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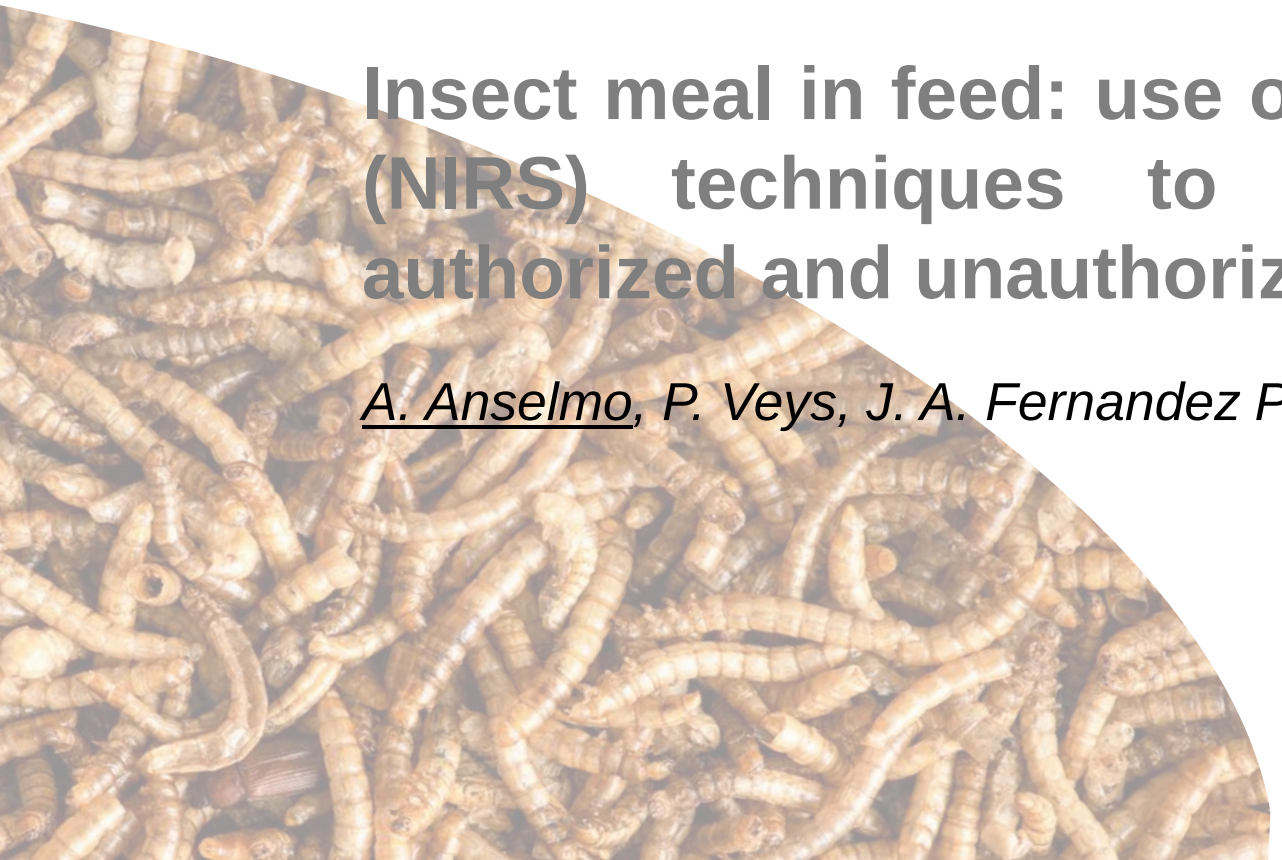

Insect meal in feed: use of Near-Infrared Spectroscopy (NIRS) techniques to support the detection of authorized and unauthorized insect species

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8th International Feed Conference
Present and Future Challenges
FEED 2023
Milan, October 9-10

Introduction

Legislative context

1986 : First case of bovine spongiform encephalopathy (BSE) in UK

→ *Ban of the use of processed animal proteins (PAPs) in 2001 and development of official methods for safety control : Light microscopy and PCR*

