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Unlocking the biological potential of proteins from edible insects through enzymatic hydrolysis: A review

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ABSTRACT

This review, focusing on studies published between 2005 and 2017, analysed the literature on the generation of bioactive peptides (BAPs) from edible insect proteins following enzymatic hydrolysis. The protein extraction and quantification methodologies used for edible insects varied considerably. While several edible insects have been evaluated for their ability to release BAPs, silkworm (*Bombyx mori*) is currently the most studied. Specifically, the angiotensin converting enzyme (ACE) inhibitory, antioxidant and antidiabetic properties of edible insect protein enzymatic hydrolysates have been studied. Potent in vitro ACE inhibitory and antioxidant hydrolysates/peptides have been reported. In certain instances, these properties were validated in small animal studies (i.e. hypotensive effects). Enzymatic hydrolysis of edible insect proteins may also enhance technofunctional properties (i.e. solubility). The wider application of enzymatic hydrolysis protocols to edible insect proteins may ultimately allow for the increased discovery and utilisation of novel BAPs as sustainable protein/peptide sources for human nutrition.

1. Introduction

It is well documented that there is an increased demand for good quality protein worldwide, both for animal feed and human nutrition (van Huis, 2013). Insect farming for animal feed production appears to be more cost effective, yielding higher biomass (higher feed conversion ratio) than conventional animal feed derived from soy and fish sources (Feng et al., 2017; Sánchez-Muros, Barroso, & Manzano-Agugliaro, 2014; van Huis, 2013, 2016). Farming insects is also generally considered as a sustainable practice as it is associated with lower levels of greenhouse gases and ammonia than traditionally farmed cattle, poultry, fish and seafood (Rumpold & Schlüter, 2013b; Sun-Waterhouse et al., 2016; van Huis, 2013, 2016; van Huis et al., 2013). Therefore, edible insects have been proposed as an alternative to conventional animal proteins for use as human food (Spiegel, Noordam, & Fels-Klerx, 2013).

Insects belong to the Arthropods (subphylum *Hexapoda*), which are the largest animal group on earth. Owing to the high number of species, insects constitute the largest Arthropod group (Bullard, Linke, & Leonard, 2002). Examples of common insects which have traditionally been exploited for their by-products include honey bees (*Apis mellifera*), silkworm (*Bombyx mori*) and cochineal (*Dactylopius coccus*) (van Huis, 2013). Up to 2000 different edible insect species have been reported (Rumpold & Schlüter, 2013b; Sun-Waterhouse et al., 2016; van Huis, 2013, 2016). A list of edible insects available in different parts of the world is curated by the University of Wageningen (The Netherlands). Its latest

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ACE, angiotensin I converting enzyme; Ala, alanine; Arg, arginine; As, arsenic; ASEAN, Association of Southeast Asian Nations; Asn, asparagine; Asp, aspartic acid; BAP, bioactive peptide; BW, body weight; Cd, cadmium; CFU, colony forming unit; Co, cobalt; CO_2, carbon dioxide; Cr, chromium; Cu, copper; DDT, dichlorodiphenyltrichloroethane; DH, degree of hydrolysis; dH_2O, deionised water; DOE, design of experiments; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPP-IV, dipeptidyl peptidase IV; dw, dry weight; EC_{50}, half maximal effective concentration; EFSA, European Food Safety Authority; EU, European Union; FAO, Food and Agriculture Organization; FDA, Food and Drug Administration; Fe, iron; FERA, Food and Environmental Research Agency; FRAP, ferric reducing antioxidant power; FSVO, Federal Food Safety and Veterinary Office; Gln, glutamine; Glu, glutamic acid; Gly, glycine; GRAS, generally recognised as safe; His, histidine; IC_{50}, half maximal inhibitory concentration; Ile, isoleucine; INFOODS, International Network of Food Data Systems; LC-MALDI-TOF, liquid chromatography matrix-assisted laser desorption ionisation-time of flight; LC-MS/MS, liquid chromatography tandem mass spectrometry; Leu, leucine; Lys, lysine; Met, methionine; MS, mass spectrometry; NaCl, sodium chloride; NaOH, sodium hydroxide; Ni, nickel; NOAEL, no observed adverse effect level; PAP, processed animal protein; Pb, lead; PCBs, polychlorinated biphenyls; Phe, phenylalanine; pI, isoelectric point; Pro, proline; QSAR, quantitative structure activity relationship; RP, reverse phase; RSM, response surface methodology; SBP, systolic blood pressure; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SE, size exclusion; Ser, serine; SGID, simulated gastrointestinal digestion; SHR, spontaneous hypertensive rat; Sn, tin; TA, titratable acidity; Thr, threonine; TOF, time of flight; Trp, tryptophan; Tyr, tyrosine; UF, ultrafiltration; UK, United Kingdom; USA, United States of America; Val, valine; WHO, World Health Organization; Zn, zinc

