipid concentrations, as low as 0.5%, can negatively affect foam formation and stability, leading to rapid destabilisation (Lomakina & Míková, 2006). Similarly, the removal of the lipid fraction significantly increased the foaming capacity of *T. molitor* hydrolysates obtained with 2% Alcalase® at pH 8 and 60°C for 3 h (Yu et al., 2021).

4.5.1. Gelling properties

The ability to form a gel is essential in a wide range of foods, including jellies, puddings, confectionery products, and processed meats. Protein from edible insects with appreciable gelling capacity can also serve as alternative protein sources in the development of innovative food products (Boye et al., 2010; Corona-Mariscal, Sanjuan, Güell, & Clemente, 2024). Gelling involves the formation of a three-dimensional protein network, often through disulphide bonds and hydrophobic interactions. This process is influenced by several factors, including protein concentration, pH, ionic strength, and thermal treatment (Yi et al., 2013). Protein hydrolysates generally exhibit limited gelling capacity because the smaller peptide fragments are unable to form a robust network (Wouters, Rombouts, Fierens, Brijs, & Delcour, 2018). Dion-Poulin et al. (2020) observed no gel formation at protein hydrolysed concentration of 3% and 10% (w/v), underscoring how low protein concentration limits gelling properties (Lamsal, Jung, & Johnson, 2007; Panyam & Kilara, 1996). The drying methods used in the production of

insect meals may contribute to protein denaturation. Protein isolate from defatted mealworm showed high gelling properties, regardless pH and concentration. Conversely, hydrolysis with Alcalase® did not induce gel formation under any conditions tested (Yu et al., 2021).

5. Beyond nutrition: Bioactive properties and health implications

The development of protein hydrolysates is a promising strategy to enhance the use of insects as a sustainable protein source. Enzymatic hydrolysis can unlock bioactive properties by generating peptides (Nongonierma & FitzGerald, 2017), which can be exploited to create novel ingredients with potential health-promoting effects in humans (Coscueta et al., 2016; Martinez-Villaluenga, Peñas, & Frias, 2017; Samaranayaka & Li-Chan, 2011).

5.1. Antioxidant and antihypertensive activities

Protein hydrolysates from *A. diaperinus* exhibited notable antioxidant capacity and antihypertensive potential. Hydrolysis was carried out using Alcalase® 2.5 L and Corolase® PP under two conditions: an E/S of 1.5% for 4 h and an E/S ratio of 3.0% for 6 h, respectively. The resulting protein hydrolysates showed antioxidant capacity, assessed by *in vitro* ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and ORAC (Oxygen Radical Absorbance Capacity) assays, and antihypertensive potential, determined by their ability to inhibit angiotensin-converting enzyme (ACE) (Sousa et al., 2020). Protein extracts from *T. molitor* hydrolysed with gastrointestinal proteases (pepsin, α-chymotrypsin, and trypsin) also contain bioactive peptides with ACE inhibitory activity, highlighting their potential to reduce blood pressure. Cito et al. (2017) observed significantly lower IC_{50} values (half maximal inhibitory concentration) in *T. molitor* pupae and larvae protein fractions after hydrolysis compared with the corresponding crude extracts. Similarly, hydrolysates from *T. molitor* and *A. domesticus* were tested for antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Messina et al., 2019). Alcalase® from *B. licheniformis* achieved the highest DH of total proteins in both insect powders. Protamex®-generated hydrolysates of *T. molitor* displayed superior DPPH radical inhibition compared with the other enzymes, while Alcalase® produced the strongest antioxidant effect in A. *domesticus* hydrolysates.

An *in vivo* study suggested that a selected peptide from the hydrolysate exhibited clear ACE activity in spontaneously hypertensive rats, producing a dose-dependent reduction in blood pressure after oral administration, with systolic pressure decreasing by up to 27 mm Hg 4 h after a 400 mg/kg dose (Dai, Ma, Luo, & Yin, 2013).

5.2. Antithrombotic activity

Given the global health issue of thromboembolic diseases, which pose serious risks to human health and can be fatal (Mackman, 2012; Wakefield, Myers, & Henke, 2008), there is increasing interest in finding alternatives to conventional anticoagulant drugs such as heparin or warfarin, which may cause adverse effects during long-term use (Einhäupl et al., 1991). Peptides from the enzymatic hydrolysis of *T. molitor* larvae proteins have shown promising *in vitro* antithrombotic effects (Chen et al., 2019). Using Response Surface Methodology (RSM) combined with a Plackett-Burman design, the authors optimised key variables, including substrate concentration (19.8 mg/mL), pepsin digestion time (1.8 h), pepsin concentration (1634 U/mL), and trypsin concentration (126 U/mL). The resulting hydrolysates exhibited antithrombotic activity of 82.8%, with no linear relationship to the DH.

