Original combination of Real Time PCR and NIRM for the detection and the speciation of animal particles

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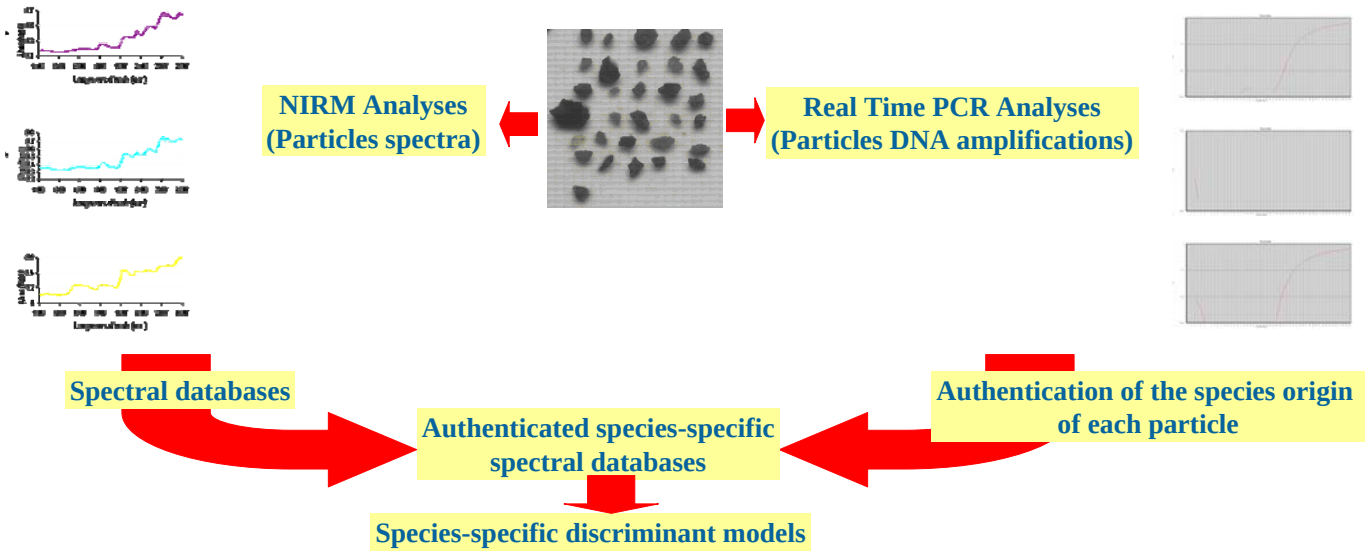
Introduction

The bovine outbreak of spongiform encephalopathy (BSE) has led the European Union to take several decisions in order to avoid the transmission through the food chain of its causal agent. The Regulation (EC) 999/2001 prohibits explicitly the feeding of mammalian PAPs to ruminants. A temporary MBM ban for all farmed animals (to avoid intra-species recycling) was established in 2001 and changed into a permanent MBM ban by amending the annex of Regulation (EC) 999/2001 through Regulation (EC) 1234/2003. The animal by-product (ABP) regulation EC 1774/2002 prohibits feeding of animals with proteins from the same species and established three categories of ABP's which reflect different levels of food safety including the risk due to Transmissible Spongiform Encephalopathy (TSE). Presently, classical optical microscopy is the only official method in the European Union used to detect constituents of animal origin in compound feeds or in their ingredients. Successfully validated, the method is nevertheless limited to the differentiation of large taxonomic groups i.e. terrestrial animal or fish. There is a tremendous need for techniques enabling to detect more routinely these highly processed animal by-products throughout the feed chain but also to identify their animal origin at the species level.

Among the alternative techniques developed for the detection of meat and bone meals, near-infrared microscopy (NIRM) and real time PCR have used by CRA-W to develop and validate original solutions. In the framework of the Belgian project FARIMAL (contract nr S-6168) both methods were combined in an original procedure to develop authenticated species specific spectral databases.

Developed analytical scheme



Performances of the discriminant chemometric models developed

Sample set

From 1100 particles measured by NIRM, 922 were authenticated by real-time PCR

Species	Number of PAPs	Number of particles
Cattle	6	229
Sheep	1	28
Pig	7	306
Chicken	2	96
Fish	8	263

and used for the building of species specific spectral databases. Discrimination models have been constructed and validated using leave-one-out cross-validation (LOOCV).

Results

Model constructed using Partial Least Squares –Discriminant Analysis (PLS-DA)
Pre-processing method: Multiple Scatter Correction (MSC)
Number of PLS factors: cattle: 8; fish: 9

CATTLE

Confusion matrix for the calibration set

Particles belonging to	Particles classified as Cattle	Particles classified as Rest
Cattle	76.70%	23.30%
Rest	15.00%	85.00%

80.85 %

Confusion matrix for the LOOCV

Particles belonging to	Particles classified as Cattle	Particles classified as Rest
Cattle	75.60%	24.40%
Rest	16.20%	83.80%

79.70 %

FISH

Confusion matrix for the calibration set

Particles belonging to	Particles classified as Fish	Particles classified as Rest
Fish	98.60%	1.40%
Rest	9.50%	90.50%

94.55 %

Confusion matrix for the LOOCV

Particles belonging to	Particles classified as Fish	Particles classified as Rest
Fish	96.80%	3.20%
Rest	10.00%	90.00%

93.40 %

Conclusions and prospects

According to these first results, the speciation of a large proportion of animal particles coming from a non sedimented sample is possible with discriminant models based on NIRM spectral databases. Moreover, the NIRM prediction can be confirmed afterwards by real time PCR thanks to a particular protocol adapted to the extraction of the DNA from a single particle. This study will be enlarged to more PAPs samples and other species-specific models (Pig vs rest, Chicken vs rest and Ruminants vs rest) will be developped.

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