

Characterization and detection of frass from *Tenebrio molitor* larvae by Near Infrared Spectroscopy techniques

A. Anselmo, A. Pissard, P. Veys, D. Michez & V. Baeten

Introduction

Overview of the European legislation

- Insects authorised for feed since 2017 (8 species)

→ Most used : Black soldier fly larvae (*H. illucens*)
and yellow mealworm (*T. molitor*)



From left to right and top to bottom : *A. domesticus*, *G. assimilis*, *G. sigillatus*, *T. molitor*, *A. diaperinus*, *M. domestica*, *B. mori*, *H. illucens*.

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Overview of the European legislation

- Insects authorised for feed since 2017 (8 species)
 - Most used : Black soldier fly larvae (*H. illucens*) and yellow mealworm (*T. molitor*)
- **Frass** : mixture of insect excrement, food substrate, insect parts and dead eggs (EU 2021)



**Prohibited in
insect meals !**

T. molitor larvae meal



Frass from *T. molitor* larvae



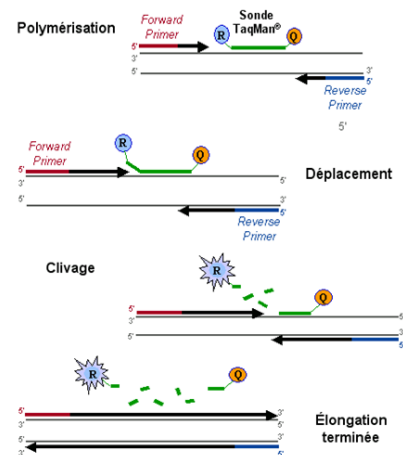
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Official methods :



Light microscopy (2003)



RT-PCR (2013)

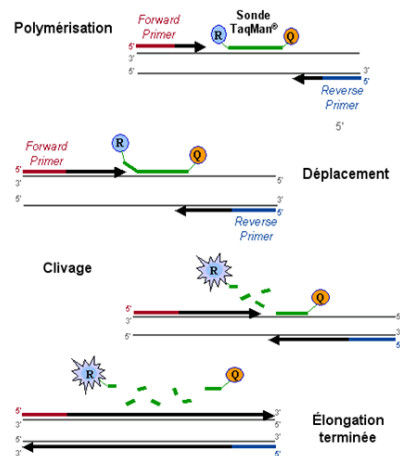
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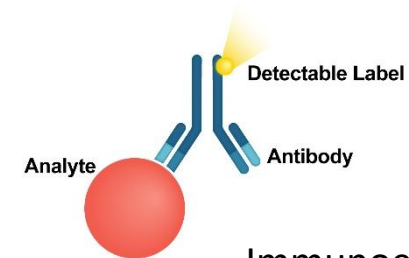
Non-official methods :



Mass spectrometry



Near Infrared Spectroscopy techniques



Immunoassay

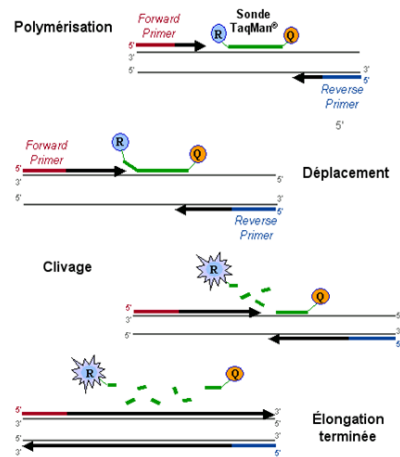
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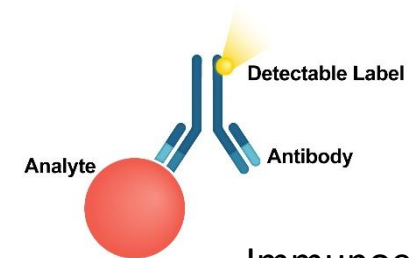
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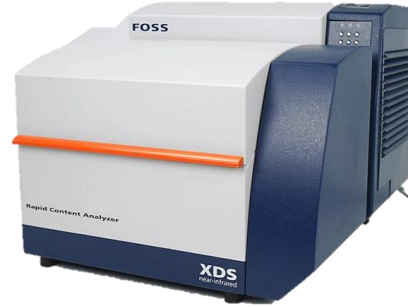
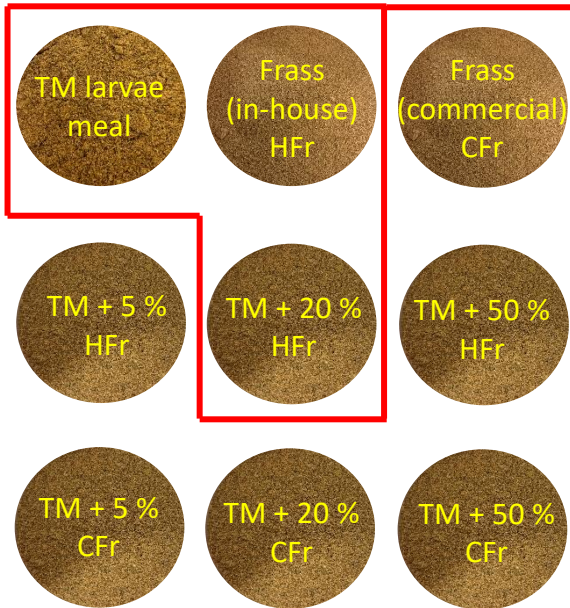
Near Infrared Spectroscopy techniques



