METHOD FOR THE ISOLATION OF PROCESSED ANIMAL PROTEINS FROM INSECTS IN FEED AND THEIR IDENTIFICATION BY MICROSCOPY

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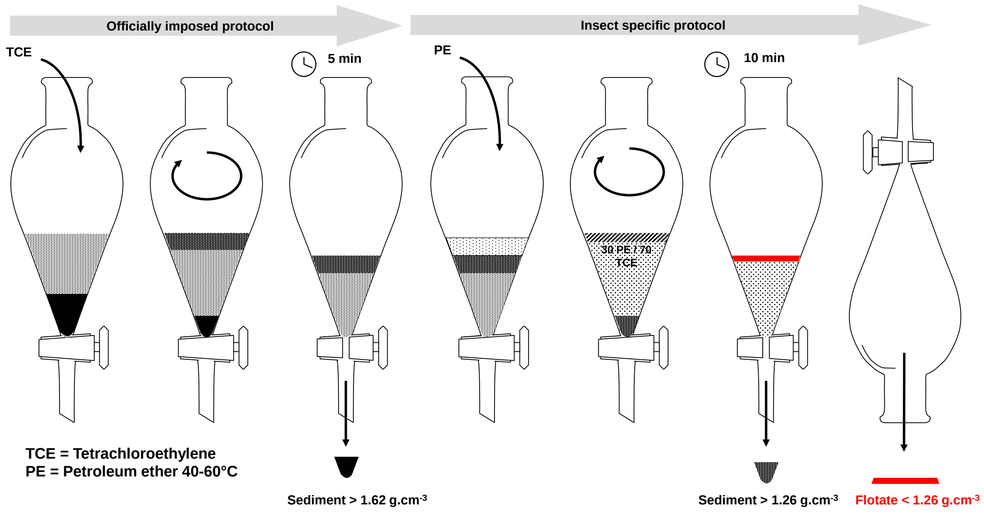
Introduction

In 2017 the European authorities agreed to introduce the use of insects and their processed animal proteins (PAPs) for feeding aquaculture animals. The question of quality control, contamination and fraud detection by efficient methods was raised. Two official methods are used for PAP detection in the European Union, light microscopy and PCR. Recently the possibility of using light microscopy and stainings for distinguishing pure insect meal against PAPs from other invertebrates such as marine arthropods was investigated (Ottoboni et al.,2017) but the detection of insect PAPs incorporated into feeds still remains unexplored. The absence of sedimentation of insect material by the official TCE settling step requires an **dedicated isolation method** before microscopic observation. In addition **reliable morphological features** are lacking for insect PAP identification, especially when such PAPs are introduced into complex matrices as feed. The EURL-AP team developed therefore an new double sedimentation protocol for concentrating insect particles from various feed matrices and proposes as well as robust identification markers for insects (Veys & Baeten, 2018).

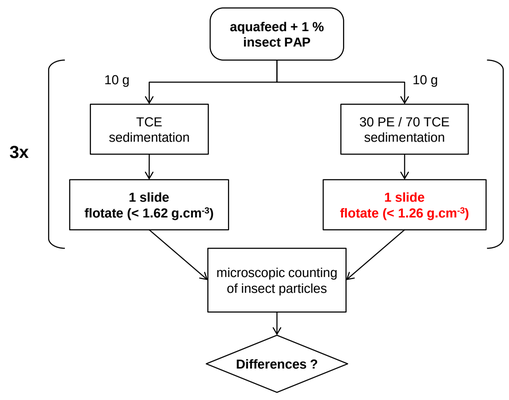
Double sedimentation to isolate insect particles