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Table 1
Summary of edible insects used for the generation of bioactive peptides (BAPs) and their protein content.

Order	Family	Genus	Species	Common name	Protein content (% (dw))	Reference
Orthoptera					22.8–65.39	Xiaoming et al. (2010)
	Gryllidae	*Gryllodes*	*sigillatus*	Banded cricket	70.0	Zielińska et al. (2015)
		Acheta	*domesticus*	House cricket	55.00–70.75	Rumpold and Schlüter (2013a)
		Amphiacusta	*annulipes*	Cricket	–	
	Acrididae	*Locusta*	*migratoria*	Locust	42.16–58.62	Mohamed (2016)
		Schistocerca	*gregaria*	Desert locust	76.0	Zielińska et al. (2015)
	Tettigoniidae	*Ruspolia*	*differens*	Grasshopper	43.10–44.30	Rumpold and Schlüter (2013a)
Coleoptera					23.20–66.20	Xiaoming et al. (2010)
	Tenebrionidae	*Tenebrio*	*molitor*	Yellow mealworm	47.18–60.20	Rumpold and Schlüter (2013a)
		Zophobas	*morio*	Superworm	43.13–46.79	Rumpold and Schlüter (2013a)
		Alphitobius	*diaperinus*	Lesser (or buffalo) mealworm	58.03	Yi et al. (2013)
Lepidoptera					14.05–68.30	Xiaoming et al. (2010)
	Bombycidae	*Bombyx*	*mori*	Silkworm	48.70–69.84	Rumpold and Schlüter (2013a)
	Saturniidae	*Samia*	*ricinii*	Eri silkworm	54.00–54.80	Rumpold and Schlüter (2013a)
Blattodea					–	–
	Blaberidae	*Blaptica*	*dubia*	Dubia cockroach	59.2	Yi et al. (2013)
		Gromphadorhina	*portentosa*	Madagascar hissing cockroach	62.52–63.35	Oonincx and Dierenfeld (2012)
Noctuidae					–	–
	Noctuidae	*Spodoptera*	*littoralis*	Cotton leafworm	–	–
Isoptera					–	–
	Termitidae	*Macrotermes*	*subhylanus*	Termites	39.34[a]	Kinyuru et al. (2013)
Hymenoptera					12.65–76.69	Xiaoming et al. (2010)
	Apidae	*Bombus*	*terrestris*	Bumble bees	–	–

–: value not available in the literature.
[a] The insects were dewinged.

version has been made accessible on the University of Wageningen website (Jongema, 2017).

Insect consumption, termed entomophagy, may allow a means to address the growing protein demand by humans worldwide. Prehistoric records have demonstrated that the evolutionary precursors of *Homo sapiens* as well as other hominins (Neanderthals, Denisovans, and *Homo heidelbergensis*) were entomophagous (Ko, 2016). Various edible insects have continued to be traditionally consumed by humans in different parts of the world, more particularly in Asia, Africa, Oceania, the Middle East and Latin America (Feng et al., 2017; Kelemu et al., 2015; van Huis, 2016; van Huis et al., 2013). In addition, examples of traditional cheeses such as, Casu marzu and Milbenkäse, which contain maggots of the cheese fly (*Piophila casei*) and cheese mites or their digestive juices are produced in Sardinia and Germany (Halloran & Münke, 2014). The most consumed insects comprise beetle, caterpillar, bee, wasp, ant, grasshopper, locust and cricket (van Huis et al., 2013). Those edible insects may potentially be used at different stages of their development, i.e. egg, larva, pupa and adult stages (Spiegel et al., 2013). Those insects that may be used as foods in the European Union (EU) are yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*) and tropical banded cricket (*Gryllodes sigillatus*). Black soldier fly (*Hermetia illucens*), *T. molitor* and common housefly (*Musca domestica*) have been proposed for use as animal feed (Spiegel et al., 2013). Currently in Europe and the United States of America (USA), different food products containing edible insects are found on the market, which are derived from crickets, *T. molitor*, *A. diaperinus* and migratory locust (*Locusta migratoria*) (van Huis, 2016). While entomophagy is still not the norm in the Western world, several studies suggest that there is potential for its increased acceptability among consumers (Caparros Megido et al., 2014; Looy & Wood, 2006; Tan et al., 2015; Tan, Fischer, van Trijp, & Stieger, 2016). More particularly, extraction of nutritional components (e.g. proteins and fats) from edible insects for subsequent use as food ingredients has been suggested as means to increase consumer acceptability (Sosa & Fogliano, 2017).

