INNOVATIVE METHODS FOR THE DETERMINATION OF THE TAXONOMIC ORIGIN OF PROCESSED ANIMAL PROTEINS IN FEED.

P. Veys, M. Ngo Njembe, M-C. Lecrenier, J. Fernández Pierna and V. Baeten*

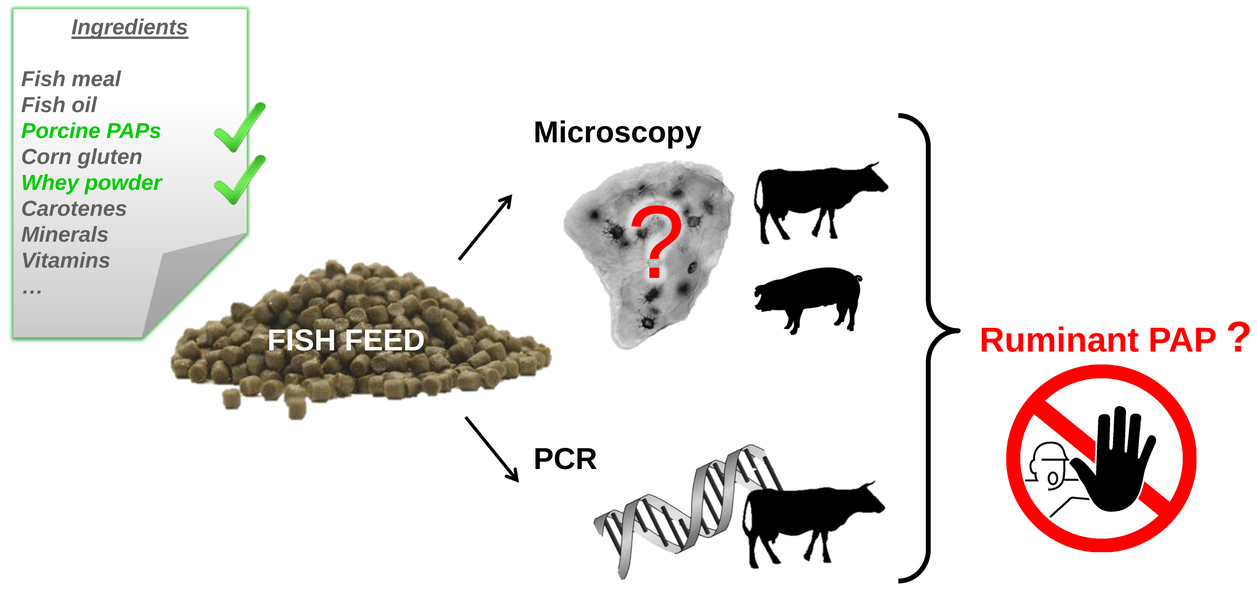
**Université Catholique de Louvain, Louvain-la-Neuve, Belgium (www.uclouvain.be)*

INTRODUCTION

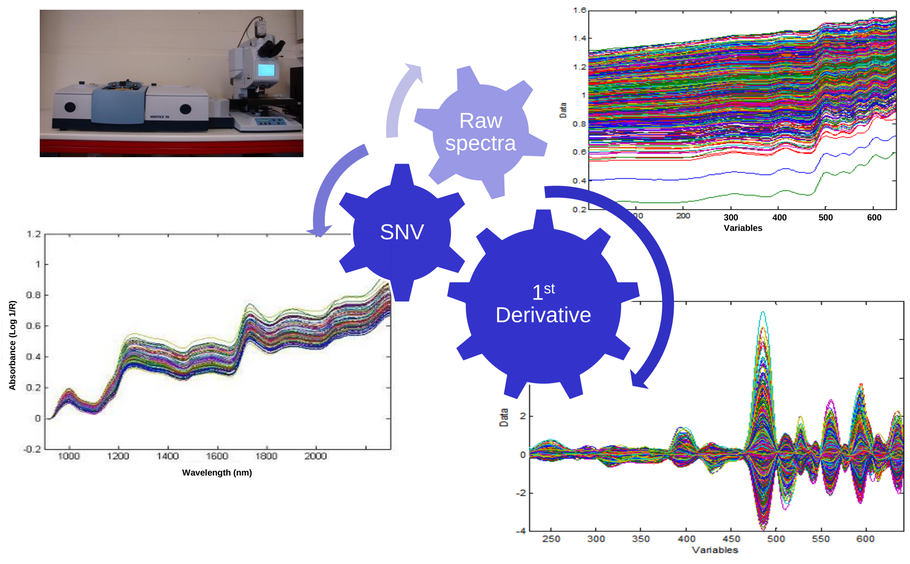
The use of animal by-products in feed depends on their nature defined by the type of tissue or body parts and the species of origin. Currently, the detection of unauthorised processed animal proteins (PAPs) is based on light microscopy and PCR methods. Nevertheless for some scenarios, even combined, these methods do not succeed in determining the taxonomic origin of the PAPs.

