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Extraction and characterisation of protein fractions from five insect species

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ABSTRACT

Tenebrio molitor, *Zophobas morio*, *Alphitobius diaperinus*, *Acheta domesticus* and *Blaptica dubia* were evaluated for their potential as a future protein source. Crude protein content ranged from 19% to 22% (Dumas analysis). Essential amino acid levels in all insect species were comparable with soybean proteins, but lower than for casein. After aqueous extraction, next to a fat fraction, a supernatant, pellet, and residue were obtained, containing 17–23%, 33–39%, 31–47% of total protein, respectively. At 3% (w/v), supernatant fractions did not form stable foams and gels at pH 3, 5, 7, and 10, except for gelation for *A. domesticus* at pH 7. At 30% w/v, gels at pH 7 and pH 10 were formed, but not at pH 3 and pH 5. In conclusion, the insect species studied have potential to be used in foods due to: (1) absolute protein levels; (2) protein quality; (3) ability to form gels.

1. Introduction

1.1. Insects as a source of food

In most developed countries, human consumption of insects is infrequent, or even culturally inappropriate, although its nutritional value is comparable to conventional meat (van Huis, 2013). In many regions and countries of the world, insects form part of the human diet and it is a misconception to believe that this is prompted by starvation (van Huis, 2013). About 1900 insect species are consumed globally as human food in the world (http://www.ent.wur.nl/UK/Edible+insects/Worldwide+species+list/).

With an increase in the world population, increased consumer demand for protein, and the amount of available agricultural land being constrained, the sustainable production of meat will represent a serious challenge for the future. Insects can be considered as an alternative protein source with less environmental impact (van Huis, 2013). Insects can be consumed as a whole. However, they can also be processed in less recognisable forms, which may increase consumer acceptability. Insects are already used as natural food ingredients, *e.g.* the red colourant carmine (E120) used in yogurt is an extract of the female cochineal insect.

1.2. Edible insects

Insects are consumed in different life stages like eggs, larvae, pupae or adults. The main species consumed are, in order of importance: beetles (Coleoptera), caterpillars (Lepidoptera), ants, bees and wasps (Hymenoptera), grasshoppers and locusts (Orthoptera), true bugs, aphids and leafhoppers (Hemiptera), termites (Isoptera) and flies (Diptera) and some others. Lepidoptera, Coleoptera, and Diptera (including flies) are commonly consumed in the larval stage; while the Orthoptera, Hymenoptera, Hemiptera and Isoptera are mainly consumed in the adult stage.

Cultivating edible insects for food consumption has several advantages: (1) Insects have a high feed conversion efficiency compared with conventional livestock. For example, the feed conversion ratio of house cricket (*Acheta domesticus*) can be calculated twice as efficient as chickens, almost 4 times more efficient than pigs and over 12 times more than cattle (van Huis, 2013), (2) Cultivating insects for protein has less environmental impact than cattle ranching, due to the lower production of greenhouse gas and NH_3 emissions (van Huis, 2013), (3) Besides the higher production yield and less environmental impact, insect feeds can be obtained from a wider range of plants than that of conventional livestock,

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such as cattle or swine (Durst & Shono, 2010). Overall, insect farming can be introduced in terms of a sustainable form of agriculture.

1.3. Proteins of edible insects

As a food source, insects are potentially nutritious, rich in protein and fat, and providing a certain amount of minerals and vitamins. Studies on protein quality, nutritional value, protein content, and the amino acid composition of various insects are available (Barker, Fitzpatrick, & Dierenfeld, 1998; Ladrón de Guevara, Padilla, García, Pino, & Ramos-Elorduy, 1995, Renault, Bouchereau, Delettre, Hervant, & Vernon, 2006). The protein content of common edible insects was around 9–25% (Finke & Winn, 2004), and the Yellow mealworm beetle larvae (24%) (Ghaly & Alkoaik, 2009), *Zophobas morio* larvae (19%) (Finke, 2002), and *A. domesticus* adult (19%) (Finke & Winn, 2004), conventional meat protein sources contain about 15–22% protein (Ghaly & Alkoaik, 2009). In addition, some insects have not only protein content comparable to meat, but also to plant protein (up to 36.5%).

People may consume insect food more easily when unrecognisable insect protein (extract) is incorporated in food in comparison to consuming whole insects. (Del Valle, Mena, & Bourges, 1982) also indicated that the extraction of proteins from insects for further use in food products is particularly relevant for countries that do not have the habit of consuming insects, such as Europe and North America.