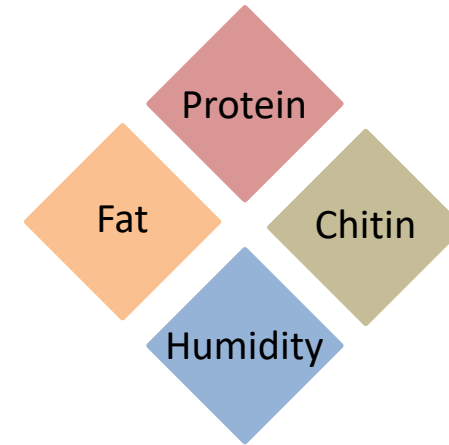
Immunoassay

What is the goal ?

Samples

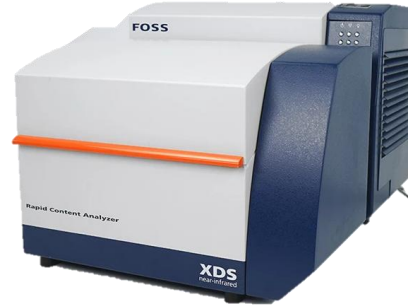
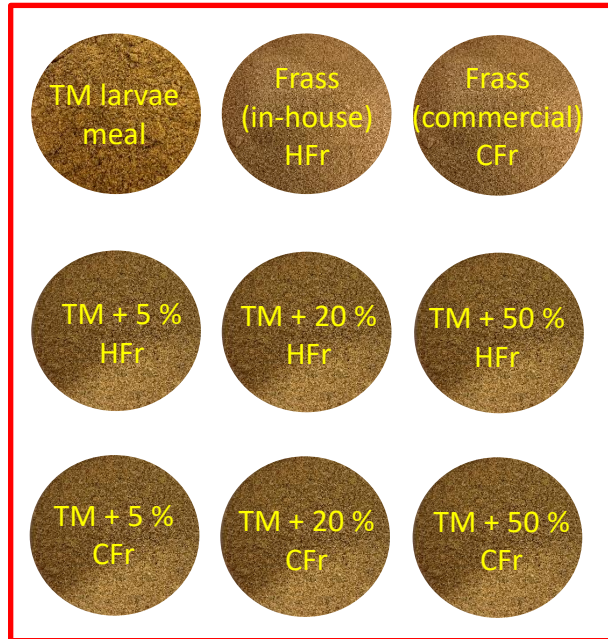


Near Infrared Spectroscopy (NIRS)
[400 – 2500 nm]

