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Insects as sources for sustainable peptide drugs against type 2 diabetes

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Abstract:
This work describes the fractionation of insect protein hydrolysates by Size Exclusion Chromatography (SEC) with focus on hydrolysates showing activity on dipeptidyl peptidase IV (DPP-IV), with the aim to identify future sustainable peptide drugs against type 2 diabetes.

Introduction

Over 1 million insect species are known and every year more than 7,000 new species are described. It is estimated that only 20 % of all insect species have been discovered so far. In total there is estimated to be 10 quintillion individual insects alive. Insects have the potential to play a main role in the fight against global warming and the growing population since they are a sustainable source of proteins. Besides producing sustainable proteins for food, they can also be used for production of suitable drugs [1, 2, 3].

Insects constitute a class in the subphylum Hexapods. Hexapods belong to the kingdom Animalia and the phylum Arthropod. The insect class is divided into several different orders and the prevalence of species within the different orders is shown in **figure 1** [1, 2].

To identify bioactive peptides from different insect species, a literature search was conducted, followed by protein extraction, enzymatic hydrolysis and fractionation by SEC.

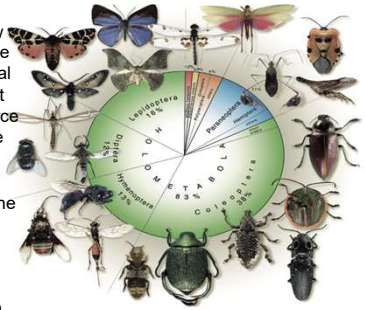

Figure 1. Prevalence of insect species within the different orders [2].

Method

Literature search – selection of insect species
To select insect species for investigation as source to bioactive peptides, a literature search was conducted to examine the reported protein content in different insect species. A flow chart illustrating the literature search is shown in **figure 2**. The protein content in 195 insect species were provided. Based on the results three insect species, mealworm (*Tenebrior molitor*), black soldier fly (*Hermetia illucens*), and house cricket (*Acheta domesticus*), was selected for further investigation.

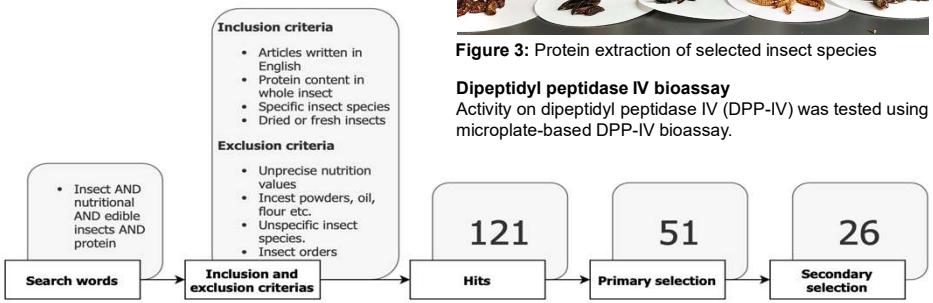

Figure 2: Flowchart of literature search

Protein extraction and enzymatic hydrolysis of insects
Protein extraction and enzymatic hydrolysis with 4 different proteases were performed for Black soldier fly (larvae, pupae, adult), mealworm (larvae) and house cricket (adult). The protein extraction is illustrated in **figure 3**.

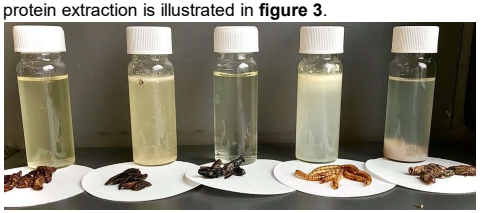

Figure 3: Protein extraction of selected insect species

Dipeptidyl peptidase IV bioassay
Activity on dipeptidyl peptidase IV (DPP-IV) was tested using a microplate-based DPP-IV bioassay.

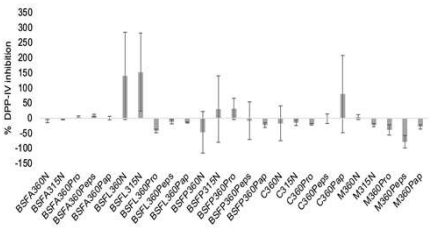

Figure 4: % inhibition of DPP-IV of the produced hydrolysates

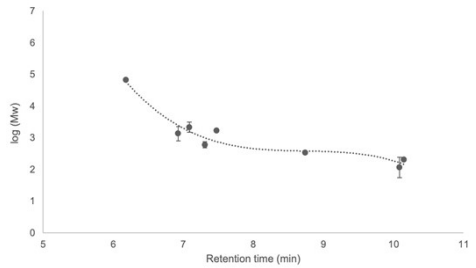

Figure 5: SEC calibration curve described as: $\log(M_w) = -0.1262t^3+3.3055t^2-28.895t+86.889$ (R^2=0.9415)

Results

Activity on DPP-IV
All hydrolysates were screened for activity on DPP-IV, and % inhibition is shown in **figure 4**. It was decided to proceed with hydrolysates of the black soldier fly at time 315 minutes (BSF315N) and at time 360 minutes (BSF360N), even though large standard deviation were seen. These were chosen because only one of the samples in the triplicate differed from the other two.

Fractionation of insect hydrolysates by SEC
SEC was performed to fractionate and estimate the average masses of the components in the hydrolysates. To do so a calibration curve was conducted shown in **figure 5**. The fractionation of BSF315N and the control sample is shown in **figure 5**. It is seen that the proteins and peptides in the hydrolysates have been cleaved as a result to the enzymatic hydrolysis.

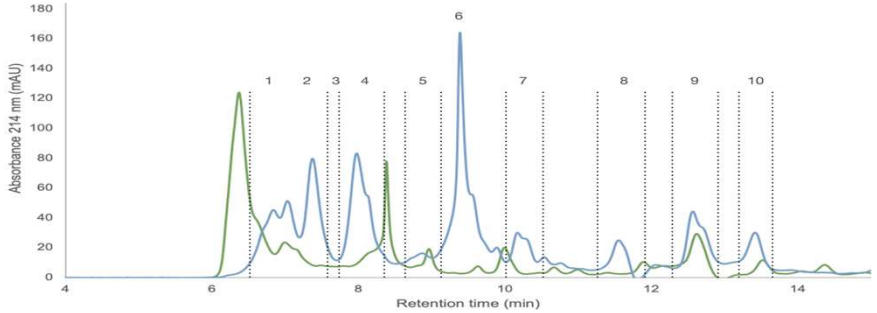

Figure 6: Fractionation of insect hydrolysates. The green graph shows the size-exclusion chromatogram (SEC) of the protein extract of black soldier fly larvae without enzyme at time 315 minutes and the blue graph shows the SEC of the protein hydrolysate of Black soldier fly at time 315 minutes with Neutrase®.

Further perspectives

The next step in the project is to screen the fractions obtained by SEC for inhibitory activity towards DPP-IV. Fractions exhibiting DPP-IV inhibitory activity will be further fractionated by RP-HPLC followed by identification of the bioactive peptides. In addition, it is desired to develop a MOR bioassay to screen for dual-acting activity.

References

[1] Stork NE. How many species of insects and other terrestrial arthropods are there on Earth? *Ann. Rev. Entomol.* **2018**, *63*, 31-45.
[2] Engel MS. Insect evolution. *Curr. Biol.* **2015**, *25*, R868-R872.
[3] Jantzen da Silva Lucas A, Menegon de Oliveira L, da Rocha M, Prentice C. Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food Chem.* **2020**, *311*, 126022.

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