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Assessment of the discrimination of animal fat by FT-Raman spectroscopy

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ABSTRACT

In recent years, there has been an increased attention towards the composition of feeding fats. In the aftermath of the BSE crisis all animal by-products utilised in animal nutrition have been subjected to close scrutiny. Regulation requires that the material belongs to the category of animal by-products fit for human consumption. This implies the use of reliable techniques in order to insure the safety of products. The feasibility of using rapid and non-destructive methods, to control the composition of feedstuffs on animal fats has been studied. Fourier Transform Raman spectroscopy has been chosen for its advantage to give detailed structural information. Data were treated using chemometric methods as PCA and PLS-DA which have permitted to separate well the different classes of animal fats. The same methodology was applied on fats from various types of feedstock and production technology processes. PLS-DA model for the discrimination of animal fats from the other categories presents a sensitivity and a specificity of 0.958 and 0.914, respectively. These results encourage the use of FT-Raman spectroscopy to discriminate animal fats.

1. Introduction

Humans are at the top of the food chain and consumer safety depends on the assessment of this entire chain in order to prevent health risks. The quality of animal-based food products is directly related to animal feeding practices [1–3], which makes the ingredients used in animal feed very important in regards of the resulting food.

In the last years, animal feeding practices have changed considerably. The increased demand of food animals has led to the inclusion of plant-based products, antibiotics, and animal by-products in animal feed. Animal fats are important animal by-products and are currently used as ingredient in feeding formulations. They are preferred by feed producers because of their positive influence on the quality of meat and taste [4]. However, the inclusion of theses ingredients can affect the safety of animal based food products and pose potential risks to human health. In the aftermath of the BSE crisis all animal by-products utilised in animal nutrition have been subjected to close scrutiny [5]. Therefore, the majority of European Union Member States have regulated the use of the animal by-products in the animal feeds. Criteria for the safe use of ruminant fat in animal nutrition in Europe are defined by the Regulation 1774/2002 [6], which requires that the material belongs to the category of animal by-products fit for human consumption, and the maximum concentration of residual insoluble impurities after purification does not exceed 0.15%. In the same time, scientists are already working on methods in order to determine and differentiate fats used in feedstuff formulations in terms of their sources [7], the production technology and their composition [8].

The need of rapid and reliable techniques for the quality control and the assessment of food and feed composition and contamination has allowed to an increase use of vibrational spectroscopic approaches, because of their rapidity and high ability to give molecular structural information. Raman spectrometry method is fast and does not require any sample preparation steps prior to analysis. It has a great potential for compositional analysis of oils and fats. Several studies have been carried out on the adulteration of oils [9,10], quantitative analysis [11], classification of oils and fats [12], and on their unsaturated fatty acids [13–15]. Animal fats used in feeding purposes were also studied to ensure the safety of the products. The classification of fats was the subject of a study realized by Bellorini et al. [7] who worked on the assessment of the abilities of various analytical methods (Fourier transform infrared spectroscopy, gas chromatography, immunoassays, and polymerase chain reaction) to differentiate the sources of fats used in feedstuff formulations and to discriminate tallow from non-ruminant fats.

Various technological processes could lead to significant differences in fats by-products, especially in their composition and properties. In this regards, the EU Feeding Fats Safety Research Project (FOOD-CT2004-007020) [16] has worked on the quality and safety

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of feeding fats obtained from co-products or by-products of the food chain, by the characterisation of the composition and the quality, and also the determination of the contamination of fat materials used in animal feed. Within the framework of this project, the group of Gasperini et al. [8] has tried to establish rules for the classification of the feeding fats by using Fourier Transform Infrared spectroscopy.

The most commonly used fat sources in feed are animal (lard, tallow), vegetable (coconut oil, palm oil, corn oil, rapeseed oil and soybean oil) and marine (fish oil). The objective of this study is to establish whether it is possible to discriminate animal fats in terms of their sources or production process using a rapid and effective analytical methodology as Fourier Transform Raman spectroscopy.

In our study, collected Raman spectra of fats were treated by Principal Component Analysis (PCA) in order to visualise the natural grouping of samples. Partial Least Square-Discriminant Analysis (PLS-DA) algorithm was applied to assess the discrimination of the animal fats.