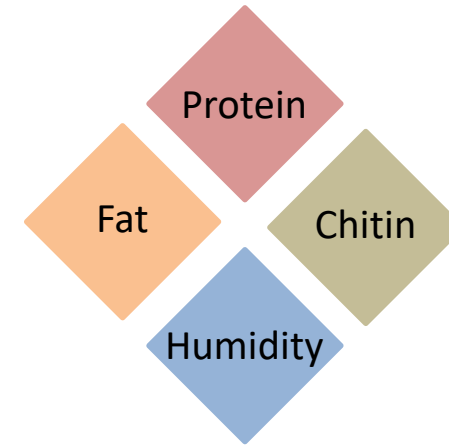


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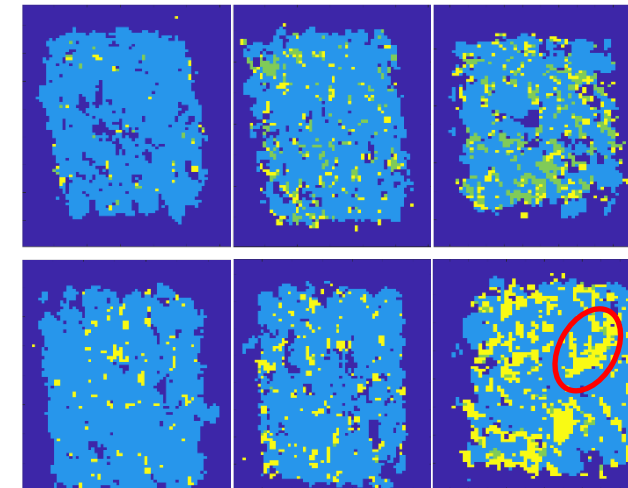
Samples



Near Infrared Spectroscopy (NIRS)
[400 – 2500 nm]



Near Infrared Microscope (NIRM)
[1111 – 2500 nm]



Presence of
frass ?

Quality control of insect meal

Composition of insect meal and frass

- Preprocessing : Standard Normal Variate (SNV) – Detrend and 1st derivative
- Partial Least Squares Regression (PLS-R) models

Sample	Humidity (%)	Protein (%)	Fat (%)	Chitin (%)	GH
TM larvae meal	6.57	63.29	7.52	9.73	0,70
Frass	10.58	15.91	- 0.63	5.16	4,61
TM larvae meal + 20% of frass	7.42	55.35	6.03	8.77	1,01
Standard Deviation	2.11	25.38	4.34	2.41	2,17
Total values	3	3	3	3	3

Humidity, protein, fat and chitin content (expressed in %) for the T. molitor larvae meal, the frass produced in-house and the T. molitor larvae meal adulterated with 20 % of frass.
GH : virtual mathematical distance used to assess the degree of similarity between the NIR spectrum of a sample and the average spectrum of the corresponding database.



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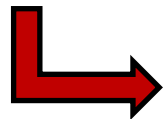
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Sample	Humidity (%)	Protein (%)	Fat (%)	Chitin (%)
Frass	16.46	16.41	0.59	16.43

