




Walloon Agricultural Research Centre

Quality and Authentication of Agricultural Products Unit
GEMBLOUX (BELGIUM)
m.lecrenier@cra.wallonie.be

Authentication of insect-based products: Proteomics-MS to highlight farmed animal proteins residues

M.-C. Lecrenier, A. Marien, A. Cordonnier, A. Anselmo, F. Debode and V. Baeten

RAFA 2026 symposium – Prague, Czech Republic – 3 November 2026

Benchmark survey of 2025

insects

MDPI

Article

Authentication of Insect-Based Products in Food and Feed: A Benchmark Survey

Aline Marien [1,*], Benjamin Dubois [2], Olivier Fumière [1], Abigaël Anselmo [1], Julien Maljean [1], Clémence Debailleul [1], Jean-François Morin [3] and Frédéric Debode [2]

Published: 17 July 2025 *



119 commercial products

- from industries or purchased online
- from EU or non EU countries
- intended of Food or Feed:

- 28 pure insect meals for food
- 27 multi-ingredient food
- 21 pure insect meals for feed
- 43 multi-ingredient feed

* Marien, A., Dubois, B., Fumière, O., Anselmo, A., Maljean, J., Debailleul, C., Morin, J.-F., & Debode, F. (2025). Authentication of Insect-Based Products in Food and Feed: A Benchmark Survey. *Insects, 16*(7), 729.

Benchmark survey of 2025

insects

MDPI

Article

Authentication of Insect-Based Products in Food and Feed: A Benchmark Survey

Aline Marien [1,*], Benjamin Dubois [2], Olivier Fumière [1], Abigaël Anselmo [1], Julien Maljean [1], Clémence Debailleul [1], Jean-François Morin [3] and Frédéric Debode [2]

Published: 17 July 2025



8 insect species
(all authorised in EU)



Tenebrio molitor
(Coleoptera)



Alphitobius diaperinus
(Coleoptera)



Acheta domesticus
(Orthoptera)



Locusta migratoria
(Orthoptera)



Gryllodes assimilis
(Orthoptera)



Gryllodes sigillatus
(Orthoptera)



Hermetia illucens
(Diptera)



Bombyx mori
(Lepidoptera)

Benchmark survey of 2025

insects

MDPI

Article

Authentication of Insect-Based Products in Food and Feed: A Benchmark Survey

Aline Marien [1,*], Benjamin Dubois [2], Olivier Fumière [1], Abigaël Anselmo [1], Julien Maljean [1], Clémence Debailleul [1], Jean-François Morin [3] and Frédéric Debode [2]

Published: 17 July 2025



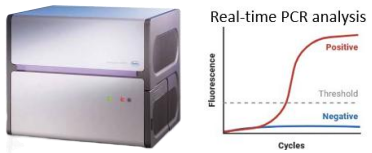
Analytical Methods:

- ➢ two DNA-based methods:
  - Real-time PCR
  - Metabarcoding
- ➢ Proteomics-MS

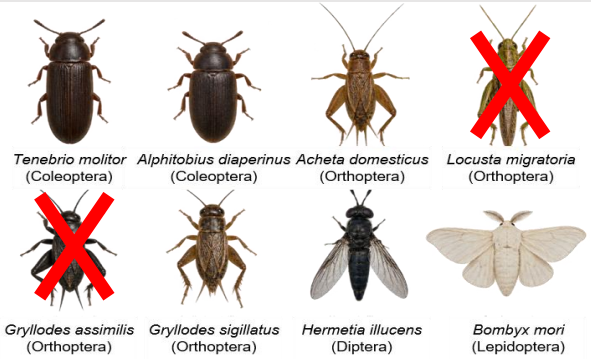
Methods

Extraction

EURL-AP Standard Operating Procedure (SOP)



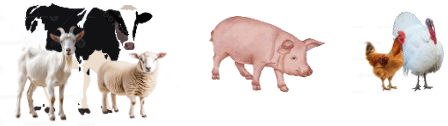
LightCycler 480 thermocycler (Roche Diagnostics Ltd.)



+

EURL Animal Proteins

Terrestrial farm animals



Ruminant | Pig | Poultry (chicken + turkey)



Extraction/digestion/ purification *

UHPLC Xevo TQ-XS (Waters)

EURL Animal Proteins

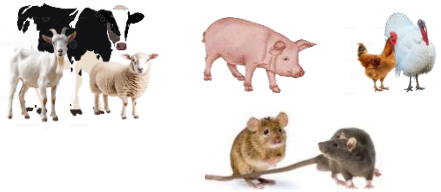


Casein & βlactoglobulin Hemoglobin Collagen | Collagen | Collagen



nanoLC timsTOF HT (Bruker)

- DDA mode
- Database:



protein sequences: 354568

+

- Plants # 294729
- Fish-SP: # 5917
- Insects: # 98639

* Lecrenier, M.-C. (2023). *JAFC, 71*(42), 15774–15784.

Real-time PCR: What did they learn?