2. Experimental

2.1. Samples

Two datasets were analyzed for this study. The first one (dataset 1), previously used in the study of Bellorini et al. [7] is composed of 29 various types of animal fats in which different sample types (poultry, pig, bovine, lamb, and fish oils) were obtained from various sources, local and industrial retailers and producers, in order to cover the variation which may occur within each sample type. In addition to these samples, mixtures of fats were also measured to assess the ability of the technique used to affect them to one group of animal fats. The description of these mixtures is given in Table 1.

The second (dataset 2) is formed by 105 fat by-products. These samples were previously used for the EU Feeding Fats Safety Research Project (FOOD-CT2004-007020). They were provided from all European countries and were classified on the basis of the nature of feedstock and their production technology and from composition data according to the list established below (Table 2). Each group is identified by an acronym describing the type of fat by-product.

Samples were pre-heated at 40 °C and presented to the spectrometer in classical glass tubes of an internal diameter of 12 mm and a length of 75 mm (Schott Duran®). Tubes were introduced into a dedicated sample holder developed at the CRA-W and made of aluminium to assure repeatable position of the sample in front of the laser beam.

Table 1
Composition of fat mixtures studied.

Acronym	Composition	Description
Mixture 1 (M1)	50% Bovine, 50% ovine–porcine	Pure animal meal fat
Mixture 2 (M2)	80% Bovine, 20% porcine	Pure animal meal fat
Mixture 3 (M3)	50% Bovine, 50% ovine–porcine–avian–former food stuffs	Pure animal meal fat
Mixture 4 (M4)	55% Bovine, 15% ovine, 30% porcine, traces of avian fat	Rendered fat

2.2. FT-Raman analysis

FT-Raman spectra were acquired on a Vertex 70 – RAM II Bruker FT-Raman spectrometer. This instrument is equipped with a Nd:YAG laser (yttrium aluminium garnet crystal doped with triply ionised neodymium) with an output at 1064 nm (9398.5 cm^{-1}). The maximum of laser power is 1.5 W. The measurement accessory is pre-aligned, only the *Z*-axis of the scattered light is adjusted to set the sample in the appropriate position regarding the local point. The RAM II spectrometer is equipped with a liquid-nitrogen cooled Ge detector.

FT-Raman spectra [3600–200 cm^{-1}] were collected with a resolution of 4 cm^{-1} by co-adding 128 scans for each spectrum. Each spectrum is then collected in 4 min. Analysis were performed in duplicate. The laser power was set at 600 mW.

The OPUS 6.0 software was used for the spectral acquisition, manipulation and transformation.

2.3. Chemometric treatments

In order to achieve a reliable differentiation between fat types, Principal Component Analysis (PCA) was applied. PCA is an unsupervised method describing dataset without *a priori* knowledge of the data structure [17]. It allows converting original and correlated variables into uncorrelated variables called principal components which contain the main information. Loading plots were used to interpret the spectral information contained in each principal component.

The Partial Least Squares – Discriminant Analysis (PLS-DA) technique [18,19] was used in order to build models permitting to discriminate between groups on the basis of the spectral information. PLS-DA is a multivariate classification method that works to explain maximum separation between defined classes in the data. It works by performing a PLS regression of the data matrix (**X**) against the dummy matrix **Y** (+/−1 for a two class model) which describes variation according to class. Variation is interpreted in terms of **X**- and

Table 2
Characteristics of the fats by-products studied.

Acronym	Name	Description
AOCHE	Acid oils from chemical refining	By-product of oils and fats refining process, carried out according to the chemical technology
AOPHY	Acid oils from physical refining	By-product of oils and fats refining process, carried out according to the physical technology
LECI	Lecithins	Mixture of polar and neutral lipids recovered from some seed oils (corn, rape, sunflower, soy) immediately after extraction by water or steam addition
RECY	Recycled cooking oils	Products coming from the collection of exhausted oils residuated from the deep frying process carried out in food industries, catering installation and sometimes, home food preparation. They are forbidden for food and feed uses
ANFA	Animal fats	Products coming from the rendering process (sterilisation, cooking and melting of animal tissues). Only fats belonging to category 3.
FISH	Fish oils	These oils are obtained by rendering of whole low value fishes of from fish wastes from the food industry, such as canned tuna fish, smoked salmon, salted sardines, dried stockfish, etc
EBE	Oils extracted from exhausted bleaching earth	Oils are coming from chemical and physical refining oils. They are recovered, generally by means of solvent extraction. They are forbidden for food and feed uses
HYBY	Hydrogenated by-products	This category is covered by fully hydrogenated acid oils from chemical refining

