

Insects in feed: challenges, identification
 and extraction method

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1 Abstract:

The EU legislator, since 2017, authorised the use of insect proteins originating from eight insect species in feed for aquaculture, poultry and swine animals. The listed insects include the black soldier fly (*Hermetia illucens*, Stratiomyidae), common housefly (*Musca domestica*, Muscidae), yellow mealworm (*Tenebrio molitor*, Tenebrionidae), lesser mealworm (*Alphitobius diaperinus*, Tenebrionidae), house cricket (*Acheta domesticus*, Gryllidae), banded cricket (*Grylodes sigillatus*, Gryllidae), and field cricket (*Gryllus assimilis*, Gryllidae). Additionally, in 2021, the silkworm (*Bombyx mori*, Bombycidae) was included. Terrestrial invertebrate constituents are extracted according to the Regulation (EU) 2022/893 with double PE/TCE sedimentation. This method could be replaced by the filth test for the extraction of insect fragments along with other particles of interest such as hair, plastic fragments, mites, etc. The filth test method was used during the PT IAG 2025, along with the official method, giving 100% correct results. Furthermore, the light filth test is a step toward sustainable methods of laboratory analysis as petroleum ether is classified as toxic to aquatic organisms and likely to cause long term effects in the environment.

2 Challenges of insect’s authorization in feed:

Identifying insects in feed presents several challenges, such as distinguishing between species—many insect fragments resemble one another, making accurate identification difficult. Additionally, processing methods like heat treatment and grinding can alter insect morphology, further complicating classification. Precise identification is essential for clear labeling, ensuring regulatory compliance and transparency for consumers. Furthermore, classifying insects enhances understanding of their nutritional properties, contributing to more effective feed formulation.

3 Identification of insects in feed:
 Toward a key of identification?

The Regulation No 2001/999 (Annex IV), amended by Regulation 2017/893 (Annex X), allows the use of insect-derived processed proteins from seven specific species in feed for aquaculture, poultry, and swine. These approved species include the black soldier fly (*Hermetia illucens*, Stratiomyidae), the common housefly (*Musca domestica*, Muscidae), the yellow mealworm (*Tenebrio molitor*, Tenebrionidae), the lesser mealworm (*Alphitobius diaperinus*, Tenebrionidae), the house cricket (*Acheta domesticus*, Gryllidae), the banded cricket (*Grylodes sigillatus*, Gryllidae), and the field cricket (*Gryllus assimilis*, Gryllidae). In addition, under Regulation (EU) 2021/1925, as part of the EU legislation on animal by-products, the use of processed animal proteins (PAPs) from silkworms (*Bombyx mori*, Bombycidae) in feed for aquaculture, poultry, and pigs was also authorized in November 2021. In feed, insects are present as particles, making it impossible to immediately distinguish whether they originate from larvae, pupae, or adults and from which species. As a result, the identification relies solely on the presence of specific fragments, which can provide insights into both the species and developmental stage (table 1). By removing head particles, tracheal segments, and cuticle fragments, we keep mainly species-specific characters such as presence or absence of wings, legs and antenna.

	Wing	Antenna	Cercus	Anal segment	Spine-like sensilla	Mandibles	Spiracle	Stigmate	Leg	Setae	urogomphi	Claw
<i>Musca domestica</i>		x				oral hooks						
<i>Hermetia illucens</i>		x		x	x		x	x		x		
<i>Tenebrio molitor</i>		x		x		x	x		x		x	x
<i>Alphitobius diaperinus</i>		x				x	x		x	x	x	x
<i>Acheta domesticus</i>	x	x	x						x			
<i>Grylodes sigillatus</i>	x	x				x			x	x		x
<i>Gryllus assimilis</i>	x	x							x	x		
<i>Bombyx mori</i>		x				x	x		x	x		x

Feature	Shape	
Adult Gryllidae (Crickets)	Long, thread-like (filiform/setaceous), multi-segmented (Many flagellomeres)	
Larvae of Tenebrionidae, Muscidae, Stratiomyidae, Bombycidae	Short, few-segmented (Tenebrionidae, Stratiomyidae, Bombycidae); Reduced to papillae/bristles (Muscidae)	

The antennae of Gryllidae (crickets) adults are significantly different from the antennae of the larvae from the preceding families (Tenebrionidae, Muscidae, Stratiomyidae, and Bombycidae) in both morphology and relative size (table2). Cricket's adult typically possesses multi-segmented antennae, often described as filiform or setaceous. They consist of a scape (basal segment), a pedicel (second segment), and a long, thread-like flagellum composed of numerous small segments called flagellomeres. Larvae of Tenebrionidae have short to moderately long antennae that are typically few-segmented (usually 3-4 segments). Muscidae Larvae have greatly reduced antennae that are often barely visible or represented by small sensory papillae or bristles on their pseudocephalon (retractile head). Larvae of Stratiomyidae have short, often conical or peg-like antennae that are typically one to a few segments long while antennae of larvae of Bombycidae are short, typically three- segmented and located near their mouthparts.

