

# Attenuated-Total-Reflection Fourier-transformed spectroscopy as a rapid tool to reveal the molecular structure of insect powders as ingredients for animal feed



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## Food security

80% of feed protein is imported from outside EU

Almost 30% of fisheries are overexploited

80% of global arable land is dedicated to livestock

GMO-feeds social concern



## Environment

98% of water used in farming is due to irrigation of feed crops

71% of South American rainforest conversion for farming → loss of biodiversity

High GHG emissions linked to fertilization, pesticides and transport

# Protein Feed Source of the future

is Asia Australia Middle East Africa Inequality Cities Global developmen

## Amazon rainforest's final frontier under threat from oil and soya



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## All seafood will run out in 2050, say scientists



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## The bean at the heart of America's trade war

By Reality Check team  
BBC News

2 August 2018



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## Waitrose ends use of GM animal feed on its farms: Critics hail decision as 'beginning of the end' for use of the crops in the UK

• Waitrose meat, milk and eggs will not come from animals on GM feed



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## Monsanto ordered to pay \$289m damages in Roundup cancer trial

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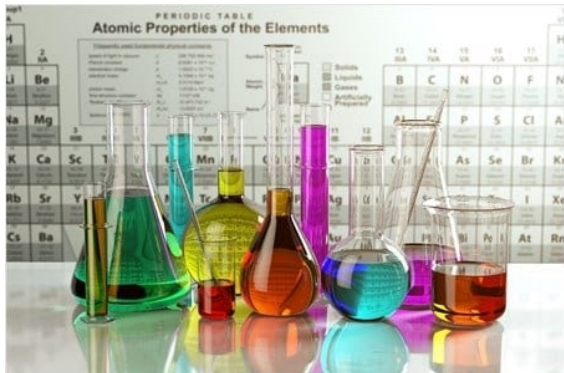


**INSECTS ARE A COMMON  
INGREDIENT IN THE DIET OF  
MONOGASTRIC ANIMALS**



# Traditional 'wet' analytical chemistry

- ✓ Usually looks for a specific known component through homogenisation of the tissue.
- ✓ Information about the spatial origin and distribution of the component of interest is lost.
- ✓ Rely heavily on the use of harsh chemicals altering the native feed structures and possibly generating artifacts.
- ✓ Require reasonable amounts of feed material.
- ✓ Time consuming, expensive and prone to errors within and between laboratories.
- Studying the secondary structure of proteins leads to an understanding of the components that make up a whole protein.
- Often vital to understanding its digestive behaviour, nutritive quality, utilisation and availability in animals.





## Application Potential of ATR-FT/IR Molecular Spectroscopy in Animal Nutrition: Revelation of Protein Molecular Structures of Canola Meal and Presscake, As Affected by Heat-Processing Methods, in Relationship with Their Protein Digestive Behavior and Utilization for Dairy Cattle

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### ANIMAL RESEARCH PAPER

## Ruminal dry matter and nitrogen degradation in relation to condensed tannin and protein molecular structures in sainfoin (*Onobrychis viciifolia*) and lucerne (*Medicago sativa*)

## Magnitude Differences in Bioactive Compounds, Chemical Functional Groups, Fatty Acid Profiles, Nutrient Degradation and Digestion, Molecular Structure, and Metabolic Characteristics of Protein in Newly Developed Yellow-Seeded and Black-Seeded Canola Lines

Katerina Theodoridou,<sup>†,‡</sup> Xuwei Zhang,<sup>†,§</sup> Sally Vail,<sup>#</sup> and Peiqiang Yu<sup>\*,†,§</sup>



Contents lists available at ScienceDirect

## Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: [www.elsevier.com/locate/saa](http://www.elsevier.com/locate/saa)



Explore protein molecular structure in endosperm tissues in newly developed black and yellow type canola seeds by using synchrotron-based Fourier transform infrared microspectroscopy

Katerina Theodoridou<sup>a</sup>, Sally Vail<sup>b</sup>, Peiqiang Yu<sup>a,c,\*</sup>



## FOOD CHEMISTRY

## Synchrotron-Based Microspectroscopic Study on the Effects of Heat Treatments on Cotyledon Tissues in Yellow-Type Canola (*Brassica*) Seeds