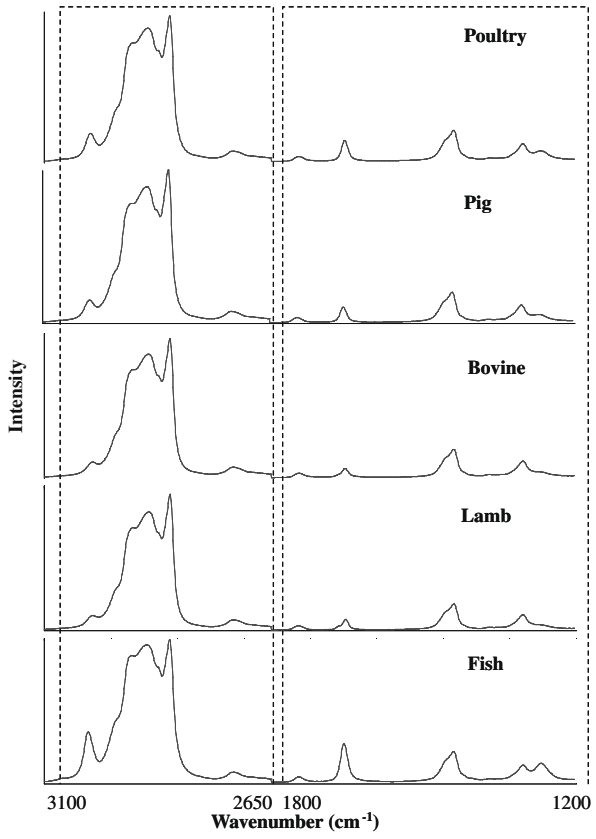

Fig. 1. FT-Raman spectra of animal fats from different sources.

Y- scores, **X**-loadings, **X**- and **Y**- weights and PLS regression coefficients. Once a PLS-DA model is calculated and validated (by cross validation), it can be used for prediction of class membership for unknown samples. When more than two classes are present, the dummy variables have to be created in another way. For the case of a three classes model: [1,0,0]; [0,1,0];...

2.4. Software

PCA application was performed by the UNSCRAMBLER software version 9.2 from CAMO (Computer Aided Modelling, Trondheim, Norway).

PLS-DA computations with internal cross-validation were carried out with programs developed in Matlab v. 7.0. (The Mathworks, Inc., Natick, MA, USA).

In both PCA and PLS-DA models, Savitzky Golay First Derivative has been applied as pre-processing technique (4 points in each side and a 2nd order polynomial) in order to correct the spectrum by separating overlapping peaks and to enhance spectral differences.

3. Result and discussion

Raman spectra of animal fats present mainly bands arising from vibrations of the hydrocarbon chains (saturated and unsaturated structures), and some contributions of carbonyl groups. Fig. 1 presents spectral data between 3100–2650 cm^{-1} and 1800–1200 cm^{-1}.

Spectra of poultry, pig, bovine, lamb fats, and fish oils seem to be similar. In order to highlight the spectral variations, a PCA was applied on the important regions of collected spectra included between 3100 and 2650 cm^{-1}, and 1800–1200 cm^{-1} which allowed the separation of the various fats. The graph on Fig. 2 built with the two first components shows the distribution of animal fats according to their composition. The first principal component represents 67% of the variance while the second one explains 24% of the variance.

Fish oils, poultry, pig and bovine fats are well distinguished. Lamb samples are close to the bovine ones which may be explained by a great similarity in their composition. It is also possible to notice that the first component expresses the variation between classes of animal fats and shows that fish oils have the highest positive values.

