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 category of the 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.

Keywords: Animal fats, discrimination, FT-Raman, PCA, PLS-DA.

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 [1,2,3], 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 category of the 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 the 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] and classification of oils and fats [12] and also on their unsaturated fatty acids [13,14,15]. 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 of feeding fats obtained from co-products or by-products of the food chain, by the characterisation of the composition, the quality and the determination of the contamination of fat materials used in animal feed. Within the framework of this project, the group of Gasperini and al. [8] has tried to establish rules for the classification of the feeding fats by using FT-IR spectroscopy.

The most commonly used fat sources in feed are animal (lard, tallow), vegetable (coconut oil, palm oil palm oil mix, 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. Material and method

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 (poultry, pig, bovine, lamb, pure, rendered fats, and fish oils), while 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 1**). Each group is identified by an acronym describing the type of fat by-product.

Table 1

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.

2.1 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.

2.2 Chemometric analysis

In order to achieve a reliable differentiation between fats 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.

Partial Least Squares – Discriminant Analysis (PLS-DA) [18] algorithm was used in order to build discriminant models permitting to discriminate between groups on the basis of the spectral information.

2.3 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 2^{nd} 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. **Figure 1** presents spectral data between 3100-2650 cm^{-1} and 1800-1200 cm^{-1}.

Figure 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 full collected spectra which allowed the separation of various fats. The graph built with the two first components on **figure 2** 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.

Figure 2

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 higher positive values.

Figure 3

From the loading plot corresponding to the first component (**Figure 3**), it is possible to determine peaks responsible of this distinction. The major bands are associated to the

unsaturated structures (**Table 2**) which is in a good agreement with fish oils composition known as highly polyunsaturated [12,19].

Table 2

After that, we have projected pure and rendered fats on the obtained PCA space. Although the origin of these samples is unknown, this procedure has permitted us to evaluate the composition of these samples. In fact, the distribution of samples presented on PCA graph of **figure 4** shows that studied pure and rendered fatss have similar composition as bovine or pig ones. This result is in a good agreement with those published by Bellorini et al. [7] who have previously worked on these samples in order to differentiate ruminant from porcine fats.

Figure 4

To confirm the obtained separation, PLS-DA was applied in order to discriminate fat samples. Results are presented in the **table 3** 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 %).

Table 3

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.

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 on **figure 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 (**Figure 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 [19] 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 4** show a good classification in function of the different groups. For animal fats, the model presents sensitivity and specificity of 0.958 and 0.914, respectively.

Table 4

The examination of PLS-DA graph permitting to discriminate animal fats from the others (Data not shown) indicates some overlapping between animal fat samples and the recycled cooking ones. 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. Also, the classification of one sample noted “HYBY” as an animal fat sample may be due to the fact that this category of fats represents hydrogenated palm fatty acid distilled and hydrogenated fatty acids of animal origin.

5. 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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6. References

[1] D.J. Capucille, M.H. Poore, G.M. Rogers, J Anim Sci, 82 (2004) 3038-3048.

[2] L.A. Galtin, M.T. See, J.A. Hansen, J. Odle, J Anim Sci ., 81 (2003) 1989-1997.

[3] A. Zaghini, G. Martelli, P. Simioli, L. Rizzi, Poult Sci., 84 (2005) 825-832.

[4] S. Woodgate, J. van der Veen, Biothechnol. Agron. Soc. Environ., 8 (4) (2004) 283-294

[5] EC(1998) Bovine spongiform encephalopathy (BSE), 3rd edn. Vademecum, 16 October 1998. European Community, Brussels.

[6] EC (2002) Regulation (EC) No 1774/2002 of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption. Off. J. Europ. Commun., L273:1-95.

[7] S. Bellorini, S. Strathmann, V. Baeten, O. Fumière, G. Berben, S. Tirendi, C. von Holst Anal. Bioanal. Chem., 382 (2005) 1073-1083.

[8] G. Gasperini, E. Fusari, D.B. Bella, P. Bondioli, Eur. J. Lipid Sci. Technol., 109 (2007) 673-681.