2013 : First relaxation of the legislation concerning the feed ban

→ *Use of fishmeal, non-ruminant blood products, collagen, gelatin, hydrolyzed proteins, di- and tri- calcium phosphate and egg and dairy products*

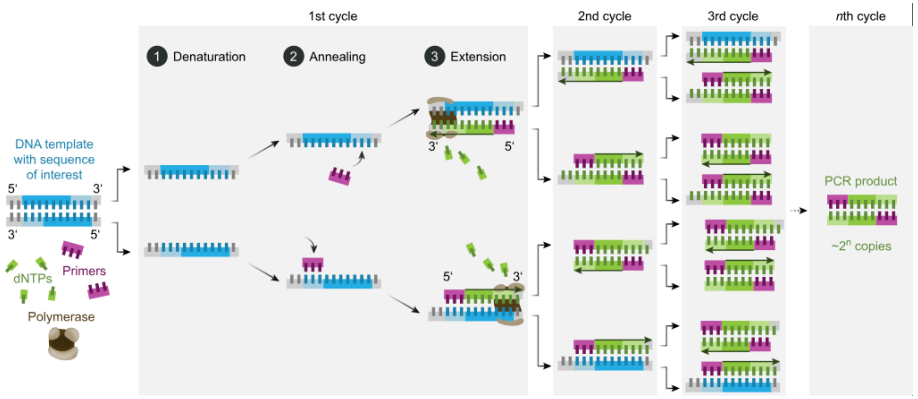
2021 : Re-authorization of PAPs and authorization of the use of insect proteins in non-ruminants

Introduction

Legislative context

- Detection in feed intended for ruminants
- Characterization and identification

Ensure the application of the legislation



Do not provide any insight into the chemical composition & requires sample pre-treatment

FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2023.2211677

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Challenges related to the application of analytical methods to control insect meals in the context of European legislation

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ABSTRACT

Since their approval for use in aquaculture in 2017, processed insect proteins have been extensively studied for their nutritional quality in animal feed. This new type of meal is highly promising but requires, as for other products used in animal feed, strict sanitary control in accordance with European legislation. Within this legal framework, light microscopy and PCR remain the official methods but have some analytical limitations that other methods could overcome. This paper aims to provide an overview of the European legislation concerning use of processed insect proteins, but also to highlight the advantages and disadvantages of the official methods for their analysis. It also points out other analytical methods, which have already proved their worth for the analysis of processed animal proteins, which could be used as complementary methods.

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KEYWORDS
Insects; legislation; feed; processed animal proteins; light microscopy; PCR; metagenomics; vibrational spectroscopy; mass spectrometry; combination of methods

Spectroscopy techniques

Development of non-invasive methods to overcome the limitations of official methods

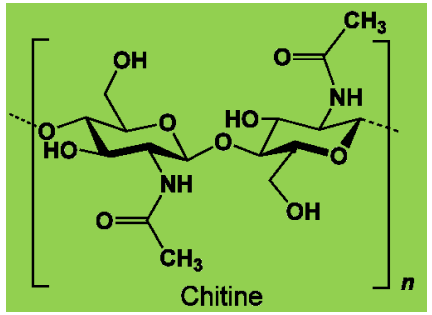
Do not provide any insight into the chemical composition & requires sample pre-treatment

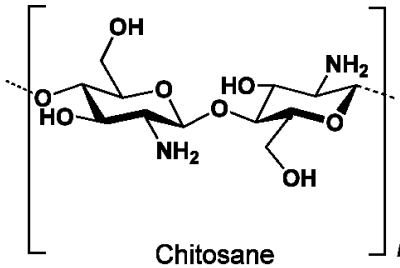
Analysis of insect meals by NIRS

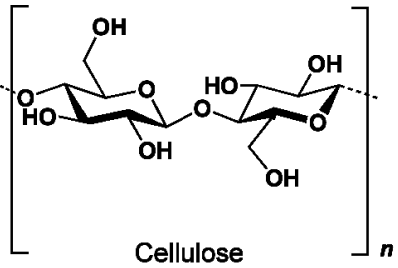
Device, pre-treatments & targeting

- XDS Spectrophotometer (FOSS), range from 408 nm to 2498 nm
- Pre-treatment : SNV detrend on raw spectra
- Reference values (wet chemistry analyses) used to calibrate models using spectral range from 1100 nm to 2498 nm