From the loading plot corresponding to the first component (Fig. 3), it is possible to determine peaks responsible of this distinction. The major bands are associated to the unsaturated structures (Table 3) which is in a good agreement with fish oils composition

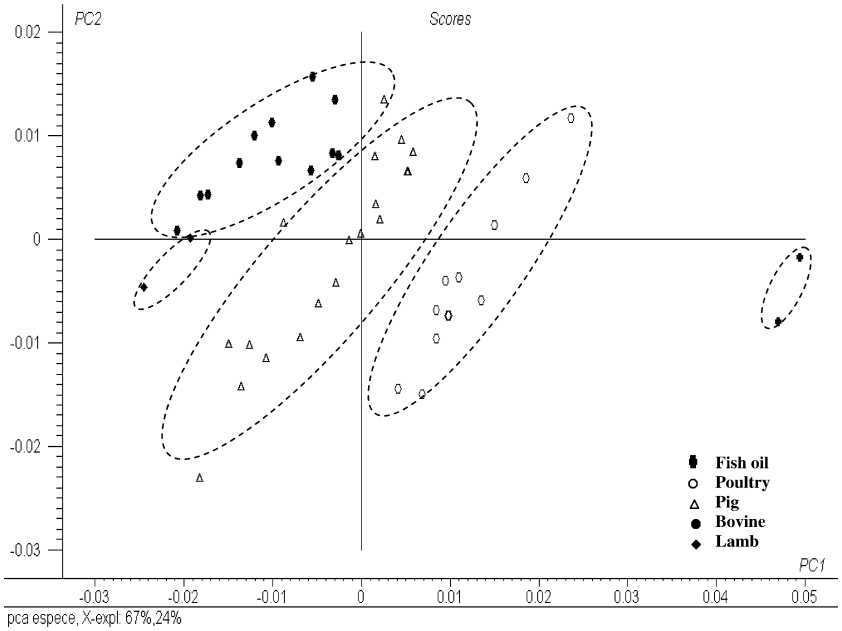

Fig. 2. PCA on FT-Raman spectra of animal fats from different sources.

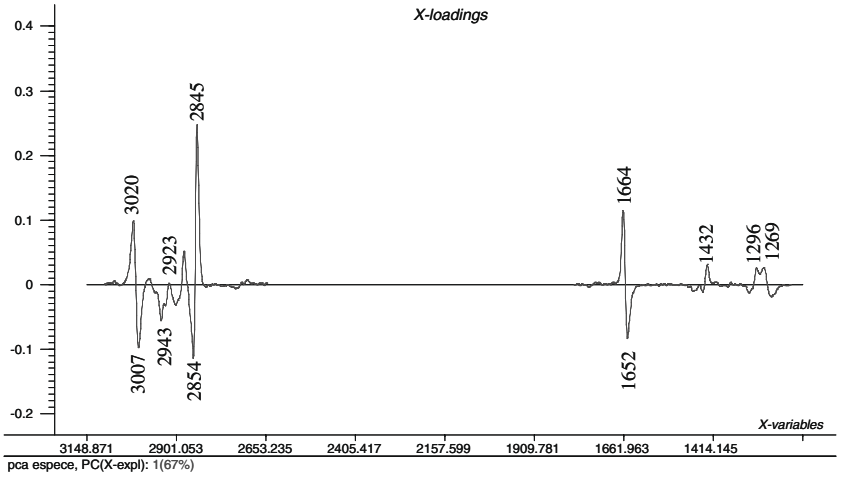

Fig. 3. Loading associated to the first principal component (PC1).

known as highly polyunsaturated [12,20]. In fact, the positive part of the loading which characterize mostly fish oils is mainly presented by the vibrations of structures with high degree of unsaturation: a band at 3020 cm^{-1} representing the asymmetric stretching of =C—H vibration group, a band at 1664 cm^{-1} which can be affected to the stretching of C=C group, and two shoulders at 1296 and 1269 cm^{-1} which can be associated to the deformation in plane of $=CH_2$ and the symmetric rocking of =C—H group, respectively. In the negative part, we can observe only fewer bands attributed to the unsaturated fractions (3007 and 1652 cm^{-1} corresponding to the symmetric stretching of =C—H and the stretching of C=C group). Moreover, on the basis of the paper published by Baeten et al. [12] where they made a correlation between the Raman shift and the degree of total unsaturation, it is possible to conclude that there are more polyunsaturated structures (peak at 3020 cm^{-1}) in the group corresponding to the positive part of the loading than for the other one that shows a peak at 3007 cm^{-1}.