A typical example is an aquafeed containing authorised porcine PAP (as per EU Regulation EC/56/2013) together with dairy products: the analysis will conclude of the potential presence of ruminant PAP.

There is a **need for** developing **methods allowing taxonomic characterisation of visual structures** such as bones and muscle fibres. For the characterisation of bones, near infrared microscopy (NIRM) has yet demonstrated its potential. However the limitation of NIRM is when the presence of bones is reduced or absent, as for bakery by-products containing pig lardons used as feed material. This study investigated the potential of **NIRM** for the determination of the taxonomic origin of **muscle fibres**. NIRM combines the advantages of light microscopy and PCR to provide a "tissue and species-specific" method.



MATERIAL & METHODS



ANIMAL MATERIAL

- 2 porcine PAPs
- 6 ruminant (bovine and ovine) PAPs
- 7 fishmeals

All materials came from the industry.

SAMPLE PREPARATION

- Sedimentation in tetrachloroethylene
- Recovery of flotate (concentration of muscle fibres)
- Drying and sieving at 250µm : fine and coarse flotate fractions
- Monolayer spreading on spectralon support
- NIRM analysis

INSTRUMENTATION

FT-NIR microscope Hyperion 3000 (Brüker Optik) with motorized platform. Near spectra (9000cm^{-1} to 4000cm^{-1} with a resolution of 16cm^{-1}) were recorded in the reflection mode. For each particle an average spectrum of 8 scans within the given range was obtained. 400 spectra were recorded per fraction.

OPUS recorded spectra were extracted and imported to MATLAB files using The Unscrambler X.

SPECTRUM ANALYSIS

Animal material spectra were differentiated from the background ones according to Von Holst *et al*. (2008).

Scatter of raw spectra were removed by standard normal variate (SNV) and subsequently by first derivative.

Principal component analysis (PCA) was applied in order to look for common patterns in the spectra. The possible cluster of similar spectra gives an indication of possible discrimination between the pairs of classes of material: ruminant *vs*. fish, porcine *vs*. fish, and ruminant *vs*. porcine.

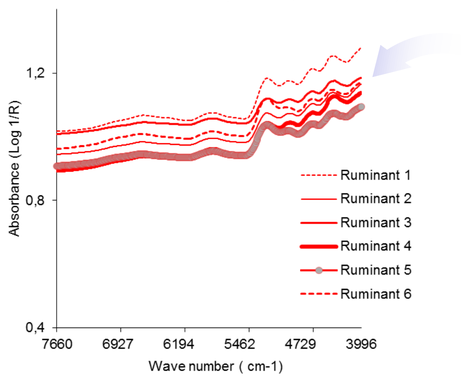
RESULTS

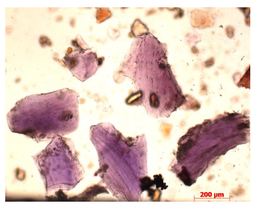
RATE OF CORRECT PAP IDENTIFICATION

About 84-85% of the spectra were recognised as from animal origin.

	Material	Nb of sample	Total Nb of spectra	Spectra identified as from animal origin	
				Nb	(%)
Coarse flotate (> 250 µm)	Fishmeals	7	2800	2175	77.68
	Ruminant PAPs	6	2400	2267	94.46
	Porcine PAPs	2	800	599	74.88
	Total	15	6000	5041	**84.02**
Fine flotate (< 250 µm)	Fishmeals	7	2800	2201	78.61
	Ruminant PAPs	6	2400	2137	89.04
	Porcine PAPs	2	800	793	99.12
	Total	15	6000	5131	**85.52**

Within classes of materials (ruminant, porcine, fishmeal) spectral profiles showed no noticeable differences since the flotate fractions consist almost exclusively of **muscle fibres** as confirmed by light microscopy.