50% of the products contained species not listed on the label or lacked the declared species*

Sample	Origin	Expected species	*Tenebrio molitor*	*Hermetia illucens*	*Alphitobius diaperinus*	*Bombyx mori*	*Acheta domesticus*	*Gryllodes sigillatus*
6 (Food)	Denmark	*Tenebrio molitor*	+ (27)	+ (32)	+ (35)	-	-	-
7 (Food)	Denmark	*Tenebrio molitor*	+ (25)	-	+ (36)	-	-	-
15 (Food)	Denmark	*Acheta domesticus*	+ (38)	-	-	-	+ (16)	-
64 (Feed)	Denmark	*Hermetia illucens*	+ (37)	+ (22)	-	-	-	-

** Data extracted from Marien et al. 2025*

Real-time PCR: What did they learn?

50% of the products contained species not listed on the label or lacked the declared species*

Sample	Origin	Expected species	*Tenebrio molitor*	*Hermetia illucens*	*Alphitobius diaperinus*	*Bombyx mori*	*Acheta domesticus*	*Gryllodes sigillatus*
6 (Food)	Denmark	*Tenebrio molitor*	+ (27)	+ (32)	+ (35)	-	-	-
7 (Food)	Denmark	*Tenebrio molitor*	+ (25)	-	+ (36)	-	-	-
15 (Food)	Denmark	*Acheta domesticus*	+ (38)	-	-	-	+ (16)	-
64 (Feed)	Denmark	*Hermetia illucens*	+ (37)	+ (22)	-	-	-	-
23 (Food)	Denmark	*Gryllodes sigillatus*	-	-	-	-	+ (22)	-
90 (multi Feed)	France	*Hermetia illucens*	+ (32)	-	-	-	-	-
100 (multi Feed)	Germany	*Tenebrio molitor*	-	+ (26)	-	-	-	-
104 (multi Feed)	France	*Bombyx mori*	+ (33)	+ (23)	-	-	-	-
105 (multi Feed)	Netherlands	*Bombyx mori*	+ (30)	+(36)	-	-	-	-
106 (multi Feed)	Netherlands	« Cricket »	+(31)	-	-	-	-	-
107 (multi Feed)	France	« Cricket »	+(34)	-	-	-	-	-

** Data extracted from Marien et al. 2025*

Real-time PCR: What did they learn?

29 % of the pure insect meals (14/49) contained DNA traces of Terrestrial farm vertebrates *

Sample	Origin	Insect species	Ruminant (cut-off at 36.6 cycles)	Pig (cut-off at 39.2 cycles)	Poultry (cut-off at 37.7 cycles)
11 (Food)	Lithuania	*Acheta domesticus*	**+(35)**	-	-
12 (Food)	Belgium	*Acheta domesticus*	**+(33)**	-	-
13 (Food)	New Zealand	*Acheta domesticus*	**+(32)**	**+(35)**	-
15 (Food)	Denmark	*Acheta domesticus*	**+(36)**	-	-
17 (Food)	United Kingdom	*Acheta domesticus*	**+(29)**	-	-
26 (Food)	USA	« Cricket »	**+(32)**	-	-
27 (Food)	USA	« Cricket »	**+(31)**	-	-
28 (Food)	USA	« Cricket »	**+(31)**	-	-
72 (Petfood)	Canada	« Cricket »	**+(33)**	-	-
61 (Petfood)	Germany	*Hermetia illucens*	**+(34)**	-	-
62 (Feed)	Netherlands	*Hermetia illucens*	**+(32)**	-	**+(25)**
63 (Feed)	Netherlands	*Hermetia illucens*	-	-	**+(19)**
67 (Petfood)	Netherlands	*Tenebrio molitor*	**+(30)**	**+(31)**	**+(35)**
70 (Petfood)	Netherlands	*Bombyx mori*	**+(36)**	-	-

* Data extracted from Marien et al. 2025

DNA source?

29 % of the pure insect meals (14/49) contained DNA traces of Terrestrial farm vertebrates



Sample	Insect species
11 (Food)	*Acheta domesticus*
12 (Food)	*Acheta domesticus*
13 (Food)	*Acheta domesticus*
15 (Food)	*Acheta domesticus*
17 (Food)	*Acheta domesticus*
26 (Food)	« Cricket »
27 (Food)	« Cricket »
28 (Food)	« Cricket »
72 (Feed)	« Cricket »
61 (Feed)	*Hermetia illucens*
62 (Feed)	*Hermetia illucens*
63 (Feed)	*Hermetia illucens*
67 (Feed)	*Tenebrio molitor*
70 (Feed)	*Bombyx mori*