In this study, there are five insect species selected based on their availability (species reared by companies in the Netherlands): three species of Coleoptera considered edible, including the Yellow mealworm (*Tenebrio molitor*), the Superworm (*Zophobas morio*), the Lesser mealworm (*Alphitobius diaperinus*) and one species of Orthoptera; the House cricket (*Acheta domesticus*) considered edible and one of the Blattodea; the Dubia cockroach (*Blaptica dubia*) not edible, but can be reared in large numbers and used for animal feed.

1.4. Objective

Although researchers from entomological and zoo-biology science have studied intact edible insects, still very little information from a food science point of view is available on characteristics and functionality of extracted insect proteins.

The aim of this study was to investigate if insects could be used as a future protein source in food. Therefore, insect protein characteristics and functionality were determined and evaluated for each of the five insect species. The specific objectives of this study were to: (a) extract proteins and characterise obtained fractions; (b) evaluate protein purity and yield of the obtained fractions; (c) establish some functional properties of the protein fractions focused on foaming and gelation; (d) study protein quality by analysis of protein content and amino acid composition.

2. Materials and methods

2.1. Insects used

T. molitor, *Z.morio*, *A.diaperinus*, *A. domesticus* and *B. dubia* were purchased from the commercial supplier Kreca V.O.F, Ermelo, the Netherlands. *Tenebrio molitor, Z. morio, A. diaperinus* species were supplied in the larvae stage, *A. domesticus and B. dubia* in the adult stage. The feed for *T. molitor*, and *Z. morio* mainly consisted of wheat, wheat bran, oats, soy, rye, corn, carrot and beer yeast. The feed for *A. diaperinus, A. domesticus* and *B. dubia* mainly consisted of carrot and chicken mash obtained from Kreca V.O.F. All insects were sieved to get rid of feed and stored alive at 4 °C for about one day before processing.

2.2. Analysis of water content, protein, and fat content

All fresh insects were frozen using liquid nitrogen and subsequently grinded using a blender (Braun Multiquick 5 (600 W), Kronberg, Germany). Frozen grinded insects were freeze-dried (GRI Vriesdroger, GR Instruments B.V., Wijk bij Duurstede, the Netherlands) to determine moisture and dry matter content. The freeze-drying process was stopped at a stable sample weight. Next, the freeze-dried insects were used for protein content analysis. Crude protein content was determined by Dumas (Thermo Quest NA 2100 Nitrogen and Protein Analyser, Interscience, Breda, the Netherlands) using a protein-to-nitrogen conversion factor of 6.25. D-Methionine (Sigma, CAS nr. 348-67-4) was used as a standard. Furthermore, fat content was determined after hexane extraction (Biosolve, CAS nr. 110-54-3) in a Soxhlet apparatus for 6 h. Afterwards, hexane was removed using a Rotary evaporator (R420, Buchi, Switzerland). Defatted insect meal was stored at −20 °C. All experiments were performed in two duplications of the same sample.

2.3. Determination of amino acid composition and protein quality

Amino acid composition of freeze-dried insect powder was analysed using ion exchange chromatography, following the International standard ISO 13903:2005. Tryptophan was determined by reversed phase C_{18} HPLC using fluorescence detection at 280 nm, according to the procedure described by International standard ISO 13904:2005. The amino acid composition of the five insect species was compared to literature data of soybean protein and casein, representing high quality proteins among vegetable and animal proteins (Sosulski & Imafidon, 1990; Young & Pellett, 1994). Protein quality was evaluated by the essential amino acid index (EAAI), which is based on the content of all essential amino acids compared to a reference protein, being values for human requirements in this case (Smith & Nielsen, 2010). EAAI gives an estimate on the potential of using insects as a protein source for human consumption without correcting for protein digestibility (Eq. (1)).

$$\mathrm{EAAI} = \sqrt[9]{\left(\frac{\text{mg of lysine in 1 g of test protein}}{\text{mg of lysine in 1 g reference protein}}\right) \times (\text{etc..for the other8essential amino acids})} \quad (1)$$

2.4. Protein extraction procedure

For protein extraction, 400 g of N_2-frozen insects was used. After adding 1200 ml demineralized water, that was mixed with 2 g ascorbic acid beforehand, blending for 1 min took place (Braun Multiquick 5 (600 W), Kronberg, Germany). Then the obtained insect suspension was sieved through a stainless steel filter sieve