Determination of moisture, protein, fat, cellulose and chitin (by ADF-ADL*) content

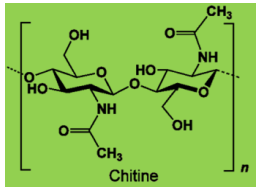






*Hahn, T.; Roth, A.; Febel, E.; Fijalkowska, M.; Schmitt, E.; Arsiwalla, T.; Zibek, S. New Methods for High-Accuracy Insect Chitin Measurement. Journal of the Science of Food and Agriculture **2018**, 98 (13), 5069–5073. https://doi.org/10.1002/jsfa.9044

Analysis of insect meals by NIRS



- Data split into a calibration set of 50 samples
- Samples chosen to contain the maximum of variability
- Partial Least Squares (PLS) regression techniques used to build calibration models

RSQ superior to 0.80 and relatively low SECV for all parameters

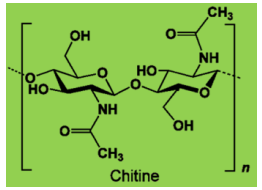
	HUM (% as_is)	MPT (% as_is)	FAT (% as_is)	CELL (% as_is)	ADF-ADL (% as_is)
n	47	46	28	46	48
MEAN	4.38	62.71	13.14	9.38	9.00
SD	1.66	9.48	7.19	3.15	3.41
SEC	0.28	1.57	0.80	0.88	1.48
RSQ	0.97	0.97	0.99	0.92	0.81
SECV	0.48	3.53	1.89	1.34	1.89
1-VR	0.92	0.88	0.93	0.82	0.72

n: number of samples in the calibration; **SD**: standard deviation; **SEC**: standard error of calibration; **RSQ**: coefficient of determination of the calibration; **SECV**: standard error of cross-validation; **1-VR**: coefficient of determination of cross-validation

Analysis of insect meals by NIRS

Test of different approaches

- **Specific** : based on insect samples only. Better specificity, but smaller size and diversification



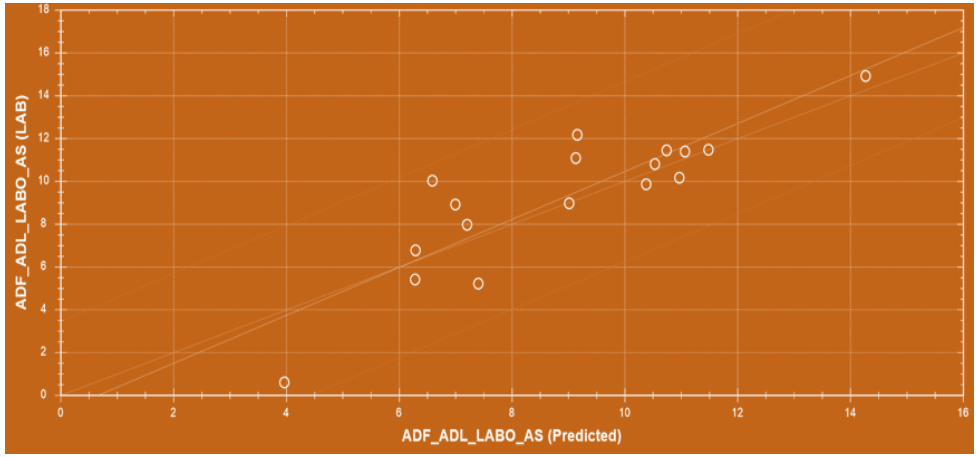
- **Global** : mix of insect samples and other feed samples. Larger size, but less specific