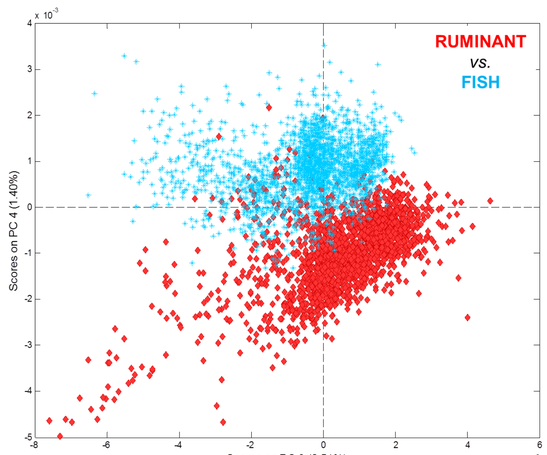
Muscle fibres stained by Fehling reagent (bright field microscopy)

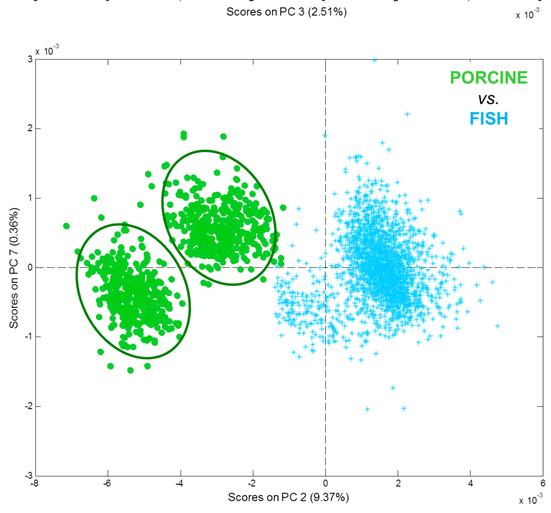
Differences in spectral absorption between classes could hardly been visually interpreted and therefore required chemometrics.

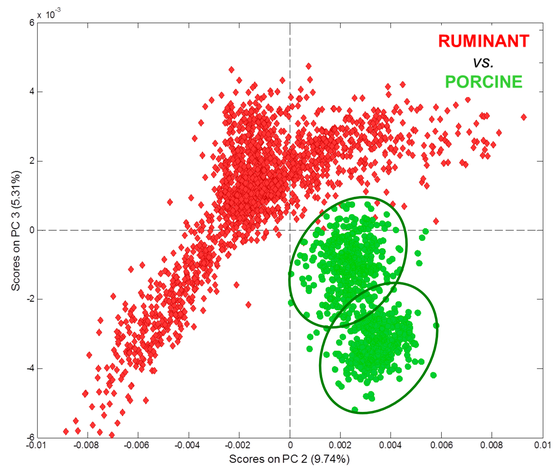
PCA ANALYSES – TAXONOMIC VALUE

Paired comparison of spectra from PAP flotates by PCA allowed to discriminate clearly different classes corresponding to their taxonomic origin : ruminant from fish, porcine from fish, and ruminant from porcine.

Ruminant spectral cluster presented more variability compared to fish. However no discrimination between ovine and bovine material could be made.







The two porcine PAPs used could be distinguished from each other (green ovals). Similar situations were noted for some ruminant material within the ruminant cluster, as well as for one fishmeal that segregated from the other fishmeals. Based on the homogeneity of tissue type of the flotate fractions (muscle) so far no explanation was found. Investigations on the industrial process of these materials might deliver some explanation.

CONCLUSION

- First NIRM study on flotate.
- **Taxonomic value of flotate** is demonstrated. Until now only the raw material or the sediment concentrating the bones was studied by NIRM. The bone NIRM based taxonomic identification was limited to terrestrial, fish and avian.
- **NIRM** allows differentiating muscle fibres from different lower taxonomic ranks: **fish, ruminant and pig**.
- In addition to this taxonomic classification, results also reveal differences inside taxonomic classes or clusters of PAPs : a door to further possibilities ?
- NIRM offers complementary information to official methods (light microscopy and PCR)

REFERENCES

Von Holst Ch., Baeten V., Boix A., Slowikowski B., Fernández Pierna J. A., Tirendi S., Dardenne P. (2008). Transferability Study of a near-infrared microscopic method for the detection of banned meat and bone meal in feedingstuffs. Analytical and Bioanalytical Chemistry 392, 313-317

This work was partly financed by the European Commission (DG SANTE)

Walloon Agricultural Research Centre, Gembloux, Belgium
Agricultural Product Valorisation Department
Food and Feed Quality Unit 15
www.cra.wallonie.be – corresponding author: p.veys@cra.wallonie.be