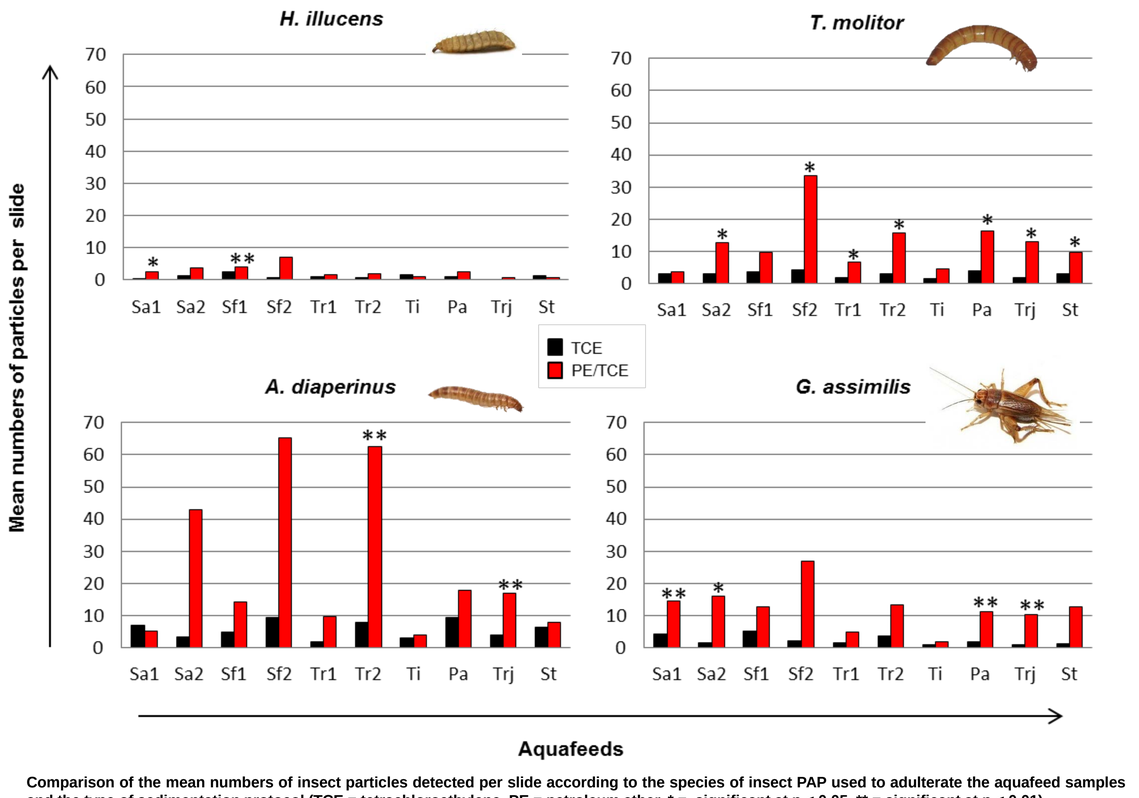


The method was tested on 40 different matrices : 10 aquafeeds fortified with 1 % of insect PAP from 4 different species (*Hermetia illucens, Tenebrio molitor, Alphitobius diaperinus* and *Gryllus assimilis*).

Slides prepared from the **flotate (< 1.26 g.cm^{-3})** obtained by the double PE/TCE sedimentation were compared to slides prepared from **flotate (< 1.62 g.cm^{-3})** obtained by current legal TCE sedimentation.

The differences in number of insect fragments were compared by t-test.





Comparison of the mean numbers of insect particles detected per slide according to the species of insect PAP used to adulterate the aquafeed samples and the type of sedimentation protocol (TCE = tetrachloroethylene, PE = petroleum ether, * = significant at p < 0.05, ** = significant at p < 0.01)

Results:

- For 92 % of samples, the number of insect particles was increased by a concentration factor of 1.22 to 12.90
- Better isolation compared to official method
- Double sedimentation comes as a complement to the official method
- Noticeable differences in number particles for *H. illucens*.

Future:

- Validation through interlaboratory study
- Challenge will be to develop identification skills of this new feed material.