[9] V. Baeten, M. Meurens, Journal of Agricultural and Food Chemistry, 44(8) (1996), 2225-2230.

[10] V. Baeten, J. A. Fernández Pierna, P. Dardenne, M. Meurens, D.L. García-González, R. Aparicio-Ruiz, Journal of Agricultural and Food Chemistry, 53(16) (2005) 6201-6206.

[11] H. Sadeghi-Jorabchi, R.H. Wilson, P.S. Belton, J.D. Edwards-Webb, D.T. Coxon, Spectrochimica Acta Part A: Molecular spectroscopy, 47 A(9/10) (1991) 1449-1458.

[12] V. Baeten, P. Hourant, M.T. Morales, R. Aparicio, Journal of Agricultural and Food Chemistry, 46(7) (1998) 2638-2647.

[13] E. F. Olsen, E-O. Rukke, A. Flåtten, T. Isaksson, Meat Science, 76 (2007) 628-634.

[14] R.C. Barthus, R.J. Poppi, Vibrational spectroscopy, 26 (1) (2001) 99-105.

[15] H. Sadeghi-Jorabchi, H. Hendra, R.H. Wilson, P.S. Belton, JOACS, 67(8) (1990) 483-486.

[16] Web site: Feeding Fats Safety Research Project (FOOD-CT2004-007020) http://www.ub.edu/feedfat/

[17] H. Martens, T. Naes, (1989), Multivariate calibration. Chichester, UK: Wiley.

[18] Massart D.L., Vandeginste B.G.M., Buydens L.M.C., De Jong S., Lewi J.P., Smeyers Verbeke J., 'Chemometrics: A Textbook', vol. 2, Elsevier, Amsterdam, (1988).

[19] Aparicio R., Baeten V., OCL, 5(3), 14-16.

Figure caption

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

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

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

Figure 4: Projection of pure and rendered samples on PCA graph.

Figure 5: FT-Raman spectra of fats from different categories.

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

Table caption

Table 1: Characteristics of the fats by-products studied.

Table 2: FT-Raman assignments

Table 3: Results of PLS-DA discrimination of animal fats (in %), using cross-validation.

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

Figure 1

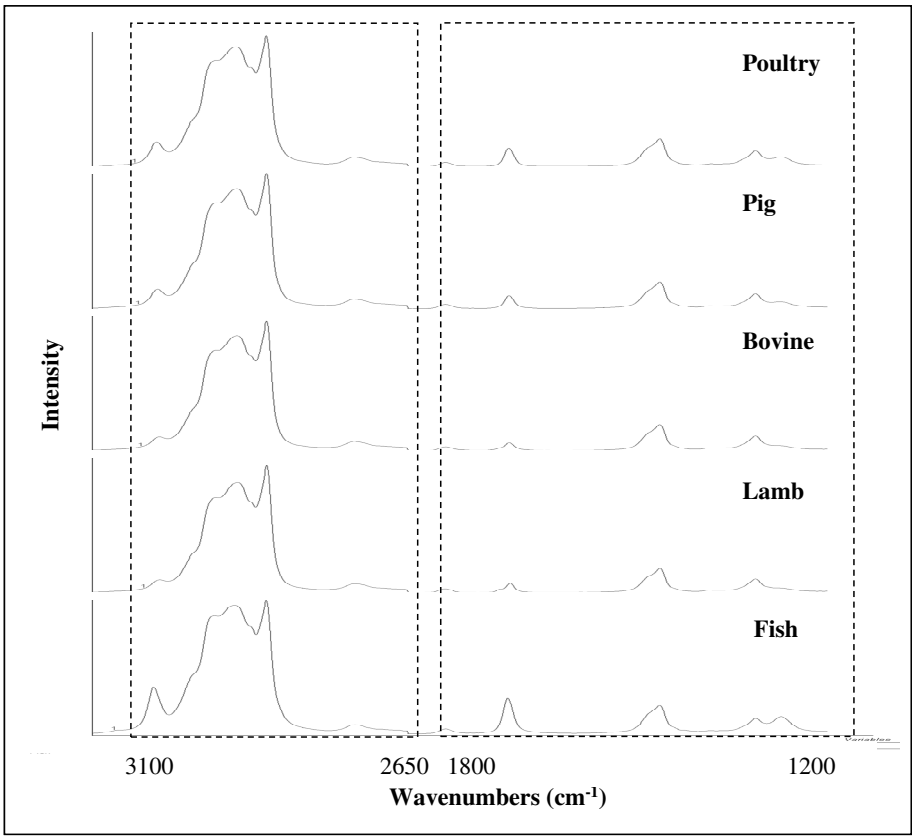
Poultry
Pig
Bovine
Lamb
Fish
Intensity
3100
2650
1800
1200
Wavenumbers (cm-1)

