Possibilities of FT-IR and PCR to discriminate species by animal fats

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Animal by-products represent yearly in the EU more than 10 million tons of materials derived from healthy animals which have to be incinerated or transformed in a variety of products used among others in human food and animal feed. A significant part of these by-products are made up of fats. The Regulation (EC) N° 1774/2002\(^1\) extends the current ruminant intra-species recycling (cannibalism) ban to other species and maintains current EU total ban on the feeding of meat and bone meal to farmed animals. The Regulation establishes clear safety rules for the production of meat and bone meal in case it is ever re-authorised for inclusion in feed for non-ruminant species, e.g. poultry and pigs. Therefore it will be necessary to clearly identify the species origin of by-products included in feed\(^2\) with efficient analytical methods.

Near Infrared (NIR) region can be used to determine the origin of the fat samples, however differences of the spectra in the Middle Infrared (MIR) region are more significant and allow to get better discrimination. The use of multivariate statistical tools (PCA, SLDA) on the full spectra allows to distinguish fish from other samples. The chemometric analysis in the fingerprint region (1000-900 cm\(^{-1}\)) allows the classification of some fat samples according to their origin. Butter and sheep are clearly separated from the other samples and a lot of the tallow samples tend to cluster together. The use of 2D graph of the values of the absorbances at two signal ratios 966/956 cm\(^{-1}\) and 990/980 cm\(^{-1}\) is a robust tool to distinguish samples.

PCR is a species-specific technique which could be a helpful complement of the spectroscopic methods. Successful attempts of DNA extraction and PCR amplification were realised at CRA-W on three industrial fat samples (lard, tallow and chicken fat). Short mitochondrial targets initially developed for MBM detection were tested in real time PCR and gave significant results\(^3\).

Based on these preliminary results, FT-IR and PCR could be used in combination for the control of the species origin of the fat incorporated in feed. The spectroscopic methods could be used for screening and PCR for confirmation as a forensic method. The results obtained with the real time PCR show that DNA is still present in such samples and significant amplification is possible with appropriate targets.

Keywords
Animal fats, animal by-products, species identification, FT-IR, spectroscopy, PCR

\(^2\) EC DG Health and Consumer Protection; MEMO/04/107 Brussels, 6 May 2004 Questions and Answers on animal by-products
\(^3\) Dubois M., Fumière O., von Holst C. & Berben G. (2002). - Meat and bone meal detection in feed by search of specific animal DNA segments. 181\(^{st}\) meeting of the Belgian Society of Biochemistry and Molecular Biology, 4\(^{th}\) of May, Katholieke Universiteit Leuven (KUL), Heverlee (Belgium), abstract nr. 7