Peiqiang Yu,<sup>\*,†,‡</sup> Katerina Theodoridou,<sup>‡</sup> Hangshu Xin,<sup>‡</sup> Pei-Yu Huang,<sup>§</sup> Yao-Chang Lee,<sup>§</sup> and Bayden R. Wood<sup>||</sup>

### RESEARCH

### Open Access



Using vibrational infrared biomolecular spectroscopy to detect heat-induced changes of molecular structure in relation to nutrient availability of prairie whole oat grains on a molecular basis

M. D. Mostafizar Rahman<sup>1,2</sup>, Katerina Theodoridou<sup>1,3</sup> and Peiqiang Yu<sup>1\*</sup>



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# Objectives of the study



Assess the potential of FTIR to reveal the nutrient molecular profile of four insect species (allowed to include in animal feed)

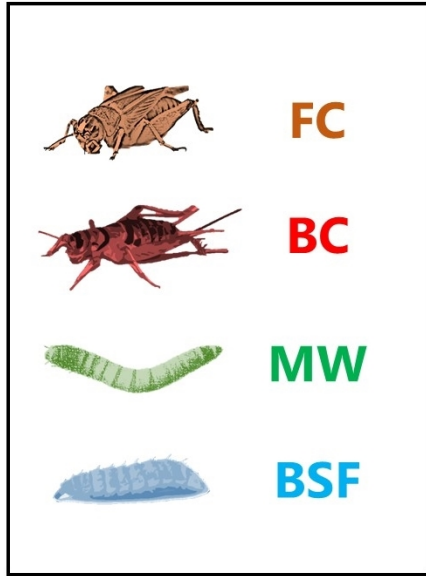


Explore the chemical profile and digestibility of those insect species



Study the relationship between the molecular structure and the nutritive value of those insect species

# Experimental Design



Field Crickets

Banded Crickets

Mealworm

Black Soldier Fly Larvae

3 batches of 4 insect species live

sieved-remove frass and litter

fasted for 12h at room temperature

Washed and freeze-dried

Milled and sieved <1mm

Stored -20°C

Chemical profile

*In vitro* DM Digestibility

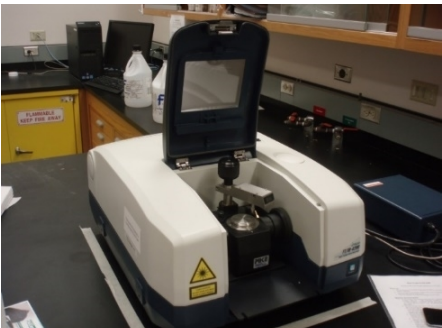
Nutrient molecular structure



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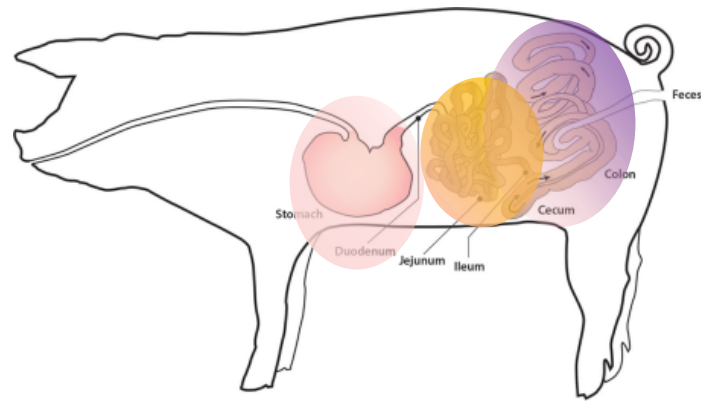
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# In vitro DM digestibility



## Insect Samples

Stomach

••Pepsin: pH 2, 2h, 39°C



Small intestine

••Pancreatin: pH 6.8, 4h, 39°C



Large intestine

••Viscozyme: pH 4.8, 18h, 39°C

*Adapted and modified from Boisen and Fernandez 1997*



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# Statistical Analysis

- One-way ANOVA was performed for the chemical composition molecular structure and digestibility using the statistical package SPSS 25.
- Significance was declared at  $P < 0.10$
- Correlation between the molecular structure profile and wet chemistry analysis in the insect samples were analyzed using Pearson's correlations
- The normality test of the residual data was conducted using the Shapiro-Wilk method

# Chemical profile

No significant differences for CP, chitin, NDF/ADF, between cricket species. MW has a higher CP content compared to the rest species (P < 0.0001).