Results of chemical analyses on the frass produced in-house

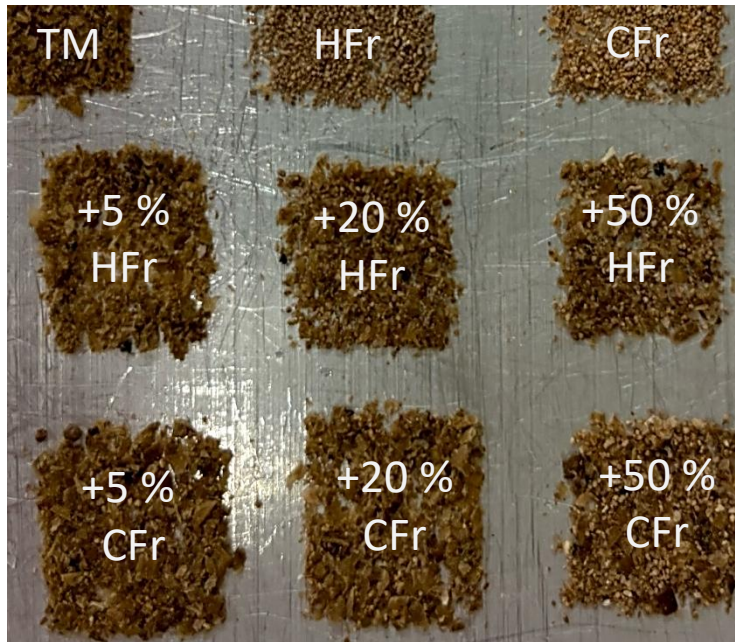
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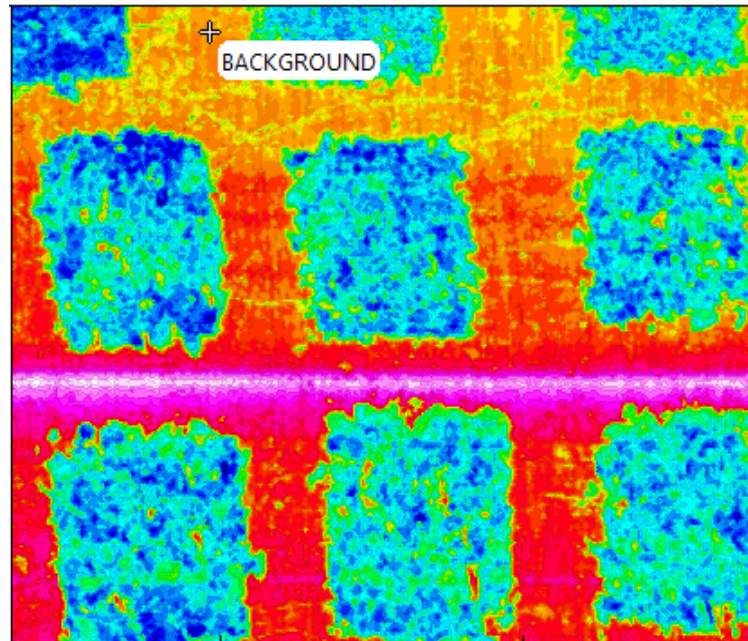
Need to improve the NIR database for frass