Table 3
FT-Raman assignments.

Wavenumber (cm^{-1})	Intensity	Type de vibration
3020	m–s	Asymmetric stretching of =C—H
3007	m	Symmetric stretching of =C—H
2943	m	Asymmetric stretching of $—CH_2$
2870–2840	s–vs	Symmetric stretching of $—CH_2$
1665–1630	m–s	Stretching of C=C
1480–1440	vw	Scissoring vibration of CH_2
1296	sh	Deformation in plane of $=CH_2$
1269	sh	Symmetric rocking of =C—H

m, medium; s, strong; vs, very strong; sh, shoulder; vw, very weak; w, weak.

After that, we have projected pure animal meal fats and rendered fats on the obtained PCA space. The procedure applied has permitted us to evaluate the composition of these samples. In fact, the distribution of samples presented on PCA graph of Fig. 4 shows that studied mixtures (pure animal meal fats and rendered fats) are

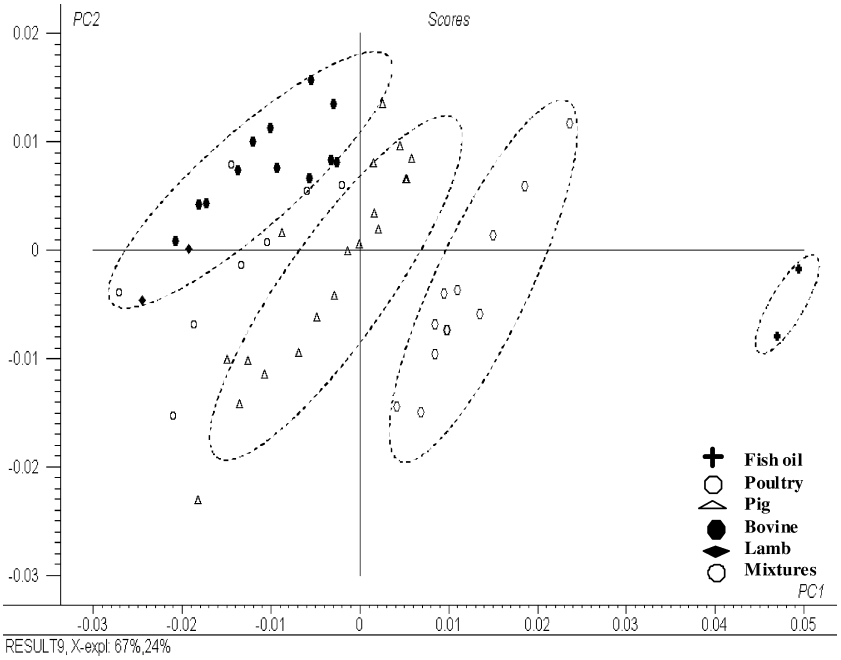

Fig. 4. Projection of pure and rendered samples on PCA graph.

located between pig and bovine samples indicating that they have similar composition as these groups of fats. The sample M2 composed of 80% of bovine and 20% of porcine is located in the cluster corresponding to bovine fats while the others (M1, M3, M4) which have approximate rates of bovine and porcine are situated between bovine and pig samples. Raman results reflect well the composition of these samples.

To confirm the obtained separation, PLS-DA was applied in order to discriminate fat samples. All samples were used for calculations but discriminant models were built only for poultry, pig and bovine groups. Lamb fats and fish oils contain a reduced number of samples so; the construction of models to discriminate each of them has no meaning. Results are presented in the Table 4 containing the values of sensitivity (*i.e.* percentage of bovine fats correctly classified as such), specificity (*i.e.* percentage of non-bovine fats correctly classified as such) and classification error (the average value of the mis-classified samples) (in%). Computations were carried out with internal cross-validation.

Table 4
Results of PLS-DA discrimination of animal fats (in %), using cross-validation.

Class	Poultry	Pig	Bovine
Sensitivity (CV)	0.917	0.900	0.923
Specificity (CV)	1.000	0.897	0.944
Classification error (CV)	0.0416	0.101	0.066

CV, cross-validation.