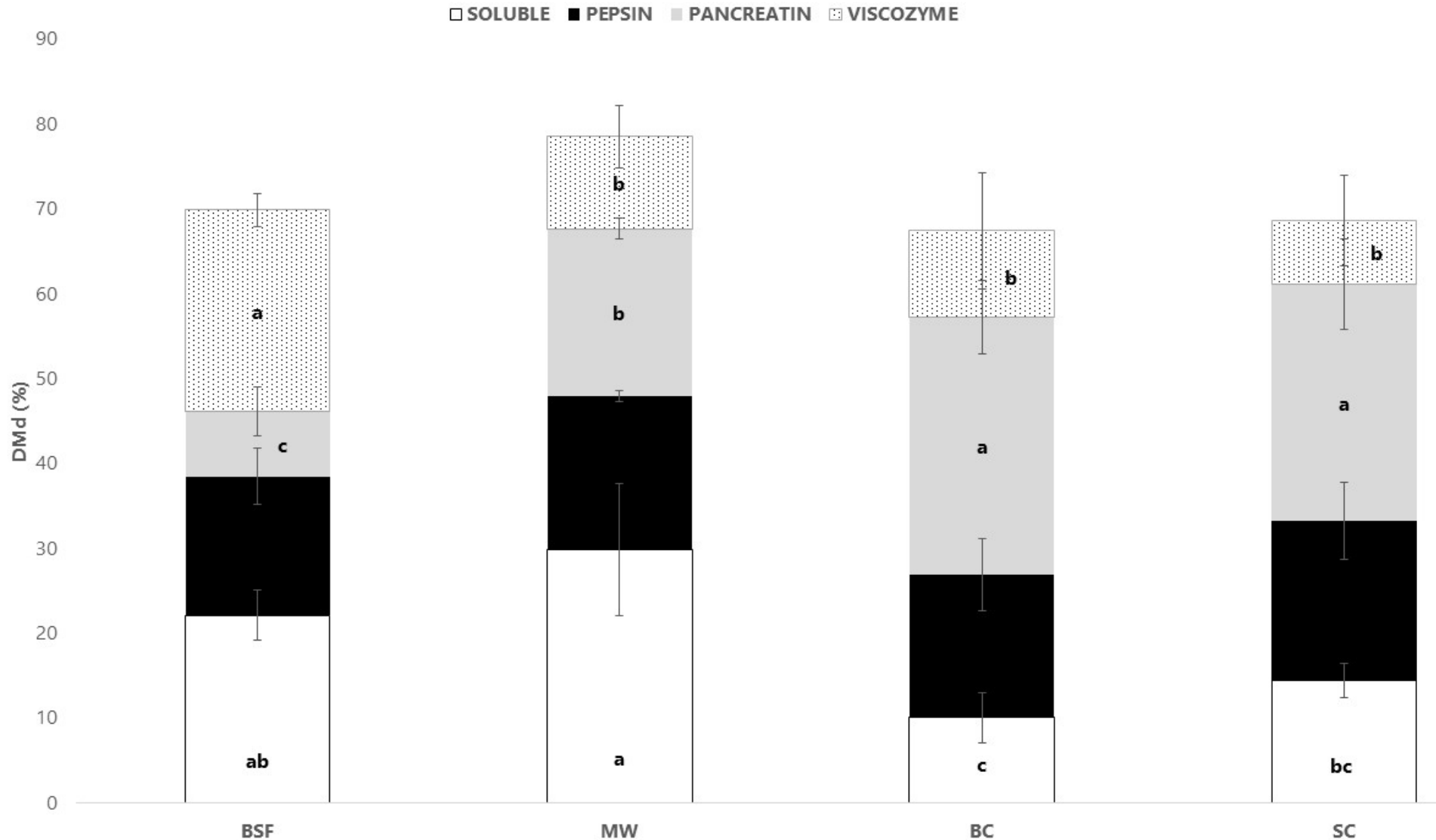
g/100g DM	Insect species								SEM	P
	BSF		MW		FC		BC			
DM (g/100g)	35.2	a	38.5	a	28.6	b	26.9	b	1.59	**
EE	31.3	ab	35.0	a	21.8	c	25.3	bc	1.82	*
CP (Nx6.25)	38.9	c	50.1	b	66.8	a	62.0	a	3.38	***
ASH	13.0	a	4.69	c	8.58	b	7.06	b	1.050	**
NDF	13.2	b	11.7	b	35.2	a	32.3	a	3.31	***
ADF	9.33	bc	8.36	c	15.6	a	12.1	ab	0.954	**
ADICP	4.06	b	4.38	b	9.81	a	5.75	ab	0.338	**
ADL (sa)	1.17		1.44		1.50		1.16		0.104	NS
Chitin	5.83	b	4.86	b	7.70	a	7.41	a	0.506	*

