In-house validation of a near infrared (NIR) hyperspectral imaging method for the detection of Meat and Bone Meal (MBM) in compound feed

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The aim of this work is to present a summary of a protocol created in order to provide a framework where a near infrared imaging method can be developed and validated as a standard accepted by regulatory authorities.

The thematic concerned is the **detection of meat and bone animal meals (MBM) in feedingstuffs**. Due to the resurgence of BSE problematic at the end of the nineties, a Council Decision was taken in 2000, which prohibited the use of all meat and bone animal meals for all farmer animals, which were kept, fattened or bred for the production of food. Classical microscopy is the reference method for this kind of analysis. However, the near infrared microscopy (NIRM) and near infrared hyperspectral imaging methods have been proposed by the CRA-W as alternative methods having clear advantages (speed, flexibility, easy to use…).

This in-house validation of the near infrared hyperspectral imaging method for qualitative MBM detection has been performed in the framework of ISO 17025.

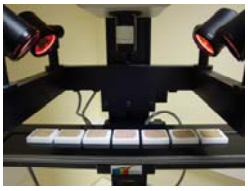
Various criteria and tests have been considered as the limit of detection, the repeatability and the risk of cross-contaminations. All the criteria and tests considered in this study have driven to a correct validation of the method for the qualitative detection of processed animal proteins in compound feed by Near Infrared Imaging.

The method is running under accreditation ISO 17025 since 2005 at the CRA-W.

Feed materials

The samples used for this study came from different EU projects and Interlaboratory studies: Stratfeed, Nutreco, IAG, VLA, DG Sanco 2003 and 2004.

All the samples have been prepared according to consolidated protocols and certified by the reference method, i.e. the classical microscopy.



NIR Imaging system

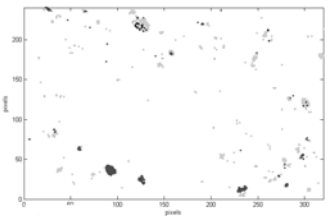
The near infrared camera used in this study is a MatrixNIR Chemical Imaging System (Malvern Instruments, Analytical Imaging, Columbia, MD, USA). Two coupled liquid crystal tuneable filters (LCTF) allow go through sequentially the reflected energy at a defined wavelength range. The LCTF have to be adjusted in order to collect the energy in the 900 nm to 1700 nm spectral range (resolution of 10 nm). After the LCTF, the reflected energy passes through an infrared focal plane arrays of size of 240 x 320 corresponding to 76 800 individual infrared detector elements (or pixels). For each pixel, the compilation of the absorbances at each wavelength gives a spectrum.

Flow chart

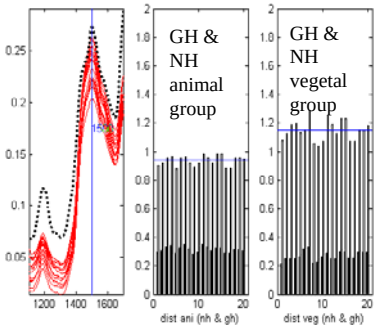
Here it is shown for the sediment fraction but the procedure has been created for both the raw and the sediment fractions.

Chemometric tool

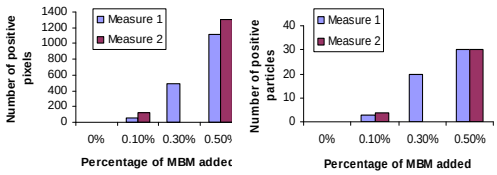
Support Vector Machines (SVM) is used as chemometric tool for discrimination. Next figure shows an example of a result using this methodology in an unknown sample.



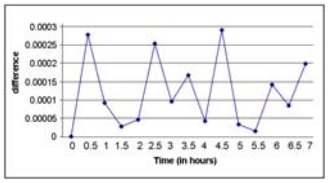
Visual observation & distances



Limit of Detection (LOD)



Stability test (repeatability)



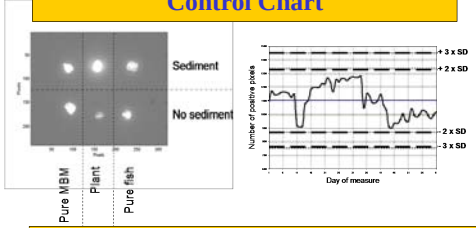
Validation tests

Next table shows the results for some positive (different % of MBM) and negative samples.

Sample	Source	% MBM real	Animal pixels detected	Conclusion
1	Stratfeed	0	0	-
2	Stratfeed	0	0	-
3	Stratfeed	0	0	-
4	Stratfeed	0	0	-
5	Stratfeed	0	2	-
6	Stratfeed	0	0	-
7	Stratfeed	0	0	-
8	Stratfeed	0	0	-
9	Stratfeed	0	4	-
10	Stratfeed	0	1	-
11	Stratfeed	0	0	-
12	Stratfeed	0	0	-
13	VLA	0	7	-
14	DGSanco 2004	0	39	+ FP
15	Stratfeed	2	111	+
16	Stratfeed	4.5	452	+
17	Stratfeed	5	41	+
18	Stratfeed	2.5	699	+
19	Stratfeed	1.5	71	+
20	Stratfeed	6	3437	+
21	Stratfeed	3.5	338	+
22	Stratfeed	4	429	+
23	Stratfeed	6	946	+
24	Stratfeed	5	1442	+
25	Stratfeed	0,1	27	+
26	IAG	pure fish	49932	+
27	IAG	fish + 0,1	61	+
28	IAG	0,1	1	- FN
29	VLA	0.1	81	+
30	VLA	0.5	91	+
31	VLA	0.1+1 fish	100	+
32	VLA	0.3	109	+
33	DGSanco 2003	0.1	38	+
34	DGSanco 2003	0.5	29	+
35	DGSanco 2003	0.1 + 5 fish	119	+
36	DGSanco 2004	5 fish	75	+
37	DGSanco 2004	0.5 feather	27	+
38	DGSanco 2004	0,1	16	+
39	DGSanco 2004	0.1 + 5 feather	456	+
40	DGSanco 2004	0,1	29	+
41	DGSanco 2004	0.1 <133	15	+

* FP: false positive; FN: false negative

Control Chart



Cross-contaminations

Sample	Theoretical percentage	Animal pixels detected	Methodology conclusion
A	5% MBM	971	Positive
B	0% MBM	0	Negative
A	5% MBM	1516	Positive
B	0% MBM	0	Negative
A	5% MBM	1530	Positive
B	0% MBM	0	Negative

Conclusion

All the criteria and tests considered have driven to a correct validation of the NIR hyperspectral imaging method for the qualitative detection of MBM in compound feed. The LOD is about 0.1% and can be even lower by enhancing the number of particles analysed. The control chart showed that for the different days of analysis, the results have always been within the limits allowed and no cross-contamination was proved. Moreover the percentage of false positive and negative results is acceptable for a screening method.

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