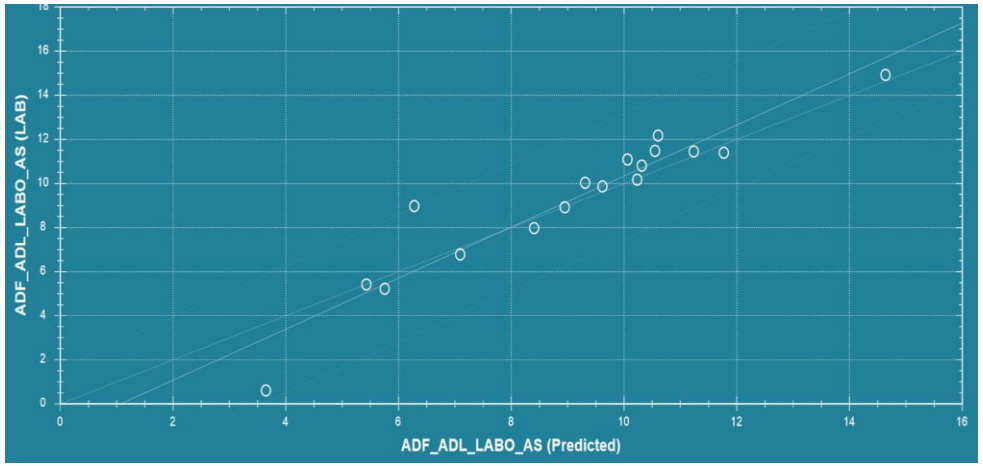
LOCAL mode

	n Val	SEP	RSQ
HUM (% as_is)	17	0.27	0.97
MPT (% as_is)	17	2.76	0.89
FAT (% as_is)	17	1.16	0.97
CELL (% as_is)	17	1.06	0.90
ADF-ADL (% as_is)	17	1.68	0.75

n Val : number of samples in the validation; **SEP** : Standard Error of Prediction; **RSQ** : coefficient of determination.

	n Val	SEP	RSQ
HUM (% as_is)	17	0.32	0.96
MPT (% as_is)	17	2.21	0.91
FAT (% as_is)	17	2.97	0.93
CELL (% as_is)	17	1.22	0.87
ADF-ADL (% as_is)	17	1.15	0.89