Targeted proteins/peptides

Targeted origin	Targeted by-products	Proteins	Peptides	Parent ion (m/z)	Product ion # 1 (m/z)	Product ion # 2 (m/z)	Product ion # 3 (m/z)
Ruminant	Milk & milk products	Casein	**FFV**APFPEVFGK	692.9	920.5	991.5	676.4
			NAVPITPTLNR	598.3	285.2	456.3	911.5
		β-lactoglobulin	**LSF**NPTQLEEQCHI	858.4	1254.6	928.4	627.8
			VLVLDTDYK	533.3	853.4	754.4	641.3
	Blood meal, haemoglobin powder & PAPs	Haemoglobin α & β-chain	**AAV**TAFWGK	475.8	709.4	608.3	537.3
			VGGHAAEYGAEALER	510.6	617.3	745.4	622.3
			EFTPVLQADFQK	711.9	523.3	1045.6	849.4
			VVAGVANALAHR	393.2	490.3	454.8	681.4
	PAPs, gelatine	Collagen I α-2 chain	**GST**GEIGPAGPpGPpGLR	824.9	1047.6	822.4	879.5
			GPpGESGAAGPTGPIGSR	790.9	841.5	1426.7	912.5
			IGQP[+16]GAVGPAGIR	604.8	910.5	299.2	669.4
Pig	PAPs, gelatine	Collagen I α-2 chain	**GFp**GSpGNVGPAGK	637.3	812.4	372.2	1069.5
			GIpGEFGLpGPAGPR	727.4	667.3	837.5	984.5
Poultry	PAPs	Collagen I α-2 chain	**GNV**GLAGPR	420.7	570.3	513.3	441.2
			GLHGEFGVpGPAGPR	488.6	667.3	797.4	698.3
			GLVGEpGPAGAK	534.8	613.3	799.4	456.2

p indicates the presence of hydroxyproline

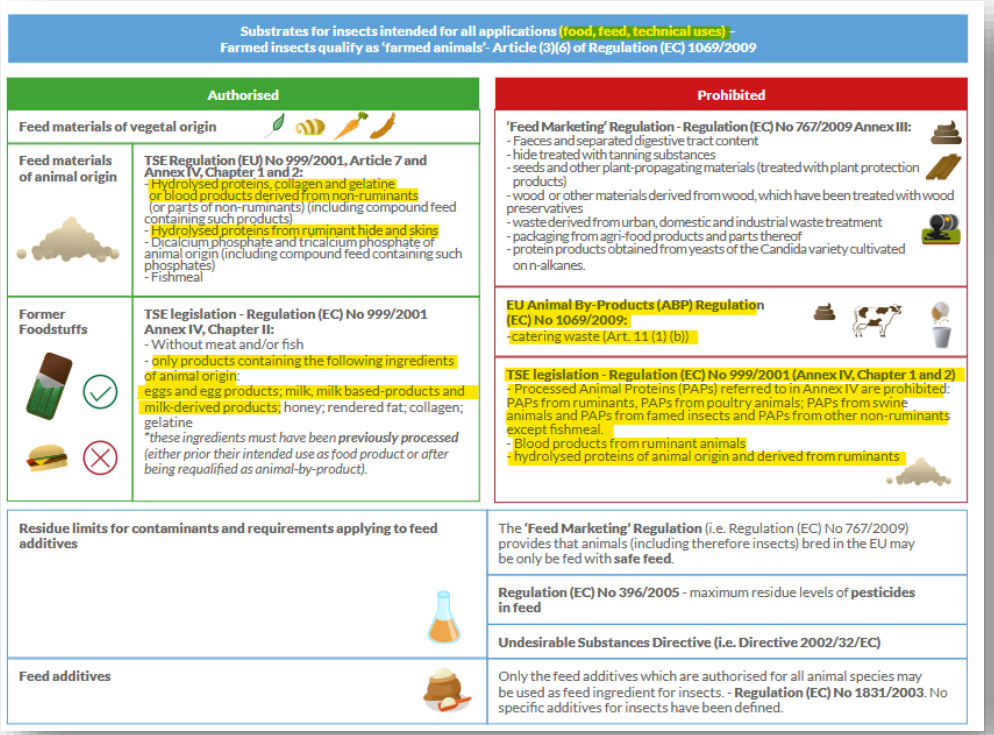
Targeted-MS Results

			PCR			Ruminant											Pig		Poultry		
						Casein ↘		βlg ↗		Hemoglobin				Collagen			Collagen		Collagen		
Sample	Insect	Origin	Rum	Pig	Po	FFV	NAV	LSF	VLV	AAV	VGG	EFT	VVA	GST	GPP	IGQ	GFP	GIP	GNV	GLH	GLV
11 (Food)	*Acheta domesticus*	Lithuania	+(35)	-	-	+	-	+	+	-	-	NI	-	-	-	-	-	-	-	-	-
12 (Food)	*Acheta domesticus*	Belgium	+(33)	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13 (Food)	*Acheta domesticus*	New Zealand	+(32)	+(35)	-	-	-	-	NI	-	NI	-	-	-	-	-	+	+	-	-	-
15 (Food)	*Acheta domesticus*	Denmark	+(36)	-	-	NI	NI	NI	NI	NI	NI	NI	NI	-	-	-	-	-	NI	NI	NI
17 (Food)	*Acheta domesticus*	United Kingdom	+(29)	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
26 (Food)	« Cricket »	USA	+(32)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
27 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+	-	+	+	+	-	-	-
28 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+	+	+	+	+	-	-	-
72 (Petfood)	« Cricket »	Canada	+(33)	-	-	-	NI	NI	NI	NI	NI	NI	NI	-	-	-	-	-	NI	-	-

NI=Not interpretable

Source of proteins ? Whey, Gelatine or PAPs ?