A first draft key to authorized species in feed was prepared as follow:

- Key to Insect Identification**
 1 particles where barely visible legs and no wing are present.....(larvae) **3**
 2 Particles where visible legs and developed wings are present (**Gryllidae; Orthoptera**)
 Light brown particles..... **house cricket (*Acheta domesticus*)**
 abdomen black(**Jamaican**) **field cricket (*Gryllus assimilis*)**
 Legs transparent.....**banded cricket (*Grylodes sigillatus*)**
 3 With thoracic and abdominal legs **Bombycidae (*Lepidoptera*)**
 Larvae (silkworms) are with a well-defined head capsule, three pairs of thoracic legs, and five pairs of abdominal prolegs (false legs) with crochets (hooks) for gripping. They are covered in small secondary setae. A characteristic feature is often a prominent horn on the dorsal side of the eighth abdominal segment.
 4 With only thoracic legs.....**Tenebrionidae (*Coleoptera*)**
 Distinct head capsule and well-developed thoracic legs. Possess a well-developed, prognathous (mouthparts directed forward) head capsule with distinct antennae and mouthparts, including mandibles for chewing.
Yellow mealworm (*Tenebrio molitor*) Lesser mealworm (*Alphitobius diaperinus*)
 5 Apodous (legless)..... (**Diptera**)
 Presence of mouthhook and D-shaped posterior spiracles with three sinuous slits ***Musca domestica* (Muscidae)**
 The last abdominal segment or anal segment with a rounded shape ***Hermetia illucens* (Stratiomyidae)**

References:
 https://www.eurl.craw.eu/publications/micrograph-collection/animal-structures/
 Regulation (EU) No 2017/893 amending Annexes I and IV to Regulation (EC) No 999/2001 (see notably Annex IV, section F) and Annexes X, XIV and XV to Regulation (EU) No 142/2011 (see notably Annex X, chapter II section 1)
 Regulation (EU) 2021/1925 of 5 November 2021 amending certain Annexes to Regulation (EU) No 142/2011 as regards the requirements for placing on the market of certain insect products and the adaptation of a containment method. Commission Implementing Regulation (EU) 2022/893 of 7 June 2022 amending Annex VI to Regulation (EC) No 152/2009 as regards the methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed



Conclusions

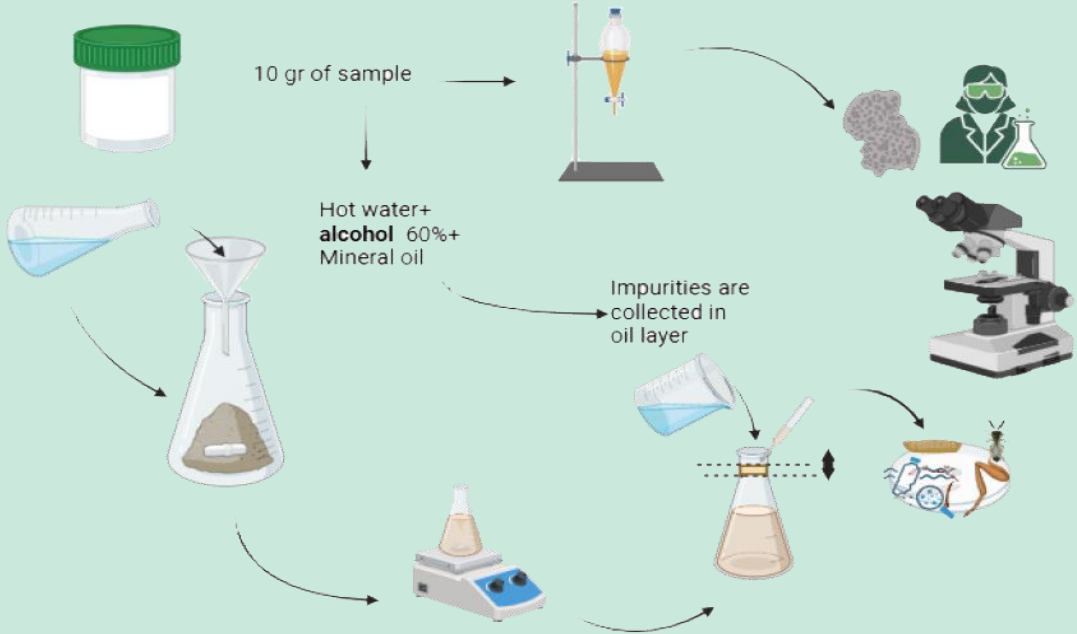
- * Light Filth test might be adopted, after method validation, to replace petroleum ether use a solvent with non-neglectable effect on the environment.
 * It is also advisable to update continuously the reference collection with all important species subject of regulation especially particles from eight authorized insect species. This might help in a future key of identification.
Petroleum ether is classified as toxic to aquatic organisms and likely to cause long term effects in the environment.