Numerous amino acid sequences have been identified within a wide range of dietary proteins, which have been linked with in vitro bioactive properties. Peptides having a biological function are generally termed bioactive peptides (BAPs). These have been identified, for example for their antihypertensive, antioxidant, antidiabetic, immunomodulatory and mineral binding properties (Cicero, Fogacci, & Colletti, 2017; Li-Chan, 2015). Milk, soy and fish proteins have been extensively studied as sources of BAPs (Jo, Khan, Khan, & Iqbal, 2017; Nongonierma, O'Keeffe, & FitzGerald, 2016; Rizzello et al., 2016). With the wider application of in silico tools (Iwaniak, Minkiewicz, Darewicz, Protasiewicz, & Mogut, 2015), BAP motifs have been identified within dietary proteins other than from milk, soy and fish (Chang & Alli, 2012; Cheung, Nakayama, Hsu, Samaranayaka, & Li-Chan, 2009; Udenigwe, 2016; Vecchi & Añón, 2009). In particular, an in silico analysis of actin from insects revealed that they contain several previously identified angiotensin I converting enzyme (ACE) inhibitory peptides (Vercruysse, Smagghe, van der Bent, et al., 2009). ACE is a metabolic enzyme which converts angiotensin I into the potent vasoconstrictor angiotensin II, and can also degrade bradikynin, a potent vasodilator (FitzGerald, Murray, & Walsh, 2004; Udenigwe & Mohan, 2014). ACE inhibition has therefore been targeted for blood pressure reduction. The identification of biologically active peptides within a range of dietary proteins has led to the concept of exploiting undervalorised/underutilised edible proteins as a source of BAPs (Lemes et al., 2016). Currently, BAPs are being reported within a wide range of protein sources and the trend appears to be on the diversification of protein starting materials in keeping up with the increased global demand for high quality food-grade protein.

A wide range of biofunctional components (e.g. chitin, polyphenols, antioxidant enzymes, antimicrobial peptides/proteins, etc.) exist in insects (Mlcek, Borkovcova, Rop, & Bednarova, 2014; Ratcliffe, Azambuja, & Mello, 2014; Sun-Waterhouse et al., 2016). However, to date, a limited number of edible insects appear to have been employed for the generation of BAPs. Examples of such insects are presented in Table 1. The biofunctional properties of *B. mori* proteins and peptides have been reviewed (Kumar, Dev, & Kumar, 2015; Xia, Ng, Fang, & Wong, 2013). Endogenous proteins and peptides from *B. mori* display a wide range of bioactive properties including antimicrobial, antihypertensive, antioxidant, antidiabetic, anticancer, hepatoprotective and many more activities (Kumar et al., 2015; Xia et al., 2013). Other bioactive effects have been demonstrated with protein hydrolysates manufactured using by-products of the silk industry (i.e. silk fibroin). Hydrolysates of cocoons or silk fibroin from *B. mori* displayed ACE (Zhou, Xue, & Wang, 2010) and α-glucosidase (Lee et al., 2011) inhibitory properties in vitro. α-Glucosidase is a metabolic enzyme involved in the breakdown of oligosaccharides into glucose in the small intestine during the post-prandial phase. Inhibition of α-glucosidase may currently be used to reduce intestinal uptake of glucose and subsequently lower post-prandial