5.3. Anti-obesity effects

Obesity is a major health risk associated with excessive fat accumulation. Peptides derived from defatted *T. molitor* powder have been shown to modulate lipid metabolism, inhibit fat accumulation, and promote weight loss through phosphorylation of AMP-activated protein kinase (AMPK). Song et al. (2020) used a combined chemical and enzymatic hydrolysis approach, employing strong acids and bases (1 M H_2SO_4, 6 M HCl, and 1 M NaOH) together with Protamex®, Neutrase®, Flavourzyme®, and Alcalase®. The effects of dietary supplementation with these peptides were evaluated in rats fed different experimental diets. Animals receiving *T. molitor* peptides showed significant reductions in body weight, blood glucose, and plasma insulin levels, resulting in decreased visceral fat and an overall anti-obesity effect.

5.4. DPP-4 Inhibition for glycemic control

Dipeptidyl peptidase-4 (DPP-4) inhibitory peptides derived from *T. molitor* proteins have been studied for their potential in glycaemia control in diabetic patients. Enzymatic hydrolysis of *T. molitor* proteins using Flavourzyme® effectively produced these peptides, with 4 h of hydrolysis resulting in the highest DPP-4 inhibitory activity ($IC_{50} = 1.64$ mg/mL). These findings indicate that *T. molitor* protein hydrolysates could serve as natural DPP-4 inhibitors for the management of blood glucose levels (Tan et al., 2022).

6. From lab to table: Applications, safety, and labelling

Protein hydrolysates obtained from edible insects are being investigated as potential ingredients in the formulation of novel food products, with the aim of improving nutritional quality and added value.

6.1. Applications of protein hydrolysates in foods

Tortillas and tortilla chips formulated with 20% (w/w) house cricket (*A. domesticus*) protein hydrolysate obtained using Alcalase® and Flavourzyme® showed a balanced in all essential amino acids and high lysine content (approximately 40%). Textural properties confirmed the interaction between peptides and corn macromolecules (protein-protein and protein-starch interactions); however, the effect is strictly correlated to the type of the enzyme used. Sensory analysis confirmed the overall acceptability of the tortilla chips as a novel food product (Luna et al., 2021).

Kim et al. (2016) incorporated pre-treated (freeze-dried, defatted, and acid-hydrolysed) mealworm (*T. molitor* larvae) into emulsion-type sausages, replacing 10% (w/w) of the lean pork. The sausages were formulated with 60% (w/w) lean pork, 20% (w/w) ice, and 20% (w/w) backfat. Inclusion of the insect ingredient significantly increased the protein content, hardness, and cooking yield of the sausages. In another study, Singh et al. (2023) investigated the effects of microwave and ultrasound pre-treatments on protein hydrolysates (Alcalase® 3%) obtained from *L. migratoria* on the quality of preserved meat emulsions. Meat emulsions incorporating pre-treated hydrolysates showed significant improvements in all quality parameters, including antioxidant potential, protein oxidation, lipid stability, and microbial quality during two weeks of refrigerated storage. Moreover, the authors highlighted a particularly notable positive effect of microwave pre-treatment on the sensory properties of the hydrolysates.

House Cricket (*A. domesticus*) protein hydrolysates (Alcalase®), pretreated with microwaves and ultrasounds, have been used to extend the shelf life of cheddar-style cheese (Lone et al., 2023). Protein hydrolysates incorporated into cheese samples at a maximum concentration of 1.5% (w/w) showed a significant positive effect on antioxidant potential, lipid stability, protein oxidation, microbial growth, and sensory quality of the cheddar cheese during three months of storage. Additionally, digestion simulation demonstrated a significant positive impact on the antioxidant activity of the cheddar cheese.

6.2. Safety, allergenicity, and consumer information

The use of edible insects as food ingredients involves several hazard categories that must be addressed, including biological, chemical, and an allergenic risk (Pali-Schöll et al., 2019).