Targeted-MS Results



Substrates for insects intended for all applications (food, feed, technical uses) + Farmed insects qualify as 'farmed animals'- Article (3)(6) of Regulation (EC) 1069/2009

Authorised	Prohibited
Feed materials of vegetal origin	**'Feed Marketing' Regulation - Regulation (EC) No 767/2009 Annex III:** - Faeces and separated digestive tract content - hide treated with tanning substances - seeds and other plant-propagating materials (treated with plant protection products) - wood or other materials derived from wood, which have been treated with wood preservatives - waste derived from urban, domestic and industrial waste treatment - packaging from agri-food products and parts thereof - protein products obtained from yeasts of the Candida variety cultivated on n-alkanes.
Feed materials of animal origin **TSE Regulation (EU) No 999/2001, Article 7 and Annex IV, Chapter 1 and 2:** - Hydrolysed proteins, collagen and gelatine or blood products derived from non-ruminants (or parts of non-ruminants) (including compound feed containing such products) - Hydrolysed proteins from ruminant hide and skins - Dicalcium phosphate and tricalcium phosphate of animal origin (including compound feed containing such phosphates) - Fishmeal	**EU Animal By-Products (ABP) Regulation (EC) No 1069/2009:** -catering waste (Art. 11 (1) (b))
Former Foodstuffs **TSE legislation - Regulation (EC) No 999/2001 Annex IV, Chapter II:** - Without meat and/or fish - only products containing the following ingredients of animal origin: eggs and egg products; milk, milk based-products and milk-derived products; honey; rendered fat; collagen; gelatine **these ingredients must have been **previously processed** (either prior their intended use as food product or after being requalified as animal-by-product).*	**TSE legislation - Regulation (EC) No 999/2001 (Annex IV, Chapter 1 and 2)** - Processed Animal Proteins (PAPs) referred to in Annex IV are prohibited: PAPs from ruminants, PAPs from poultry animals; PAPs from swine animals and PAPs from famed insects and PAPs from other non-ruminants except fishmeal. - Blood products from ruminant animals - hydrolysed proteins of animal origin and derived from ruminants
Residue limits for contaminants and requirements applying to feed additives	The **'Feed Marketing' Regulation** (i.e. Regulation (EC) No 767/2009) provides that animals (including therefore insects) bred in the EU may be only be fed with **safe feed**. **Regulation (EC) No 396/2005** - maximum residue levels of **pesticides in feed** **Undesirable Substances Directive (i.e. Directive 2002/32/EC)**
Feed additives	Only the feed additives which are authorised for all animal species may be used as feed ingredient for insects. - **Regulation (EC) No 1831/2003**. No specific additives for insects have been defined.

IPIFF Guide on Good Hygiene Practices for European Union (EU) producers of insects as food and feed- updated February 2024

Insects reared within the European Union fall within the category of 'farmed animals' as defined in the EU Animal By-Products' (ABP) legislation (i.e. Article 3(6)[16] of Regulation (EC) No 1069/2009). Consequently, these may only be fed with feed materials which are eligible for the above category of animals. These '**restrictions' apply, no matter the destination of the insect derived products**, including if those are intended e.g. as feed for pet food, fur animal or for technical uses (e.g. biofuel production, cosmetic, biochemistry).

COMMISSION IMPLEMENTING REGULATION (EU) 2022/188

of 10 February 2022

authorising the placing on the market of frozen, dried and powder forms of *Acheta domesticus* as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470

(10) The Authority in its opinion also considered that the consumption of frozen, dried and powder forms of *Acheta domesticus* may cause allergic reactions in persons that are allergic to crustaceans, molluscs and mites. Furthermore, the Authority noted that additional allergens may end up in the novel food, if these allergens are present in the substrate fed to insects. Therefore, it is appropriate that frozen, dried and powder forms of *Acheta domesticus* made available to the consumer as such and foods containing them are appropriately labelled following the requirements in accordance with Article 9 of Regulation (EU) 2015/2283.

Targeted-MS Results

PLoS One. 2020 Jan 24;15(1):e0227400. doi: 10.1371/journal.pone.0227400

Self-selection of food ingredients and agricultural by-products by the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae): A holistic approach to develop optimized diets

Juan A Morales-Ramos [1,*], M Guadalupe Rojas [1], Aaron T Dossey [2], Mark Berhow [3]

Editor: Nicoletta Righini[4]

Author information · Article notes · Copyright and License information

[1]USDA-ARS National Biological Control Laboratory, Stoneville, MS, United States of America

[2]All Things Bugs LLC

[3]USDA-ARS National

[4]Universidad Nacion

MEXICO

REVIEW article

Front. Sustain. Food Syst., 31 January 2022

Sec. Nutrition and Sustainable Diets

Volume 5 - 2021 | https://doi.org/10.3389/fsufs.2021.810421

A Literature Review of the Use of Weeds and Agricultural and Food Industry By-Products to Feed Farmed Crickets (Insecta; Orthoptera; Gryllidae)

Camille Kuo · Brian L. Fisher *

Department of Entomology, California Academy of Sciences, San Francisco, CA, United States