\* P < 0.05  
\*\* P < 0.001  
\*\*\* P < 0.0001

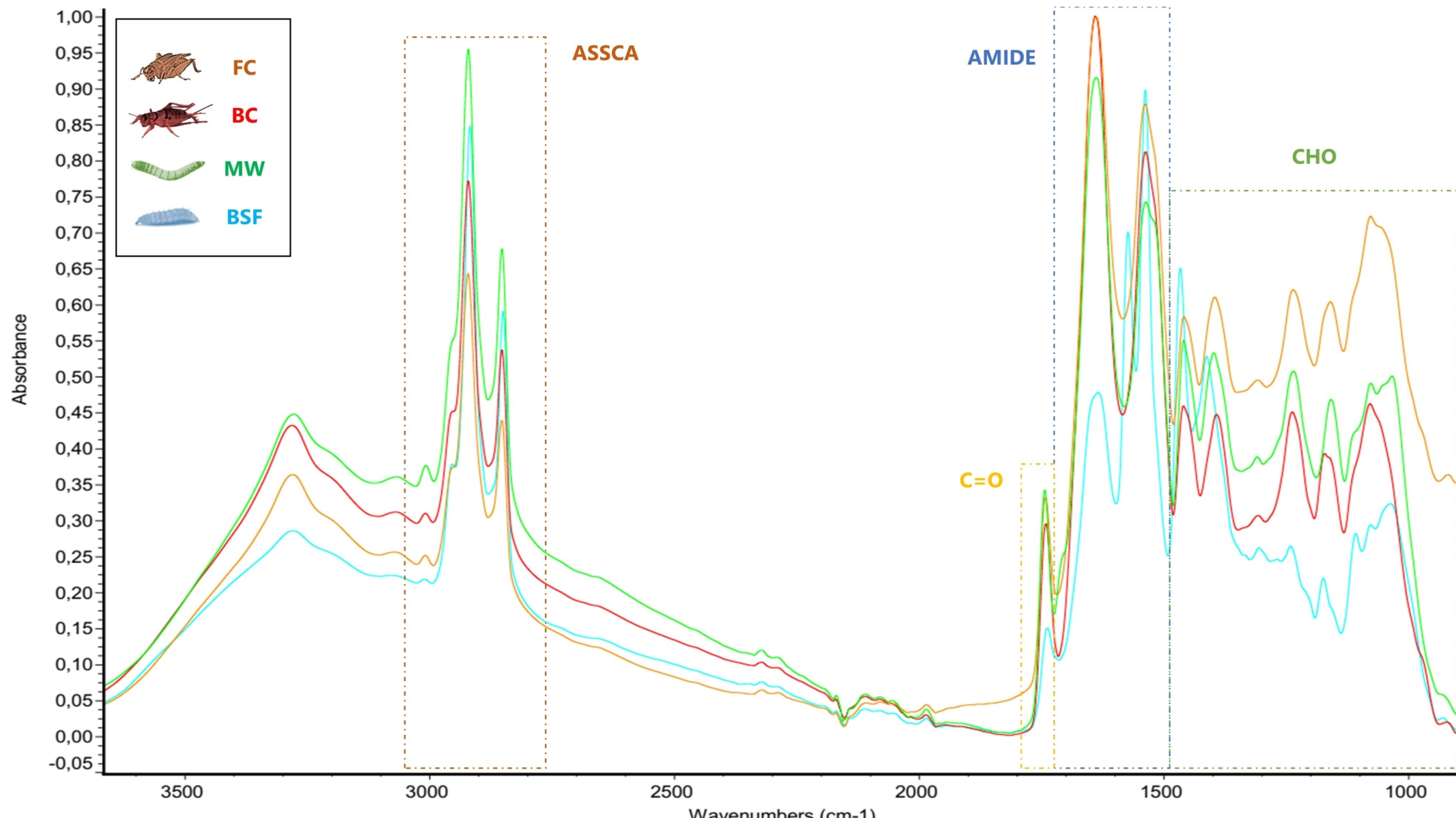


## *In vitro* DM digestibility

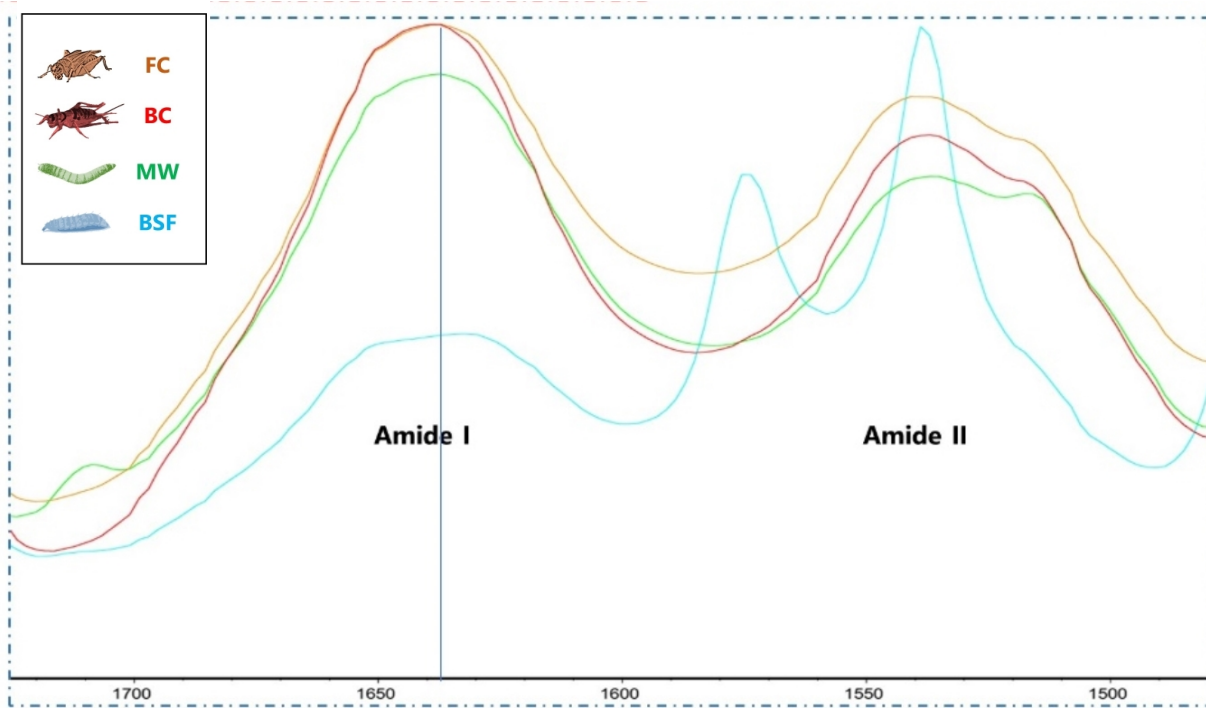
MW showed the highest ( $P<0.05$ ) DMd compared to other species. Pancreatin DMd was higher in both cricket species compared to MW or BSF.