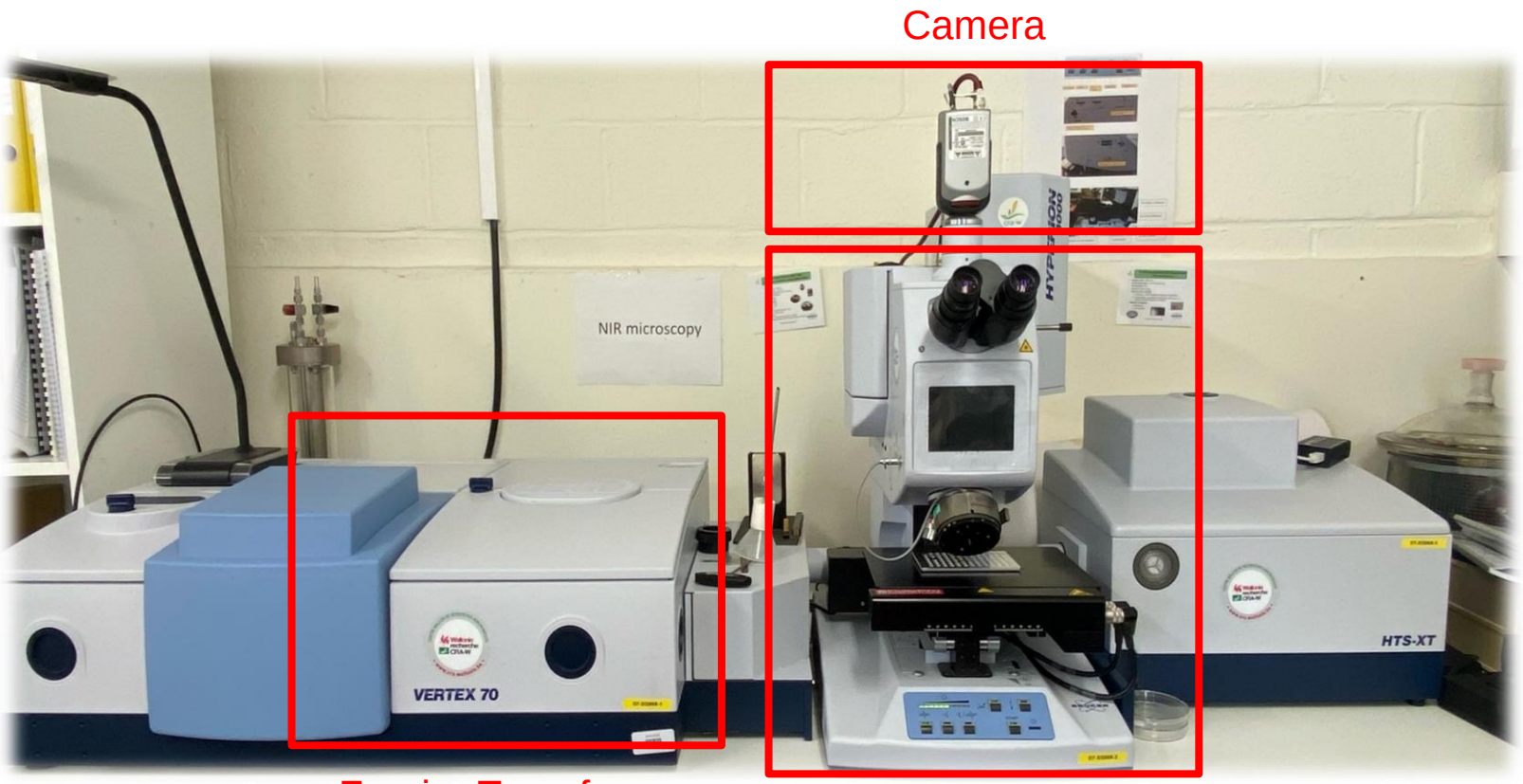




Detection of insect meal at 1 % in a feed by NIRM

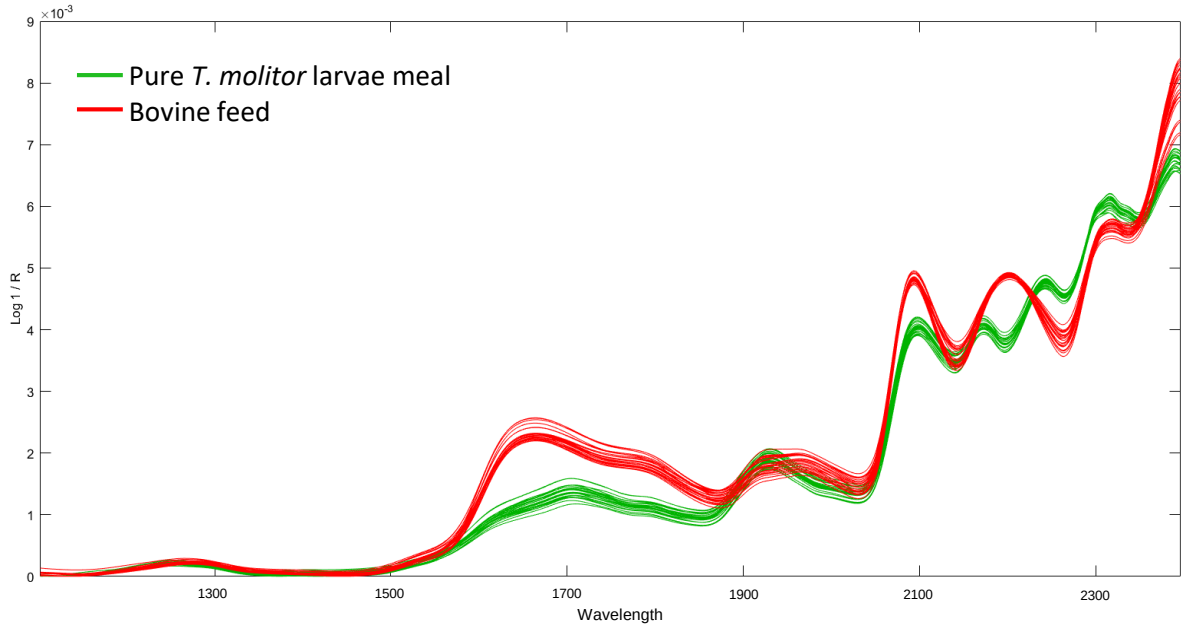
Device, samples & parameters

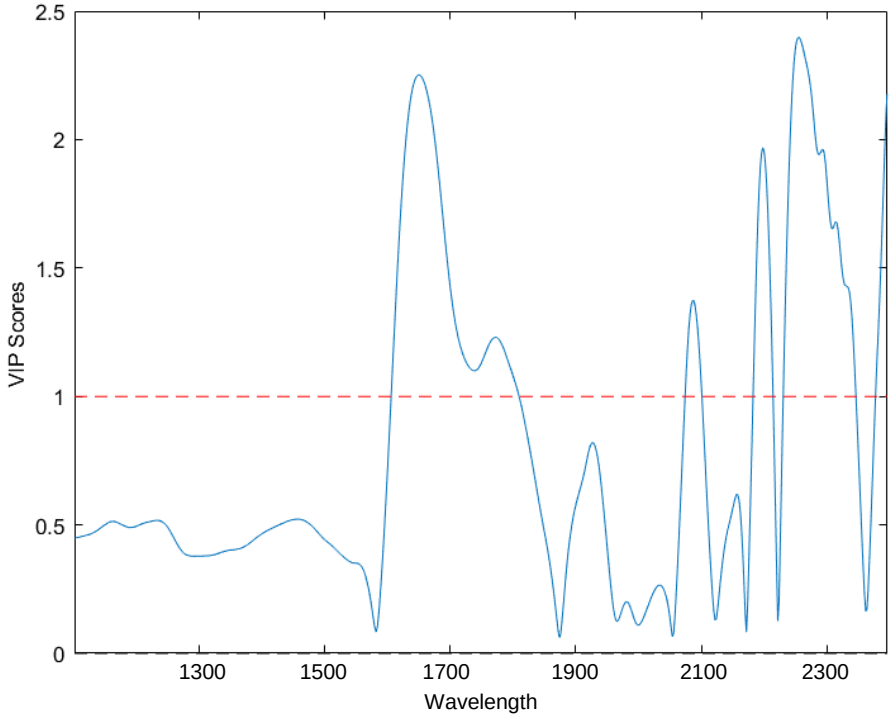
- Use of a Bovine feed and 2 insect meals (*H. illucens* and *T. molitor*)
- 3175 spectra : 100 used for the calibration set (pure insect meal and pure bovine feed) and 3075 for the validation set (bovine feed + 1 % of insect meal)
- Pre-treatment : 1st derivative (Savitzky-Golay) and SNV
- Used of Partial Least Squares – Discriminant Analysis (PLS-DA)



Detection of insect meal at 1 % in a feed by NIRM

Results





Confusion matrix

Predicted as Samples (Latent variables)	Bovine feed	*H. illucens* larvae	*T. molitor* larvae	Accuracy	Sensitivity	Specificity
Bovine feed + 1 % of *H. illucens (5)*	3064	11		0.997	0.996	0.996
Bovine feed + 1 % of *T. molitor (6)*	3067		8	0.998	0.997	0.997

Conclusion

Analysis by NIRS :

- Assessing the chemical composition of insect-based samples is promising.
- NIRS coupled with PLS regression or LOCAL method : good tool for predicting the humidity, protein, fat, cellulose and chitin content with a relatively low error of prediction.
- Both approaches (Specific and Global) seem to perform similarly to predict the chemical composition.

Perspectives : insect database should be provided with new samples to increase its variability and improve the predictive performances.

Analysis by NIRM :

- NIRM coupled with PLS-DA method can detect insect meal at 1 % in a feed.
- No sample pre-treatment was required to perform the analysis.

Perspectives : try to detect the presence of insect meals at 0.5 % or even 0.1 % to reach the detection threshold of official methods.

Detection of the presence of FRASS (insect breeding residues), and with simultaneously detection of PAPs

Thanks for your attention !