Quality control of insect meal

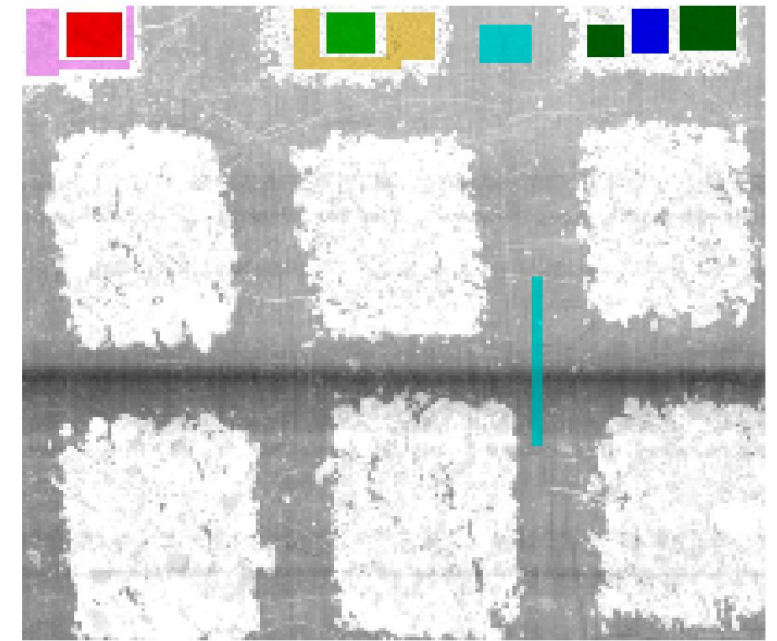
Detection of frass in insect meal



Analysed by NIRM

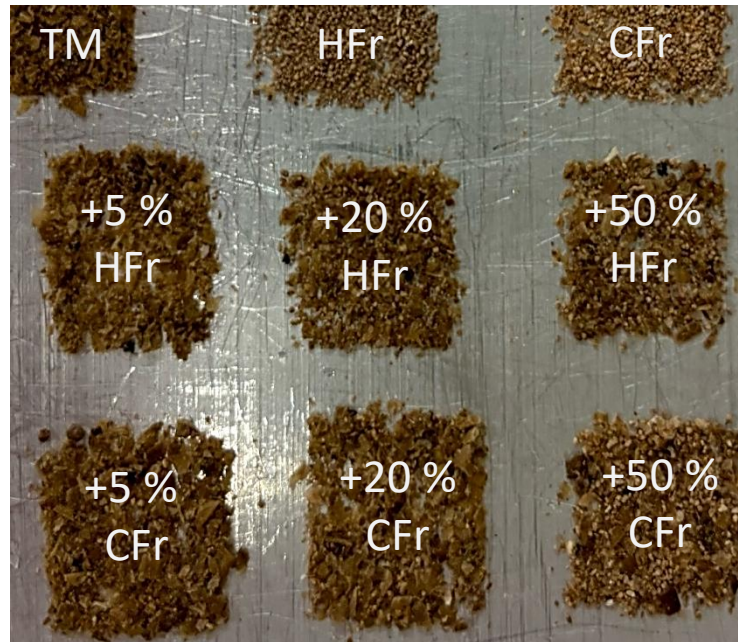


Construction of spectral libraries

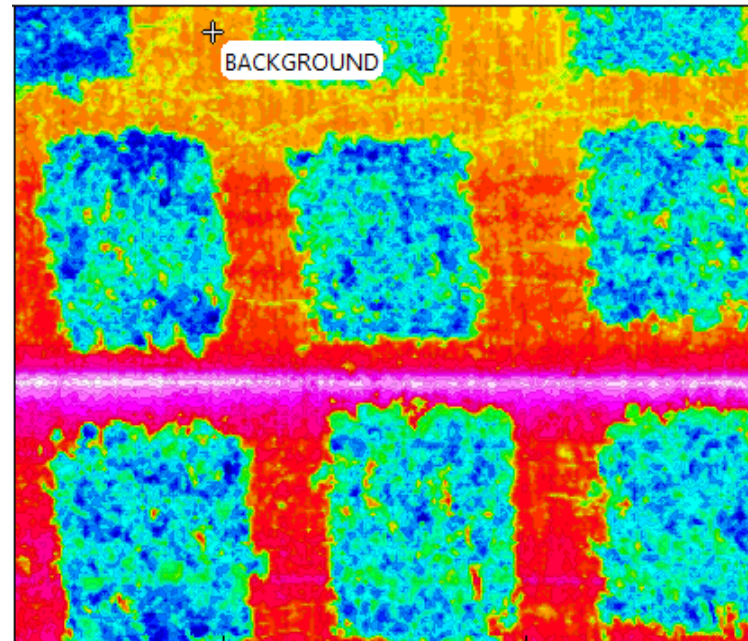


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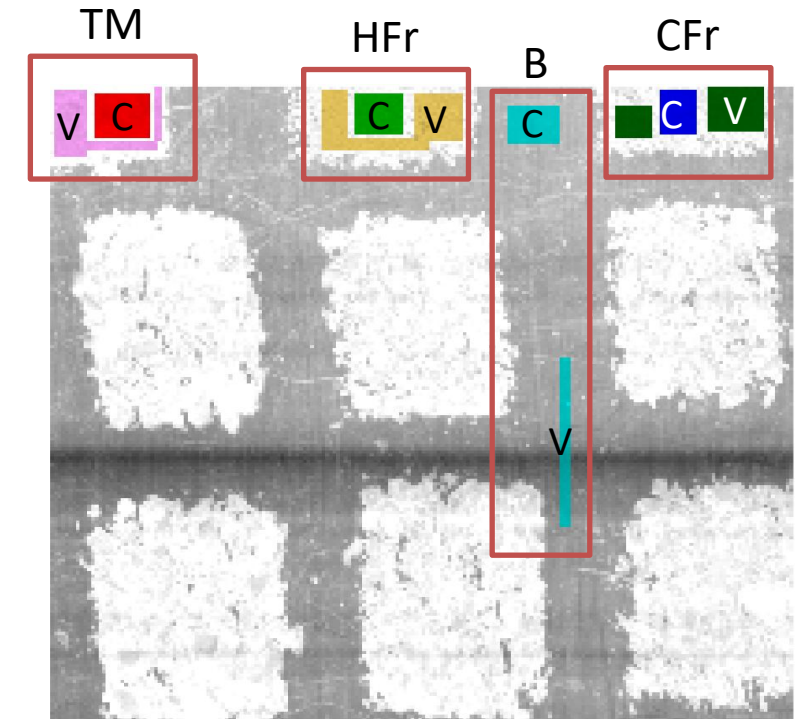
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- 2 Partial Least Squares – Discriminant Analyses (PLS-DA) models : one to remove background, and one to detect HFr and CFr in insect meal
- Preprocessing : SNV and Savitzky-Golay (1st derivative, 2nd order and window of 15)