INGREDIENTS

Grain Products, Plant Protein Products, Processed Grain Byproducts, Animal Protein Products, Forage Products, Cane Molasses, Calcium Carbonate, Dicalcium Phosphate, Salt, Dl-Methionine, Vitamin A Acetate, Vitamin D3 Supplement, Vitamin E Sup… Riboflavin Supplement, Niacin Supplement, Calcium Pant… Sodium Bisulfate Complex, Folic Acid, Pyridoxine Hydroch… Selenite, Biotin, Manganese Sulfate, Zinc Sulfate, Ferrous … Cobalt Sulfate



GUARANTEED ANALYSIS · INGREDIENTS · FEEDING DIRECTIONS

Ground Corn, Wheat Middlings, Dehulled Soybean Meal, Ground Soybean Hulls, Porcine Meat and Bone Meal, Ground Wheat, Fish Meal, Porcine Animal Fat Preserved with BHA and Citric Acid, Cane Molasses, Ground Oats, Wheat Germ, Brewers Dried Yeast, Dehydrated Alfalfa Meal, Corn Protein Meal, Dried Plain Beet Pulp, Soybean Oil, Calcium Carbonate, Salt, Dried Whey, Fish Oil, Dicalcium Phosphate, Sugar, DL-Methionine, Cholecalciferol (Vitamin …e, Zinc Oxide, Thiamine Mononitrate, Ferrous Carbonate, Preserved with Mixed …tract, Menadione Sodium Bisulfite Complex (Vitamin K), Calcium Pantothenate, Vitamin …icotinic Acid (Niacin), Riboflavin-5-Phosphate, Zinc Sulfate, Calcium Iodate, Cobalt



CRICKET & WORM FEED

Cricket and Worm Feed can be used both as a bedding and food source.

We recommend you put a small slice of potato with the feed to give the worms needed moisture.

GUARANTEED ANALYSIS:

Crude Protein, Min.	20.00%
Crude Fat, Min.	3.00%
Crude Fiber, Max.	7.00%

INGREDIENTS:

Wheat Middlings, Soybean Meal, Poultry By Product Meal, Fish Meal, Dehydrated Alfalfa Meal, Dried Whey Product, Animal Fat Stabilized with BHA (a Preservative), Salt, Ground Limestone, Monocalcium Phosphate, Dicalcium Phosphate, Vitamin B-12 Supplement, Vitamin A Supplement, Vitamin D-3 Supplement, Choline Chloride, D-Pantothenic Acid, Riboflavin Supplement, Ethoxyquin (a Preservative), Manganous Oxide, Magnesium Oxide, Copper Sulfate, Cobalt Carbonate, Ferrous Sulfate, Zinc Oxide and Calcium Iodate.



Animal meal





INGREDIENTS

Wheat Middlings, Dehulled Soybean Meal, Cane Molasses, Porcine Animal Fat Preserved with BHA, Ground Corn, Fish Meal, Ground Soybean Hulls, Ground Oats, Dehydrated Alfalfa Meal, Ground Wheat, Porcine Meat Meal, Dried Beet Pulp, Wheat Germ, Dicalcium Phosphate, Corn Gluten Meal, Salt, Calcium Carbonate, Soybean Oil, Magnesium Oxide, Cholecalciferol, Choline Chloride, Thiamine Mononitrate, Folic Acid, Riboflavin, Nicotinic Acid, Menadione Sodium Bisulfite Complex, Pyridoxine Hydrochloride, DL-Methionine, Calcium Pantothenate, Vitamin B-12 Supplement, Vitamin A Acetate, DL-Alpha Tocopheryl Acetate, Manganous Oxide, Ferrous Carbonate, Copper Sulfate, Zinc Sulfate, Calcium Iodate, Cobalt Carbonate, Sodium Selenite.

Discovery-MS Results

			PCR			Ruminant											Pig		Poultry		
						Casein ↘		βlg ↗		Hemoglobin				Collagen			Collagen		Collagen		
Sample	Insect	Origin	Rum	Pig	Po	FFV	NAV	LSF	VLV	AAV	VGG	EFT	VVA	GST	GPP	IGQ	GFP	GIP	GNV	GLH	GLV
11 (Food)	*Acheta domesticus*	Lithuania	+(35)	-	-	+	-	+	+	-	-	NI	-	-	-	-	-	-	-	-	-
12 (Food)	*Acheta domesticus*	Belgium	+(33)	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13 (Food)	*Acheta domesticus*	New Zealand	+(32)	+(35)	-	-	-	-	NI	-	NI	-	-	-	-	-	-	-	-	-	-
15 (Food)	*Acheta domesticus*	Denmark	+(36)	-	-	NI	NI	NI	NI	NI	NI	NI	NI	-	-	-	-	-	NI	NI	NI
17 (Food)	*Acheta domesticus*	United Kingdom	+(29)	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
26 (Food)	« Cricket »	USA	+(32)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
27 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+	-	+	+	+	-	-	-
28 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+	+	+	+	+	-	-	-
72 (Petfood)	« Cricket »	Canada	+(33)	-	-		NI	NI	NI	NI	NI	NI	NI	-	-	-	-	-	NI	-	-

Beta-lactoglobulin OS=Bos taurus OX=9913 GN=PAEP PE=3 SV=1

Database:	InSpectrum-Rumi
Score:	58
Monoisotopic mass (M_r):	34527
Calculated pI:	6.02

Sequence similarity is available as an NCBI BLAST search of A0AAF6Z0B1 against nr.