Figure 2

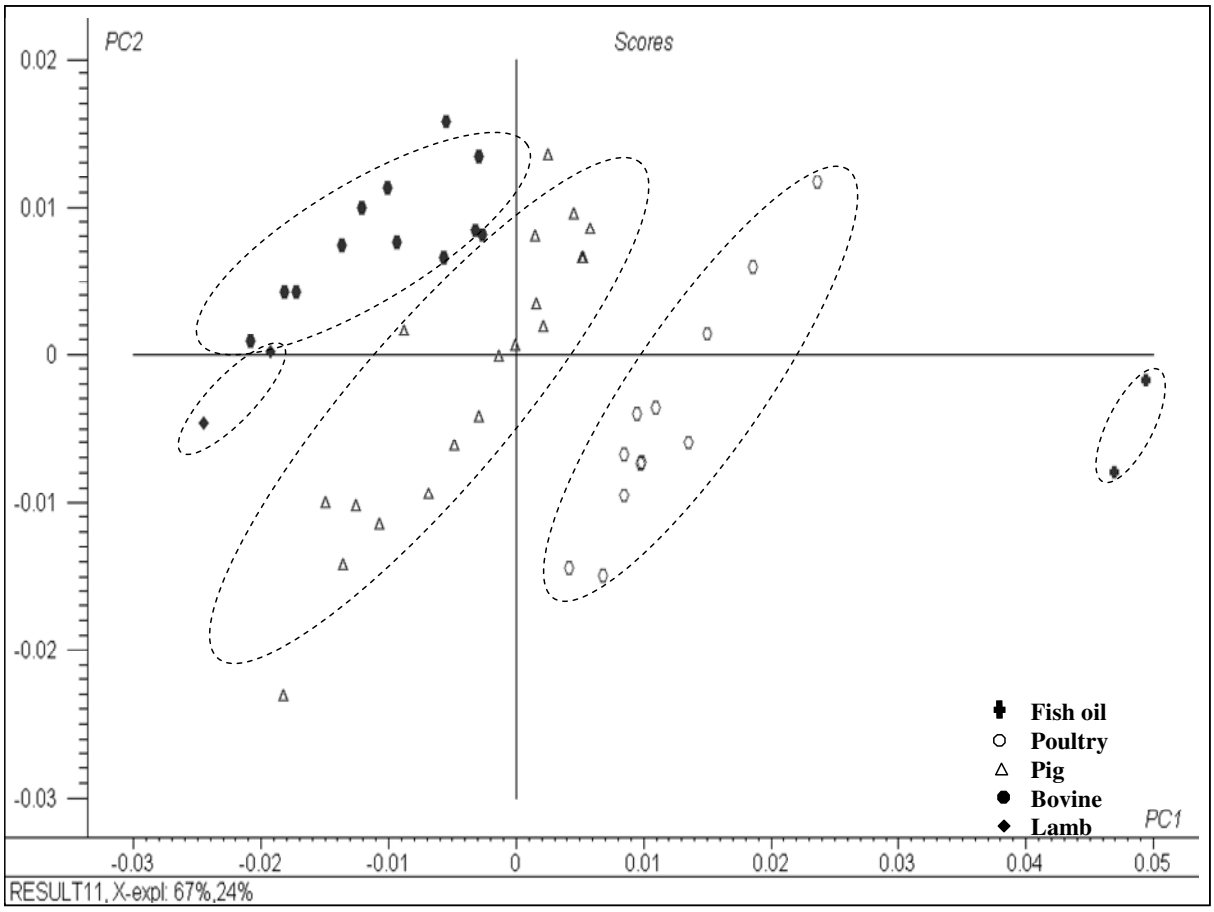
PC2
Scores
0.02
0.01
0
-0.01
-0.02
-0.03
-0.03
-0.02
-0.01
0
0.01
0.02
0.03
0.04
0.05
PC1
Fish oil
Poultry
Pig
Bovine
Lamb
RESULT11, X-expl: 67%,24%