	Calibration		Cross-validation		Validation	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
TM larvae meal	1	0.994	1	0.990	1	0.996
HFr	1	0.954	1	0.951	0.844	0.970
CFr	0.950	0.966	0.929	0.960	0.972	0.884

Results of the 3-class PLS-DA model for calibration, cross-validation and validation of T. molitor (TM) larvae meal, frass produced in-house (HFr) and commercial frass (Number of latent variables : 4)

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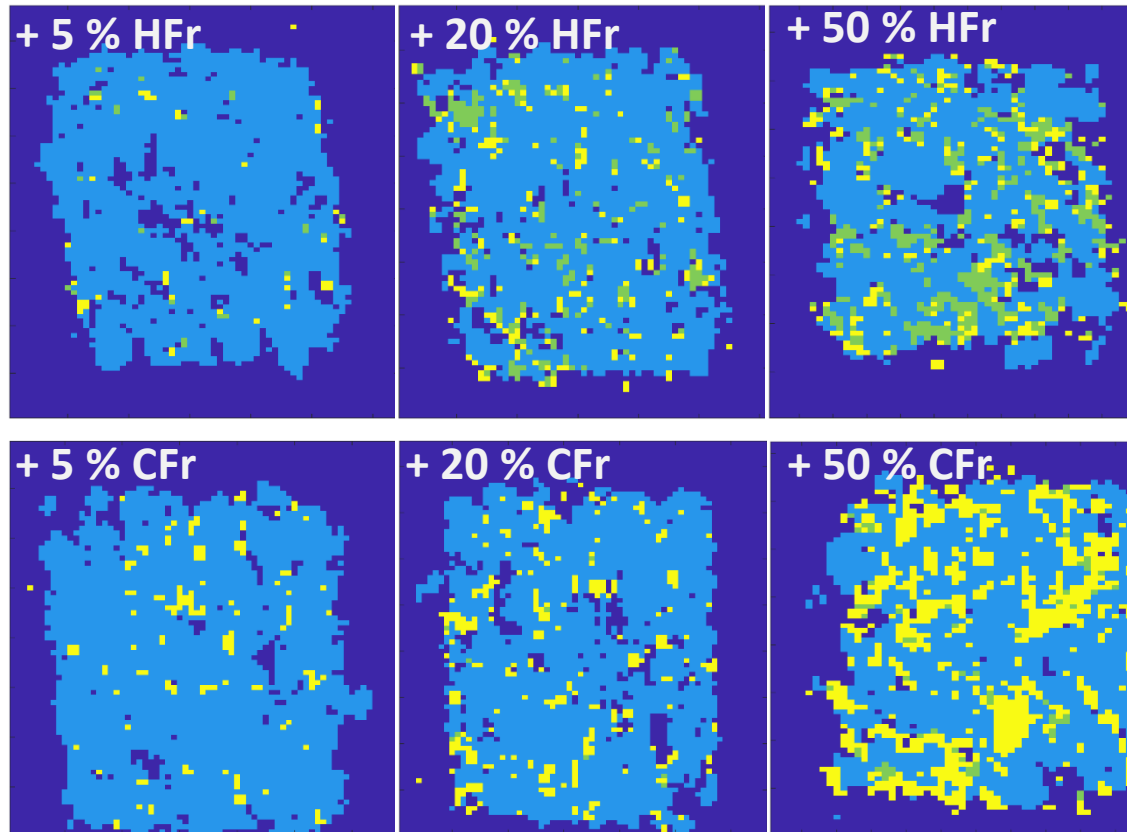
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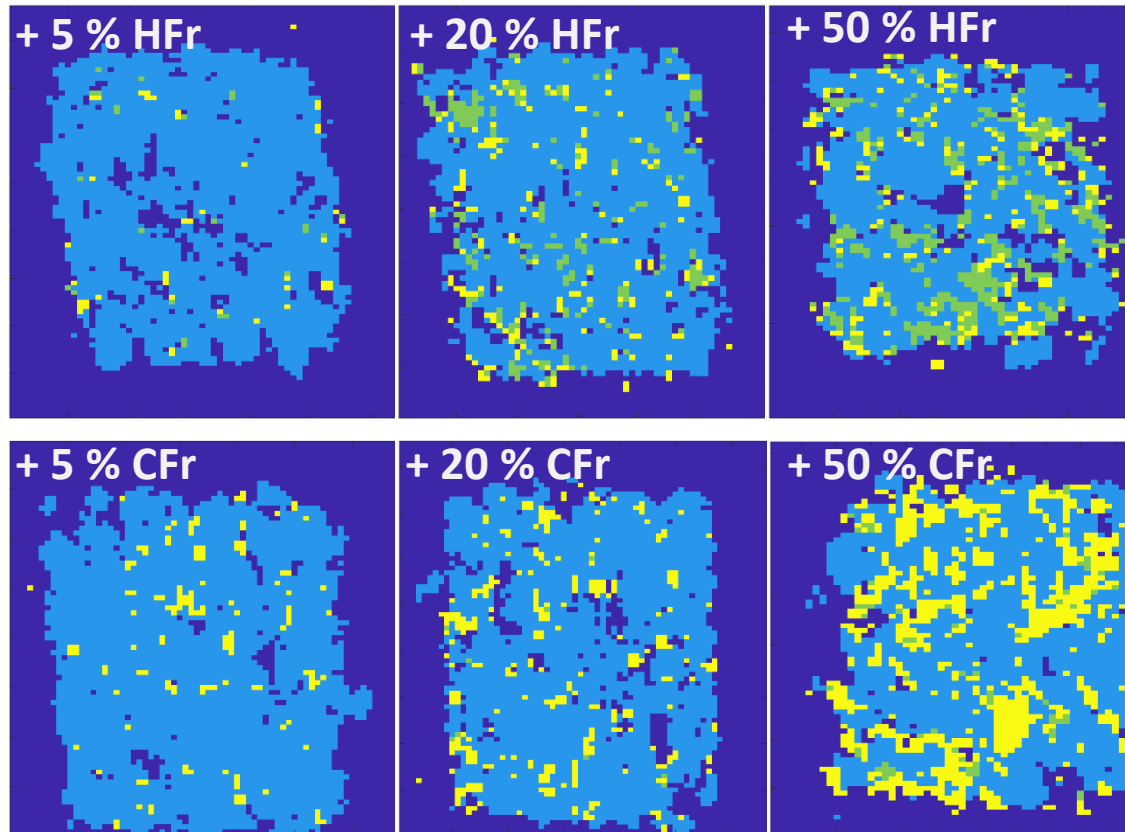