Search parameters

MS data file:	D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA·
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications:	Carbamidomethyl (C)
Variable modifications:	Acetyl (N-term), Oxidation (M)

Protein sequence coverage: 13%

Matched peptides shown in ***bold red***.

```
  1 MVPKAEATLQ DTPAPSAGSD LSLSKRLTPE VFLALAASLD PEPGHPLRPH
 51 FWGVPGTVQA QRGPSCLASN GRRPPIVLVE EATPGPKDRP GGIRGTAWLG
101 ARPGLAGWPS SCIRPRAHCL SPPLPAELRS VTPAAAMKCL LLALALTCGA
151 QALIVTQTMK GLDIQKVAGT WYSLAMAASD ISLLDAQSAP LRVYVEELKP
201 TPEGDLEILL QKWENGECAQ KKIIAEKTKI PAVFKIDALN ENKVLVLDTD
251 YKKYLLFCME NSAEPEQSLA CQCLVRTPEV DDEALEKFDK ALKALPMHIR
301 LSFNPTQLEE QCHI
```

Beta-lactoglobulin OS=Bos taurus OX=9913 GN=PAEP PE=3 SV=1

Database:	InSpectrum-Rumi
Score:	291
Monoisotopic mass (M_r):	34527
Calculated pI:	6.02

Sequence similarity is available as an NCBI BLAST search of A0AAF6Z0B1 against nr.

Search parameters

MS data file:	D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications:	Carbamidomethyl (C)
Variable modifications:	Acetyl (N-term), Oxidation (M)

Protein sequence coverage: 18%

Matched peptides shown in ***bold red***.

```
  1 MVPKAEATLQ DTPAPSAGSD LSLSKRLTPE VFLALAASLD PEPGHPLRPH
 51 FWGVPGTVQA QRGPSCLASN GRRPPIVLVE EATPGPKDRP GGIRGTAWLG
101 ARPGLAGWPS SCIRPRAHCL SPPLPAELRS VTPAAAMKCL LLALALTCGA
151 QALIVTQTMK GLDIQKVAGT WYSLAMAASD ISLLDAQSAP LRVYVEELKP
201 TPEGDLEILL QKWENGECAQ KKIIAEKTKI PAVFKIDALN ENKVLVLDTD
251 YKKYLLFCME NSAEPEQSLA CQCLVRTPEV DDEALEKFDK ALKALPMHIR
301 LSFNPTQLEE QCHI
```

Alpha-lactalbumin OS=Bos taurus OX=9913 GN=LALBA PE=1 SV=1

Database:	InSpectrum-Rumi
Score:	39
Monoisotopic mass (M_r):	19858
Calculated pI:	5.00

Sequence similarity is available as an NCBI BLAST search of A0A452DI34 against nr.

Search parameters

MS data file:	D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA-I
Enzyme:	Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications:	Carbamidomethyl (C)
Variable modifications:	Acetyl (N-term), Oxidation (M)

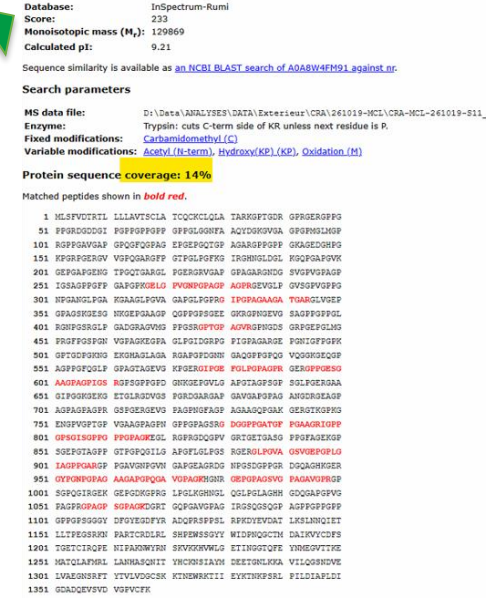
Protein sequence coverage: 14%

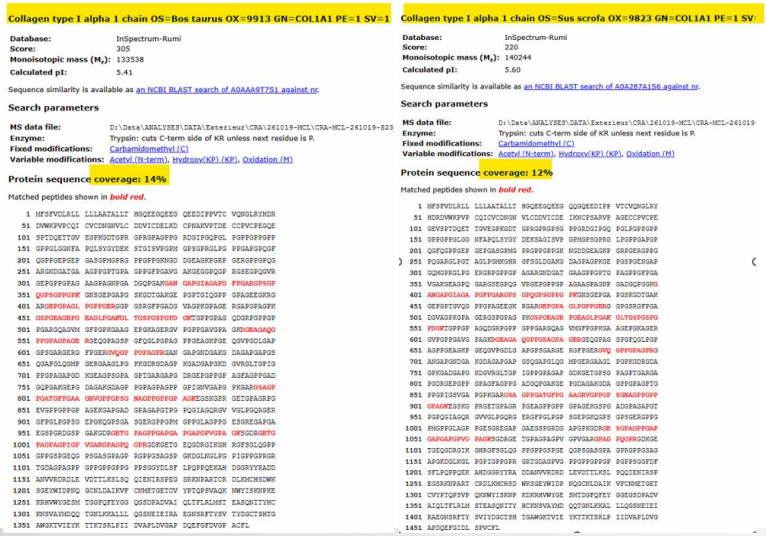
Matched peptides shown in ***bold red***.

```
  1 MPGPLRLFPQ IKGGEQCGDP ISESWGVTKM MSFVSLLLVG ILFHATQAEQ
 51 LTKCEVFREL KDLKGYGGVS LPEWVCTTFH TSGYDTQAIV QNNDSTEYGL
101 FQINNKIWCK DDQNPHSSNI CNISCDKFLD DDLTDDIMCV KKILDKVGIN
151 YWLAHKALCS EKLDQWLCEK L
```