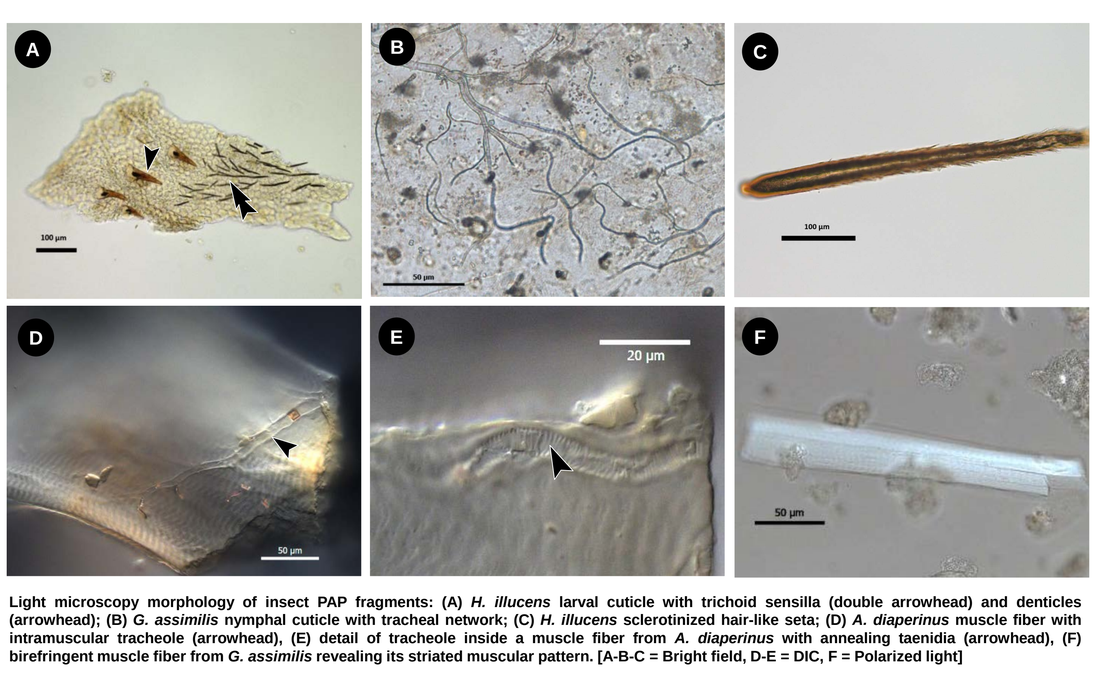
Identification of insect fragments

Exoskeleton

- Setae and appendices
- Cuticular fragments
- Tracheal system

Muscle fibers

- Pattern of sarcomers
- Intramuscular tracheoles and taenedia = confirmatory



Light microscopy morphology of insect PAP fragments: (A) *H. illucens* larval cuticle with trichoid sensilla (double arrowhead) and denticles (arrowhead); (B) *G. assimilis* nymphal cuticle with tracheal network; (C) *H. illucens* sclerotinized hair-like seta; (D) *A. diaperinus* muscle fiber with intramuscular tracheole (arrowhead), (E) detail of tracheole inside a muscle fiber from *A. diaperinus* with annealing taenidia (arrowhead), (F) birefringent muscle fiber from *G. assimilis* revealing its striated muscular pattern. [A-B-C = Bright field, D-E = DIC, F = Polarized light]

Taxonomic identification of insects ?

	Diptera	*Coleoptera*	*Orthoptera*
Developmental stages in PAP	Holometabolous (larval instars + pre-pupae)	Holometabolous (larval instars)	Hemimetabolous (nymphs + imagos)
Fragment features	*Few differentiated* • unsclerotinized, • **denticles** in spinose bands	*More differentiated* • sclerotinized +, • mouth parts, legs,	*Most differentiated* • tracheal system in cuticle • mouth parts, wings, legs, antennae, appendages • **birefringent** muscles
Taxonomic value	++ (→ species level)	?	+
Issues	Lack of reference	Confusion with pests possible (but different stages in pest contamination)	

Future:

- Training of staff for skills improvement.
- Development of reference picture libraries

References :
- Ottoboni M, Tretola M, Cheli F, Marchis D, Veys P, Baeten V & Pinotti L (2017). Light microscopy with a differential staining technique for the characterization and discrimination of insects versus marine arthropods in processed animal proteins. Food Additives & Contaminants: Part A, 34 (8), 1377-1383.
- Veys P & Baeten V (2018). Protocol for the isolation of processed animal proteins from insects in feed and their identification by microscopy. Food Control, 92: 496-504.

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