Heat treatments are well known to be effective in mitigating microbiological hazards (Fröhling, Bußler, Durek, & Schlüter, 2020; Kooh et al., 2020). Emerging technologies such as pulsed electric fields, cold atmospheric plasma, and high hydrostatic pressure have also demonstrated efficacy in reducing microbial risk (Bolat, Ugur, Oztop, & Alpas, 2021; Bußler et al., 2016; Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019), as shown in studies involving other food preparations and raw materials (Mandal, Singh, & Pratap Singh, 2018; Perinban, Orsat, Lyew, & Raghavan, 2022). However, when applied, it is essential to evaluate their potential impacts on proteins, lipids, and minerals (Foligni et al., 2022; Mannozzi et al., 2018). Concerning the chemical hazard, the presence of heavy metals such as cadmium, mercury, lead, and arsenic remains a significant chemical hazard, as noted by EFSA (Ribeiro, Sousa-Pinto, Fonseca, Fonseca, & Cunha, 2021).

Edible insects pose also an allergenic risk, primarily due to the proteins named tropomyosin and arginine kinase, which are common in these matrices and are recognised as key triggers of allergic reactions (Francis et al., 2019; F. Hall, Reddivari, & Liceaga, 2020; Verhoeckx et al., 2014). These proteins are considered pan-allergens and can cause cross-reactivity with homologous proteins in crustaceans and house dust mites (de Gier & Verhoeckx, 2018; De Marchi et al., 2021). This means that individuals with allergies to arthropods or crustaceans face a high risk of cross-reactive allergic responses when consuming insects. On the best of our knowledge, no studies report the effect of hydrolysis on allergenic risk of insect protein. However, based on the available literature, it could be supposed that hydrolysis may reduce the allergenicity thanks to the formation of small peptide fragments, which are usually not recognized by the IgE-binding receptors of mast cells and improving therefore the safety of the ingredients (Calcinai et al., 2022; Pang et al., 2024; Pi, Sun, Fu, Wu, & Cheng, 2021).

To safeguard consumers, clear and accurate labelling that explicitly states the presence of insect-derived ingredients is imperative, as imposed by the European Commission (European Commision, 2017). Innovative approaches such as using QR codes can provide consumers with additional, easily accessible allergen information.

7. Conclusions

This review has highlighted the properties and potential applications of protein hydrolysates derived from EU-approved edible insects, namely *Tenebrio molitor, Locusta migratoria, Acheta domesticus*, and *Alphitobius diaperinus*. Variability in protein composition among different insect species requires tailored enzyme selection and precise control over operating conditions (pre-treatments), processing parameters (pH, time, and temperature) and technologies (HHP, ultrasounds) to unlock specific technological and functional properties. Future research should focus on exploring new combinations of enzymatic treatments and process parameters to further understand their impact on technological and functional properties of insect proteins insect hydrolysates. Additionally, investigating the potential of emerging technologies, such as pulsed electric fields, microwave processing, and cold atmospheric plasma, would be valuable.

The improvements in solubility and emulsifying capacity make insect proteins hydrolysates promising ingredients for beverages and emulsion products (dressings, mayonnaise, sauces). Their enhanced water absorption capacity could benefit the creation of juicy and texturally appealing meat analogues, while their combined oil absorption and emulsifying abilities offer potential for a wide range of products, including ice cream, desserts, bakery products, dressings, instant sauces, and high-protein snacks and beverages.

Besides, interesting health-promoting effects in humans open to the development of new functional food with bioactive properties. However, the current body of evidence is dominated by *in vitro* studies and a small number of animal experiments. Future research should focus on peptide identification, bioavailability, dose–response relationships, and well-designed human intervention studies to clarify their actual contribution to cardiovascular health and oxidative stress modulation in realistic dietary contexts.

As research progresses, edible insect protein hydrolysates are set to become a valuable, healthy and sustainable component of the modern food industry. Therefore, deepening the understanding of their potential integration into food production processes is vital for the creation of innovative products for human consumption.

Human and animal rights

The authors declare that the work described has not involved experimentation on humans or animals.

Informed consent and patient details

The authors declare that the work described does not involve patients or volunteers.

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Roberta Foligni: Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Cinzia Mannozzi:** Writing – review & editing, Investigation. **Virginia Teresa Glicerina:** Writing – review & editing, Investigation. **Sofia Melchior:** Writing – review & editing, Visualization. **Federica Flamminii:** Writing – review & editing, Visualization. **Agnieszka Orkusz:** Writing – review & editing. **Wojciech Kolanowski:** Writing – review & editing. **Massimo Mozzon:** Writing – review & editing, Visualization, Validation.

Declaration of competing 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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Data availability

Data will be made available on request.

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