PLS-DA image processing of the six samples of *T. molitor* larvae meal adulterated with 5 % of HFr or CFr (**left**), 20 % of HFr or CFr (**middle**) and 50 % of HFr or CFr (**right**).
Dark blue : background; **Light blue** : *T. molitor* larvae meal; **Green** : HFr; **Yellow** : CFr



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Actual class	Predicted as			Total number of spectra
	<i>T. molitor</i> larvae meal	HFr	CFr	
<i>T. molitor</i> larvae meal + 5 % HFr	2523	24	44	2591
<i>T. molitor</i> larvae meal + 20 % HFr	2141	201	162	2504
<i>T. molitor</i> larvae meal + 50 % HFr	1624	381	230	2235
<i>T. molitor</i> larvae meal + 5 % CFr	3019	0	141	3160
<i>T. molitor</i> larvae meal + 20 % CFr	2760	9	307	3076
<i>T. molitor</i> larvae meal + 50 % CFr	1849	68	805	2722

Confusion matrix showing the number of spectra classified in each class (i.e. *T. molitor* larvae meal, HFr and CFr) in samples of *T. molitor* larvae meal adulterated with different concentrations (5 %, 20 % or 50 %) of HFr and CFr

Conclusion

- A **first step** in **insect meal quality control**
- **NIR spectroscopy** to be further **developed** : construction of a more robust database with additional frass samples to improve prediction models
- **NIR Microscopy** : a **useful** tool for **detecting frass**
- **Further research** : see what can be done with **official methods** (light microscopy and RT-PCR)



***Thanks for your
attention !***