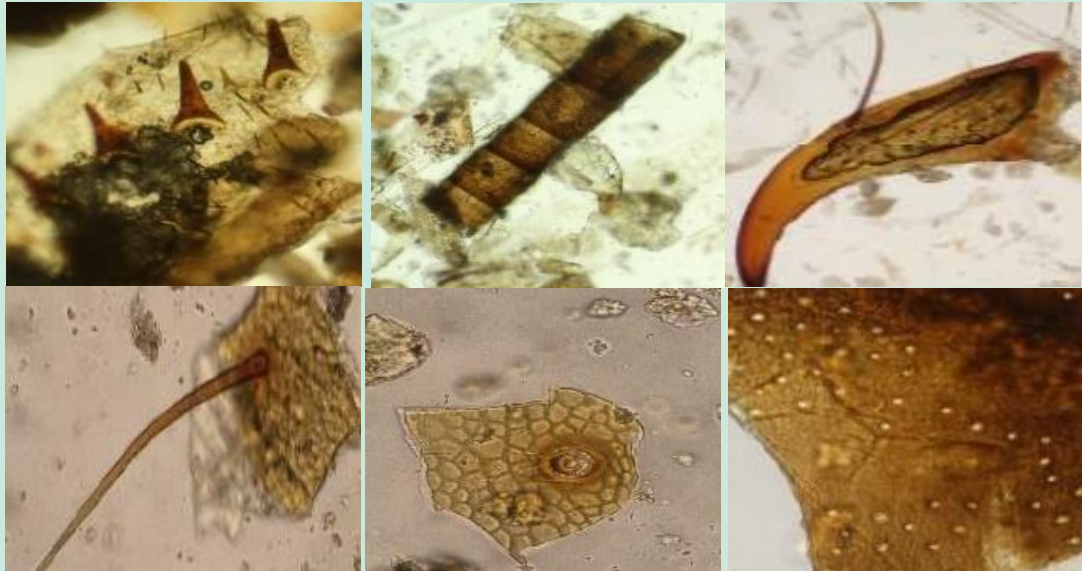
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Light filth test to extract insect
 and other animal particles

The extraction method of insect particles was detailed in the Regulation (EU) 2022/893 of 7 June 2022 amending Annex VI to Regulation (EC) No 152/2009 as regards the methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed listed in the regulation. The extraction should be carried out using a mixture of 30% Petroleum Ether (PE) and 70% Tetrachloroethylene (TCE).
 The light filth test (fig.1) include:
 Digestion step: Digest the product into small particles (Hot water, Alcohol)
 By adding hot water, alcohol 60% and mineral oil, filth particles composed mainly in insect fragments, hair, plastic fragments, mites ... are captured in the neck of the flask. The oil and interface layers are cleanly separated from the aqueous phase and can be removed with Pasteur pipette.
 The final step of most flotation methods is to transfer the oil and extracted filth to a fast draining filter paper for microscopic examination.
 It is not possible to check the presence of muscles with light filth test as the material will be dissolved in water but this issue can be solved by the examination of the raw material of the sample.
 In conclusion, a first step using Tetrachloroethylene will be necessary to extract mineral /heavy material, where bones and other heavy particles should be. The remaining superficial layer will be left to dry and then will be later used to extract insects by light filth test. A further raw examination should be added to detect the presence of other particles such as blood, milk, muscles etc.



Particles from authorized Insects



Other particles / impurities that
 can be found with light filth test