This table shows that all classes present good values of sensitivity and specificity (close to 1), which indicates a good discrimination of animal fats from different studied sources.

For poultry, the results represent the PLS-DA model between poultry samples and the rest (pig, bovine, lamb fats, and fish oils). A sensitivity and specificity of 0.917 and 1.000, respectively, show a clear separation of this class against the rest. For pig and bovine samples, the classification errors are equal to 0.101 and 0.066, respectively, showing a good discrimination of these groups with a minimal error.

Feeding fats of different categories (acid oils from chemical or physical refining, Lecithins, recycled cooking oils, hydrogenated fats from by-products fats, oils extracted from exhausted bleaching earth, fish oils, and animal fats) were analyzed by FT-Raman spectroscopy. Spectra shown in Fig. 5 present some differences.

The collected data have been analyzed by the application of PCA in order to visualise variations occurring between samples from different types of feedstock and various production technologies. The score plots of PC1 versus PC4 (Fig. 6) presents the spatial distribution of the samples in such space. Some samples grouped together creating clusters that corresponds to the fish and the HYBY. The rest of samples overlap making them difficult to distinguish.

The clear separation of fish oils and HYBY fats can be explained by the fact that fish oils are very rich on polyunsaturated structures

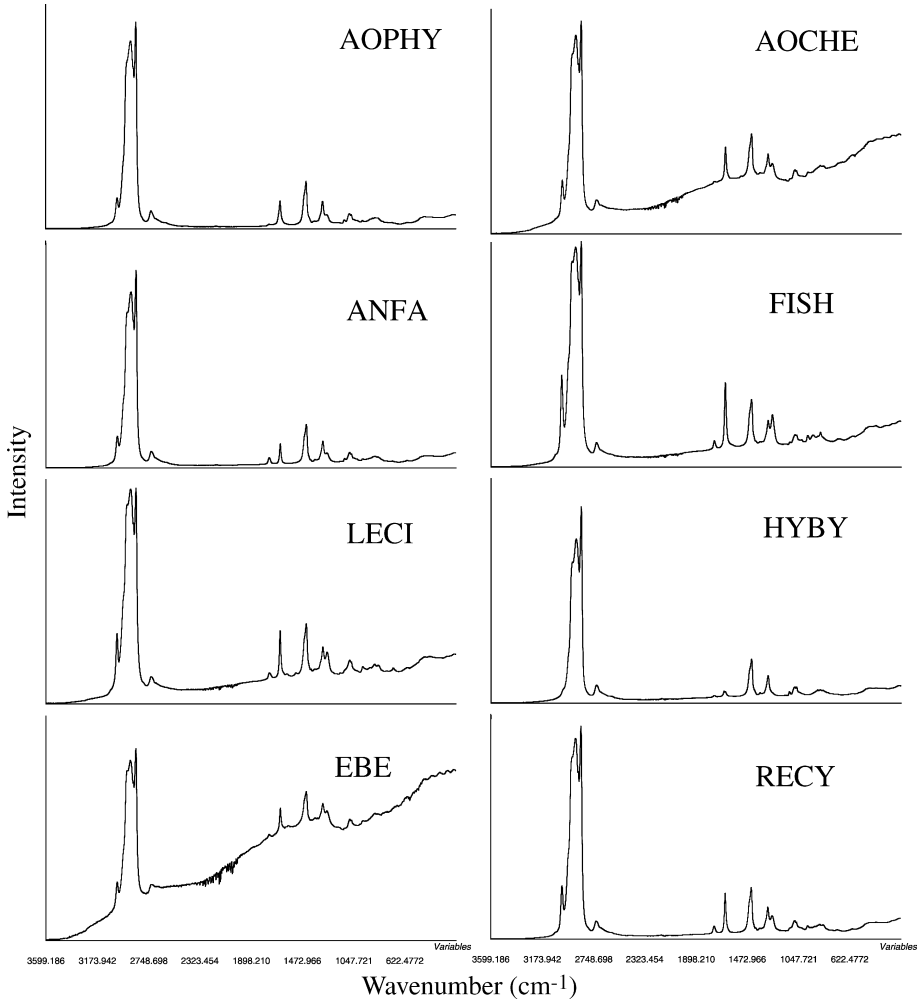

Fig. 5. FT-Raman spectra of fats from different categories.