Discovery-MS Results

			PCR			Ruminant										Pig		Poultry		
						Casein ↘		βlg ↗		Hemoglobin				Collagen				Collagen		Collagen		
Sample	Insect	Origin	Rum	Pig	Po	FFV	NAV	LSF	VLV	AAV	VGG	EFT	VVA	GST	GPP	IGQ		GFP	GIP	GNV	GLH	GLV
11 (Food)	*Acheta domesticus*	Lithuania	+(35)	-	-	+	-	+	+	-	-	NI	-	-	-	-		-	-	-	-	
12 (Food)	*Acheta domesticus*	Belgium	+(33)	-	-	+	+	+	+	-	-	-	-	-	-	-		-	-	-		-
13 (Food)	*Acheta domesticus*	New Zealand	+(32)	+(35)	-	-	-	-	NI	-	NI	-	-	-	-	-		+	+		-	-
15 (Food)	*Acheta domesticus*	Denmark	+(36)	-	-	NI	NI	NI	NI	NI	NI	NI	NI	-	-	-		-	-	NI	NI	NI
17 (Food)	*Acheta domesticus*	United Kingdom	+(29)	-	-	+	-	-	-	-	-	-	-	+	+	+		+	+	-	-	-
26 (Food)	« Cricket »	USA	+(32)	-	-	-	-	-	-	-	-	-	-	+	+	+		+	+	-	-	-
27 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+		+		+	+	-	-	-
28 (Food)	« Cricket »	USA	+(31)	-	-	-	-	-	NI	-	NI	-	-	+	+	+		+	+	-	-	-
72 (Petfood)	« Cricket »	Canada	+(33)	-	-	-	NI	NI	NI	NI	NI	NI	NI		-	-		-	-	NI	-	-





Targeted-MS Results

Sample	Insect	Origin	PCR			Ruminant											Pig		Poultry		
						Casein		βlg		Hemoglobin				Collagen			Collagen		Collagen		
			Rum	Pig	Po	FFV	NAV	LSF	VLV	AAV	VGG	EFT	VVA	GST	GPP	IGQ	GFP	GIP	GNV	GLH	GLV
61 (Petfood)		Germany	+(34)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62 (Feed)	*HI*	Netherlands	+(32)	-	+(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
63 (Feed)		Netherlands	-	-	+(19)	-	-	-	-	-	NI	-	NI	-	-	-	-	-	+	+	+
67 (Petfood)	*TM*	Netherlands	+(30)	+(31)	+(35)	-	-	-	-	NI	-	-	NI	+	+	+	+	+	+	+	+
70 (Petfood)	BM	Netherlands	+(36)	-	-	-	-	-	NI	NI	NI	NI	NI	-	-	-	-	-	-	-	-

NI=Not interpretable

HI = Hermetia illucens;
TM = Tenebrio molitor;
BM = Bombyx mori

Source of proteins ? Gelatine or PAPs ?

Discovery-MS Results

Sample	Insect	Origin	PCR: Rum	PCR: Pig	PCR: Po	Ruminant: Casein FFV	Casein NAV	βlg LSF	βlg VLV	Hemoglobin AAV	Hemoglobin VGG	Hemoglobin EFT	Hemoglobin VVA	Collagen GST	Collagen GPP	Collagen IGQ	Pig: Collagen GFP	Collagen GIP	Poultry: Collagen GNV	Collagen GLH	Collagen GLV
61 (Petfood)		Germany	+(34)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62 (Feed)	*HM*	Netherlands	+(32)	-	+(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
63 (Feed)		Netherlands	-	-	+(19)	-	-	-	-	-	NI	-	NI	-	-	-	-	-	+	+	
67 (Petfood)	*TM*	Netherlands	+(30)	+(31)	+(35)	-	-	-	-	NI	-	-	NI	+	+	+	+	+	+		
70 (Petfood)	BM	Netherlands	+(36)	-	-	-	-	-	NI	NI	NI	NI	NI	-	-	-	-	-	-	-	-

Myosin light chain, phosphorylatable, fast skeletal muscle OS=Gallus gallus OX=9031 GN=MYLPF PE=4 SV=1

Database: InSpectrum-Rumi
Score: 1363
Monoisotopic mass (M_r): 18923
Calculated pI: 4.77

Sequence similarity is available as an NCBI BLAST search of A0A8V0WZV4 against nr.