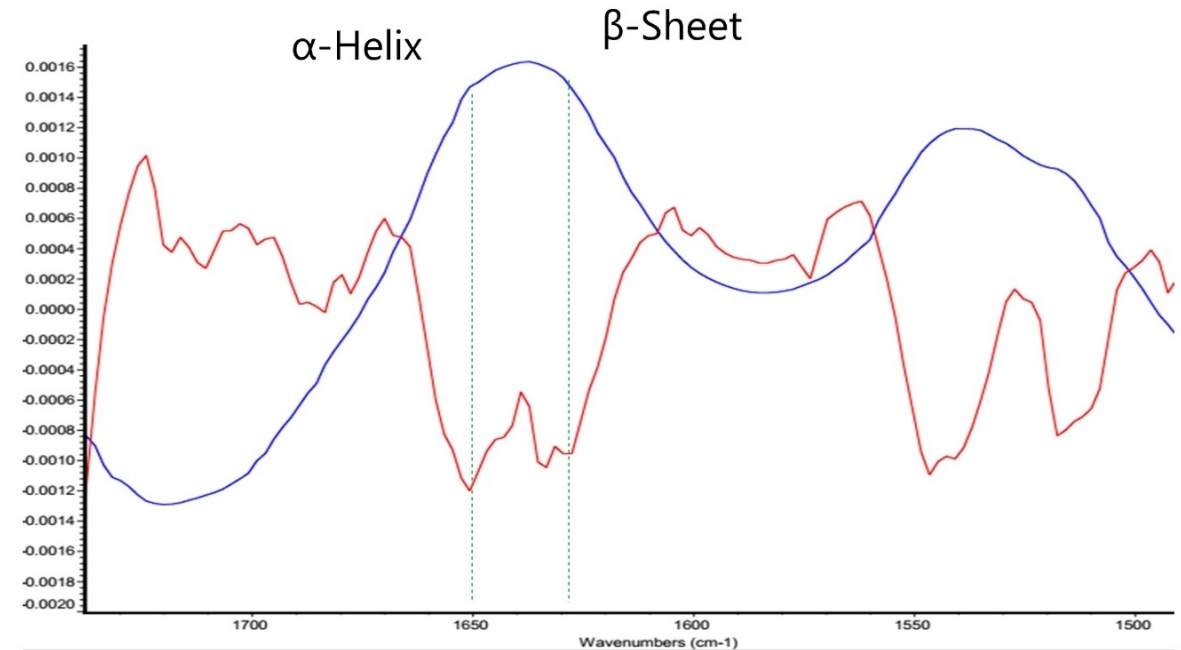
# Mid-infrared spectra (3600-900 $\text{cm}^{-1}$ )



## Primary protein structure: Amide I and Amide II



## Secondary protein structure: $\alpha$ -helix and $\beta$ -sheet



Amide I band was lower ( $P < 0.0001$ ) for BSF compared to the other species. Amide region of BSF presented an extra peak.

Pattern of  $\alpha$ -helix and  $\beta$ -sheet: FC>BC> MW>BSF ( $P < 0.0001$ )



## Correlations between spectra parameters and nutrition profiles

	AmideA	AM I	AM II	AM III	Aml/AmlI	$\alpha$ H	$\beta$ S	$\alpha$ H/ $\beta$ S
CP	0.96	0.95	-0.62	0.65	0.82	0.96	0.95	0.51
NDF	0.79	0.72	-0.28	0.29	0.52	0.73	0.72	0.40
ADF	0.70	0.65	-0.16	0.30	0.41	0.68	0.66	0.51
CHITIN	0.46	0.42	-0.10	0.12	0.26	0.46	0.42	0.58
PancDMd	0.88	0.91	-0.69	0.68	0.85	0.91	0.90	0.46
ViscDMd	-0.71	-0.76	0.75	-0.67	-0.80	-0.75	-0.74	-0.26

Strong positive correlation ( $r > 0.95$ ;  $P < 0.001$ ) was found between the height of the Aml,  $\alpha$ H,  $\beta$ S, AmideA with the CP content.

No correlation ( $P > 0.05$ ) was found with the chitin content.

PancDMd positively correlated ( $P < 0.01$ ) with CP ( $r = 0.91$ ) and NDF ( $r = 0.80$ ), which was reflected in the significant correlations found between these chemical constituents and spectral features, especially Aml,  $\alpha$ H,  $\beta$ S.



*\*Take  
home message*

- ✓ Application of FTIR demonstrated the potential to identify nutrient molecular structural differences between insect species rapidly.
- ✓ Correlations between chemical constituents and spectral features, revealing the possibility of using FTIR for the proximal composition profiling of insect powders.
- ✓ FTIR is a rapid technique to predict the chemical composition and quality of insect powders intended to be included in animal feed formulations, as well as to prevent fraud and adulterations.
- ✓ Further analysis focusing on the effect of the insect's life stage, species, feed substrate, and rearing conditions would maximize the possibilities of ATR-FITR applicability.



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Future work: 4-year project starts October 2023

***‘Insects for sustainable animal feed: Livestock farming in a climate change challenged world’***





# Thank you for your attention