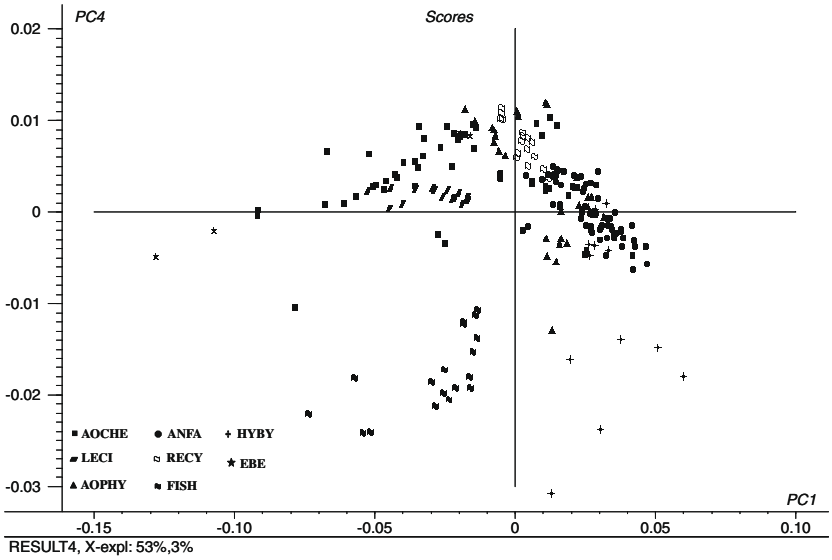

Fig. 6. PCA on FT-Raman spectra of fats from different categories.

Table 5
Results of PLS-DA discrimination of various fats (in %), using cross-validation.

Class	AOPHY	AOCHE	HYBY	RECY	ANFA	EBE	FISH	LECI
Sensitivity (CV)	0.964	0.911	1.000	0.875	0.958	1.000	1.000	1.000
Specificity (CV)	0.913	0.910	0.920	0.872	0.914	0.966	1.000	0.990
Classification error (CV)	0.061	0.089	0.040	0.127	0.064	0.017	0.000	0.005

AOPHY, acid oils from physical refining; AOCHE, acid oils from chemical refining; HYBY, hydrogenated by-products; RECY, recycled cooking oils; ANFA, animal fats; EBE, oils extracted from exhausted bleaching earth; FISH, fish oils; LECI, lecithins; CV, cross-validation.

while HYBY samples are hydrogenated and thus contain more saturated compounds.

As the objective of this study is to discriminate animal fats from the other categories, we have decided to treat spectra by a supervised method PLS-DA, which has permitted us to discriminate well the different classes. In fact, values of sensitivity, specificity and classification errors reported in Table 5 show a good classification in function of the different groups. Models were created for each of the classes studied but in this paper we will focus on animal fats ANFA for which the model presents a sensitivity and a specificity of 0.958 and 0.914, respectively. The classification error is equal to 0.064 showing the performance of the model to classify well animal fats studied.

The examination of PLS-DA graph permitting to discriminate animal fats from the others (Data not shown) indicates some overlapping between animal fat samples ANFA and the recycled cooking ones RECY. This may be explained by the fact that the recycled cooking oils, as given by the description of the sampling of the project Feeding Fat Safety [16], are coming from exhausted oils discarded by food industries and catering kitchen, which may contain certain quantities of fats from animal origin. One sample noted "HYBY" was classified as an animal fat. This may be due to the fact that this category of fats includes hydrogenated palm fatty acid distilled but also hydrogenated fatty acids of animal origin.

4. Conclusion

In this study, we investigated the ability of FT-Raman spectroscopy for the discrimination of fats with a focus of animal fats.

The method permits to classify rapidly animal fats in terms of their origin and informs us on the unsaturated structure of studied fats. Application of PCA and PLS-DA has allowed us to well classify animal fat samples. In addition, PLS-DA allowed us to discriminate terrestrial animal fats from the other categories and types of feeding fats like fish oils, and vegetable fats formed by physical or chemical refining.

Results make it possible to consider this analytical methodology as a preliminary study for the development of a rapid and reliable way for the discrimination of animal fats used in feeding stuffs.

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