Application of FT-NIR microscopy for animal origin constituents in animal feed

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Introduction

The fear of bovine spongiform encephalopathy (BSE) in cattle, induced the European Union, at the end of 1994**,** to prohibit the administration of transformed animal proteins to cattle which are kept, fattened or bred to produce food for human consumption. Therefore, in Italy, health institutions were set up to have a capillary control of animal feed.

The official analyses of animal feed (Ministerial Decree 30/09/1999) are made with a microscopy exam with a qualitative expression of the results. This method comply with health requirements, but is complicated by the length of execution and the need for expert staff.

Numerous studies have been carried out including immmunoassay tests, biomolecular analysis, such as polimerase chain reaction (PCR) and completely innovative FT-NIR microscopy.

This technique joins microscopy research with the wealth of data from spectrum analyses. As vegetable and animal components in the feed have a typical NIR spectrum, the use of this technique to analyse bone fragments allows for objective answers.

Materials and methods

40 samples of feed of different typologies were analysed with FT-NIR microscopy of which, 30 resulted positive for bone fragments and 10 negative with official method (Ministerial Decree 30/09/1999).

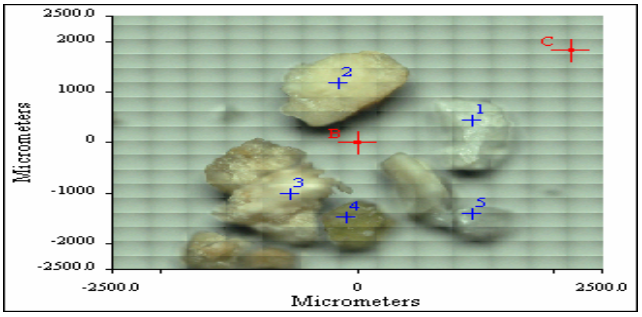

Figure 1. Visible image of analysed sample area. The markers represent the suspect bone fragments which are analysed by NIR spectroscopy.

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

The analytical determinations were made with a classic Fourier transformed spettrometry in the near infrared spectral region (FT-NIR), which was interfaced with an optic microscope equipped with a camera. These links allow for either the observation of the sample in the visible field, or the analysis of NIR spectra in the 6000–4000 cm^{-1} range. The infrared radiation of the instrument was deviated by some mirrors and lenses in the microscope optical system and then sent on the sample.The microscope includes a camera and viewing system which magnifies the visible-light image of samples so that it is possible to isolate a fragment and radiate these to collect spectra.[1] The image of the sample was displayed on the PC monitor (Figure 1).

