OFFICIAL MICROSCOPIC EXAMINATION AND NEAR-INFRARED ANALYSIS: A ROUTINE SAMPLES COMPARISON

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INTRODUCTION

Following the outbreak of BSE, processed animal proteins (PAP) were banned in animal feedstuffs in the EU and each individual member state was required to implement a feed quality programme to enforce this ban. An essential aspect of these programmes was the adoption of EU-approved methods for detecting PAP in feed. The official analytical method for the detection of processed animal proteins in feedstuffs is the microscopic examination technique described in Directive 2003/126/EC (EU, 2003). Usually, during the microscopic analysis, bone fragments are the most easily identifiable among the particles of animal origin found in feedstuffs and bone characterization is based mostly on the characteristics of lacunae. Since 1998, new methods has been proposed for the detection of meat and bone meal (MBM) in feedingstuffs to confirm and support the microscopic analysis, as near infrared microscopy (FT-NIR).

AIM OF STUDY: the aim of this study is to compare the routine analyses carried out by microscopic method to a near-infrared analysis (NIR): here is 4 months of routine control results in Piedmont (Italy), performed with both methods.

MATERIALS AND METHODS

From 11.05.07 to 10.09.07, 113 routine control samples for the identification of constituents of animal origin in animal feedingstuffs were carried out both with classic microscopic examination, according to the Directive 2003/126/EC, and with FT-NIR technique, for a qualitative identification of PAP.

MICROSCOPIC ANALYSIS

Each sample was examined using the microscopic method, according to the Directive 2003/126/EC. Sediment and sieved fractions were analyzed. Any potential bone fragments were characterized by similar morphological features (colours, shape, lacunae shape, lacunae distribution, etc.), as shown in the following figures.

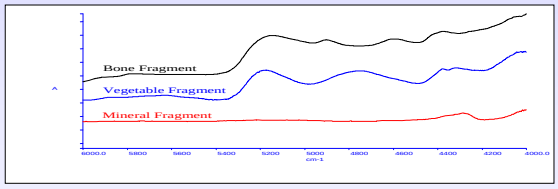
MAMMAL BONE

CHICKEN BONE

FISH BONE

NEAR INFRARED ANALYSIS (NIR)

Samples were processed applying a complete NIR protocol, that was developed and validated in order to analyze the raw and the sediment fractions of the feedingstuffs. The recognition of bone fragments among other constituents was made treating their vibrational spectra by a mathematics algorithm based on least-squares, resulting from the comparison between the spectrum of unknown sample and spectra of reference materials. Spectra examined were obtained in the reflectance mode, with 10000-4000 cm-1 range. By setting a 95% level of similarity as acceptance threshold, good results were obtained in the detection of bone fragments.



RESULTS

Microscopic examination detected 4 positive samples for fish, tag declared, and two positive specimens, for not allowed terrestrial animals. These two not compliant samples were avian feed, MBM contaminated (Figure 1). The positive samples for fish are feedingstuffs to non-ruminant farmed animals where the fish meals are allowed as in 1292/2005/EC derogations. The FT-NIR analysis showed six positive results, as in our experience this method fails to discriminate between fish meal and terrestrial animal (mammals and/or poultry meal), as shown in Figure 2..

Figure. 1 Results with microscopic method

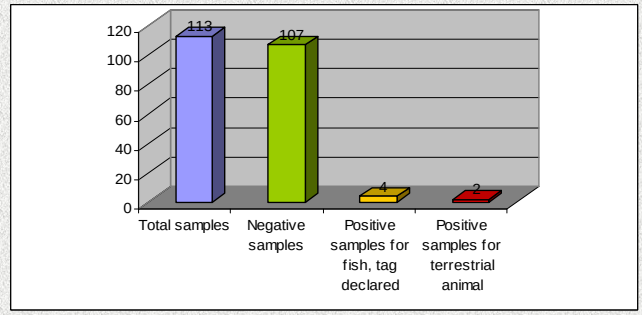
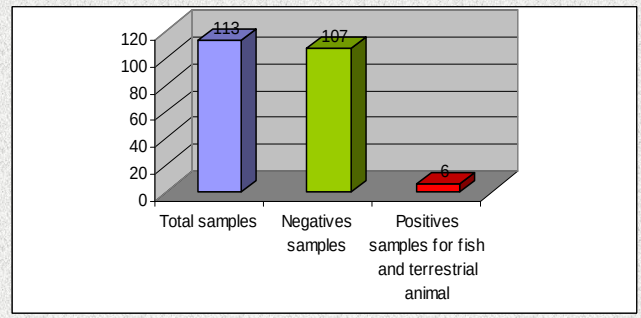


Figure 2. Results with near-infrared method



CONCLUSIONS

The results of analyses of routine samples by NIR and classical microscopy demonstrate that there is no difference between the outcome of both techniques, although the near infrared microscopy does not identify different kind of animal meal. The principal particles of animal origin that might be present in feeds are bones and muscle fibres. Additionally, cartilage, hairs, feather filaments, egg shells, fish scales and ligaments may also be present. Most of these particles show a limited number of characters, and fine structures are visible after microscopic inspection at different magnifications.Thus, besides the animal/non animal recognition, it is very important to distinguish bone tissues of different zoological origin. Experimental samples carried out by using a soft independent modelling of class analogies-SIMCA seemed to show a good differentiation between different species. The next future challenge could be how to apply these techniques to the daily routine samples.

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