Search parameters

MS data file: D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA-MCL-261019-S35_Slot1-11_1_7030.d\CRA-MCL-261019-
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Acetyl (N-term), Oxidation (M)

Protein sequence coverage: 70%

Matched peptides shown in ***bold red***.

```
  1 IAPKKAKRRA AEGSSNVFSM FDQTQIQEFK EAFTVIDQNR DGIIDKDDLR
 51 ETFAAMGRLN VKNEELDAMI KEASGPINFT VFLTMFGEKL KGADPEDVIM
101 GAFKVLDPDG KGSIKKSFLE ELLTTQCDRF TPEEIKNMWA AFPPDVAGNV
151 DYKNICYVIT HGEDKEGE
```

Animal meal



Tropomyosin alpha-1 chain OS=Gallus gallus OX=9031 GN=TPM1 PE=1 SV=2

Database: InSpectrum-Rumi
Score: 546
Monoisotopic mass (M_r): 32803
Calculated pI: 4.70

Sequence similarity is available as an NCBI BLAST search of P04268 against nr.

Search parameters

MS data file: D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA-MCL-261019-S3
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Acetyl (N-term), Oxidation (M)

Protein sequence coverage: 40%

Matched peptides shown in ***bold red***.

```
  1 MDAIKKKMQM LKLDKENALD RAEQAEADKK AAEERSKQLE DELVALQKKL
 51 KGTEDELDKY SESLKDAQEK LELADKKATD AESEVASLNR RIQLVEEELD
101 RAQERLATAL QKLEEAEKAA DESERGMKVI ENRAQKDEEK MEIQEIQLKE
151 AKHIAEEADR KYEEVARKLV IIEGDLERAE ERAELSESKC AELEEELKTV
201 TNNLKSLEAQ AEKYSQKEDK YEEEIKVLTD KLKEAETRAE FAERSVTKLE
251 KSIDDLEDEL YAQKLKYKAI SEELDHALND MTSI
```

Hemoglobin subunit alpha-A OS=Gallus gallus OX=9031 GN=HBAA PE=1 SV=2

Database: InSpectrum-Rumi
Score: 1252
Monoisotopic mass (M_r): 15533
Calculated pI: 8.54

Sequence similarity is available as an NCBI BLAST search of P01994 against nr.

Search parameters

MS data file: D:\Data\ANALYSES\DATA\Exterieur\CRA\261019-MCL\CRA-MCL-261019-S3
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Acetyl (N-term), Oxidation (M)

Protein sequence coverage: 57%

Matched peptides shown in ***bold red***.

```
  1 MVLSAADKNN VKGIFTKIAG HAEEYGAETL ERMFTTYPPT KTYFPHFDLS
 51 HGSAQIKGHG KKVVAALIEA ANHIDDIAGT LSKLSDLHAH KLRVDPVNFK
101 LLGQCFLVVV AIHHPAALTP EVHASLDKFL CAVGTVLTAK YR
```

TAKE-HOME MESSAGE – Benchmark survey



50% of the products contained insect species not supposed to be there.

→ Not problematic if it remains minimal and involves species that are also authorized in the EU.

→ Problematic if substantial presence of another species, or if contained insects not allowed in the EU.



29 % of the pure insect meals (14/49) contained traces of Ruminant, pig and/or poultry DNA

→ Proteomics-MS approach can help to distinguish presence of authorized / non authorized products.

→ Likely originating from the substrates on which the insects were reared.

→ Non authorized products:

- → major risk for products coming from non-EU country; particularly in “Cricket” products,
- → 2 cases in *Hermetia illucens* and 1 in *Tenebrio molitor* from EU.

→ Residues of authorized products => Allergenic risk.

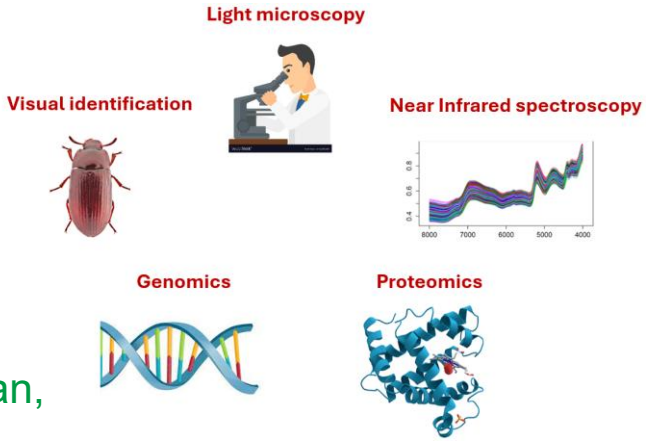


Thank you for your attention

People involved in the task force Authentication of insects species in food and feed at CRA-W

Abigaël Anselmo, Alexandra Cordonnier, Clémence Debailleul, Frédéric Debode, Delphine Delhotte, Benjamin Dubois, Olivier Fumière, Marie-Caroline Lecrenier, Julien Maljean, Aline Marien, Lisa Plasman, Pascal Veys

Marc Dieu



Questions? More infos? Contact me!