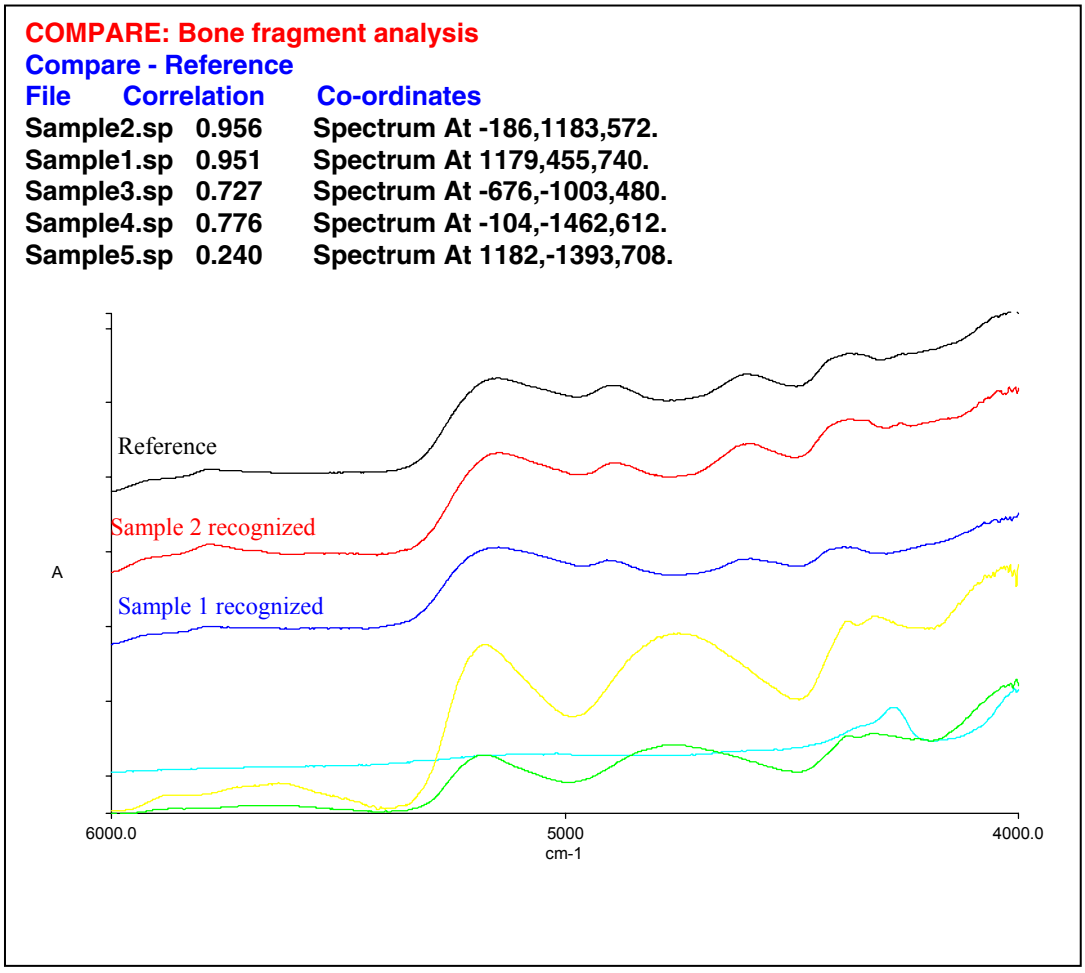

Figure 2. Comparison between reference spectrum and unknown spectra using COMPARE function.

The mathematical elaboration of NIR spectra gives information on the structural peculiarities of analysed fragment.

To identify bone fragments a computerised comparison (using the COMPARE function) was made between the unknown bone fragment spectrum and reference spectra. The result should be a correlation factor as close as possible to one (Figure 2). The reference spectra were acquired in the same instrumental conditions as the samples, from bone fragments of confirmed animal origin. These were extracted (Ministerial Decree 30/09/1999) from meal only produced from animal components, both of different (chicken, bovine, swine, equine) and single species. For the intrinsic limits of the technique and the aims of analysis accepted values were set at ≥ 0.90 (90% similarity).

In particular this value was determined by comparing bone fragment spectra and vegetable, minerals and other animal constituent spectra such as collagen, protein form and animal fats.

Operative parameters:

Source:	NIR
Slit:	50–50 μm
IR Type:	FT
Accumulations:	16
Measurement:	Reflectance
Resolutions:	4 cm^{-1}
Range:	6000–4000 cm^{-1}
Calibration:	Background on the reference material (Spectralon®)

Results and discussion

All 30 positive samples (according to the official method) were confirmed with NIR analysis. The samples were analysed only in diffused reflectance (for example, part of the radiation is absorbed, a part reflected and then detected and transformed in a spectral datum) this is as they are solids which NIR rays have difficulty in passing through.[2]

The COMPARE® function identifies a bone fragment from another interference fragment (for example, vegetables) but does not allow the animal species of the bone fragment to be discriminated with sufficient accuracy and precision. This is due to the structural similarities of the bones, especially between bones of terrestrial animals (and even more so with mammal bones), and, in part, to the scattering phenomena which is due not only to the irregular morphology of the fragments but also to the intrinsic characteristics of the diffused reflectance. A more accurate identification of bone fragments of different species is possible through a different qualitative analysis which involves the construction of families of spectra which are applied, specific chemometric operations such as Principal Components Regression. Slight spectral differences, but which are associated to spectra of a single family (for example, the spectra family of bovine bone fragments), allow greater specificity of analysis. The studies carried out to date show the separation between different species in function of the spectral characteristics of a bone fragment (Figure 3). However, some problems remain on the validation of the method in terms of accuracy and precision.

In conclusion, FT-NIR microscopy offers an efficacious innovative technique to identify and analyse animal constituents, particularly bone fragments, in complex matrices, such as feed, ascribing numeric and objective data to a structural characteristic. This technique leaves room for further interesting developments for more precise structural studies and possible quantifications of particular constituents (for example, animal proteins), and applications in proteomic fields.

Verification Diagnostic Report

Critical probability level: 0.01
Inter class distances

	BOVINE	FISH
BOVINE	-	14.35

	% Recognition rate	% Rejection rate
BOVINE	100(34/34)	100(31/31)
FISH	100(31/31)	100(34/34)

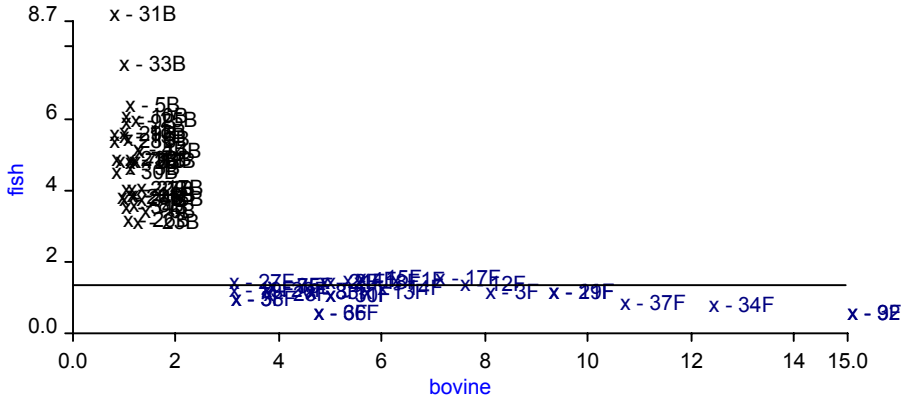

Figure 3 Qualitative analysis by chemometric operations (PCR) to identify bone fragments of different species.

References

1. F. Piraux and P. Dardenne, *Biotechnol. Agron. Soc. Environ.* **4(4),** 226 (2000).
2. L.G. Weyer, *Appl. Spectrosc. Rev.* **21(1&2),** 1 (1985).