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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.

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 (Tenebrio molitor), black soldier fly (Hermetia illucens), and house cricket (Acheta domesticus), was selected for further investigation.

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.

Inclusion criteria
• Articles written in English
• Protein content in whole insect
• Specific insect species
• Insect orders

Exclusion criteria
• Unspecific nutrition values
• Insect powder, oil, meal
• Insect species

Inclusion and exclusion criteria

121
51
26
Search words
Inclusion and exclusion criteria
Hits
Primary selection
Secondary selection

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.

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