Figure 3

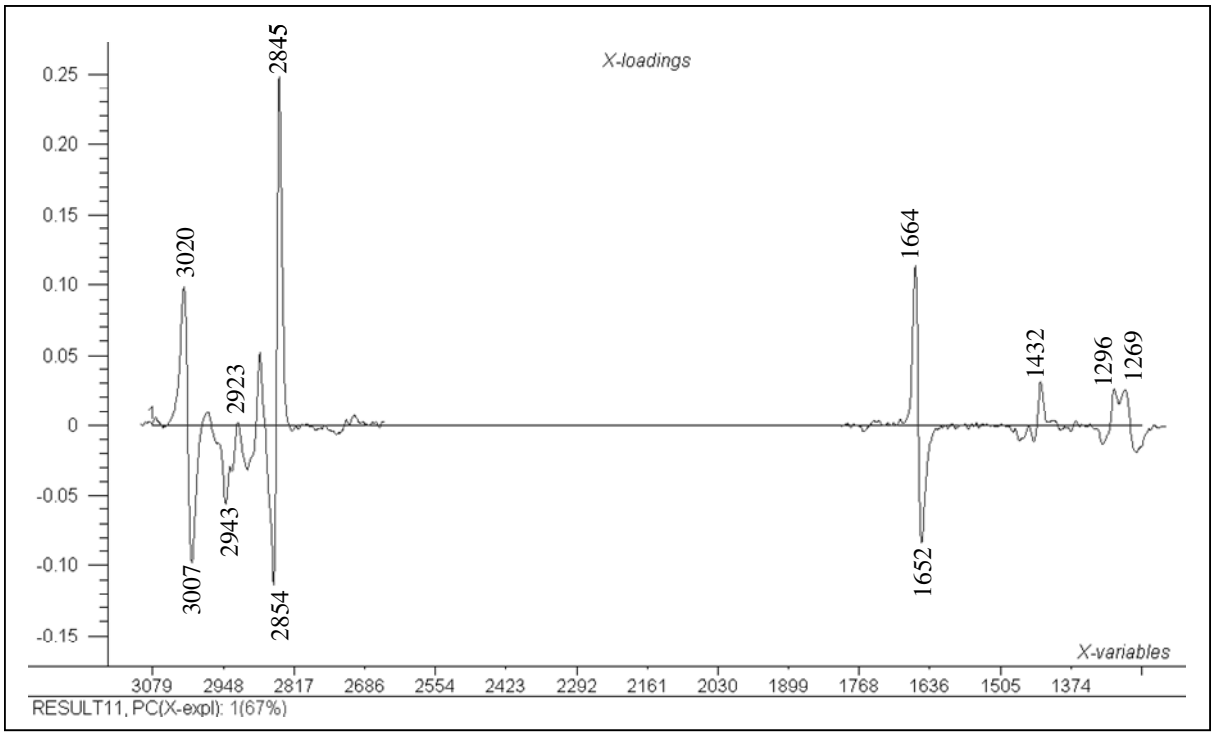
X-loadings
0.25
0.20
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
3020
2845
2923
2943
3007
2854
1664
1652
1432
1296
1269
X-variables
3079
2948
2817
2686
2554
2423
2292
2161
2030
1899
1768
1636
1505
1374
RESULT11, PC(X-expl): 1(67%)

Figure 4

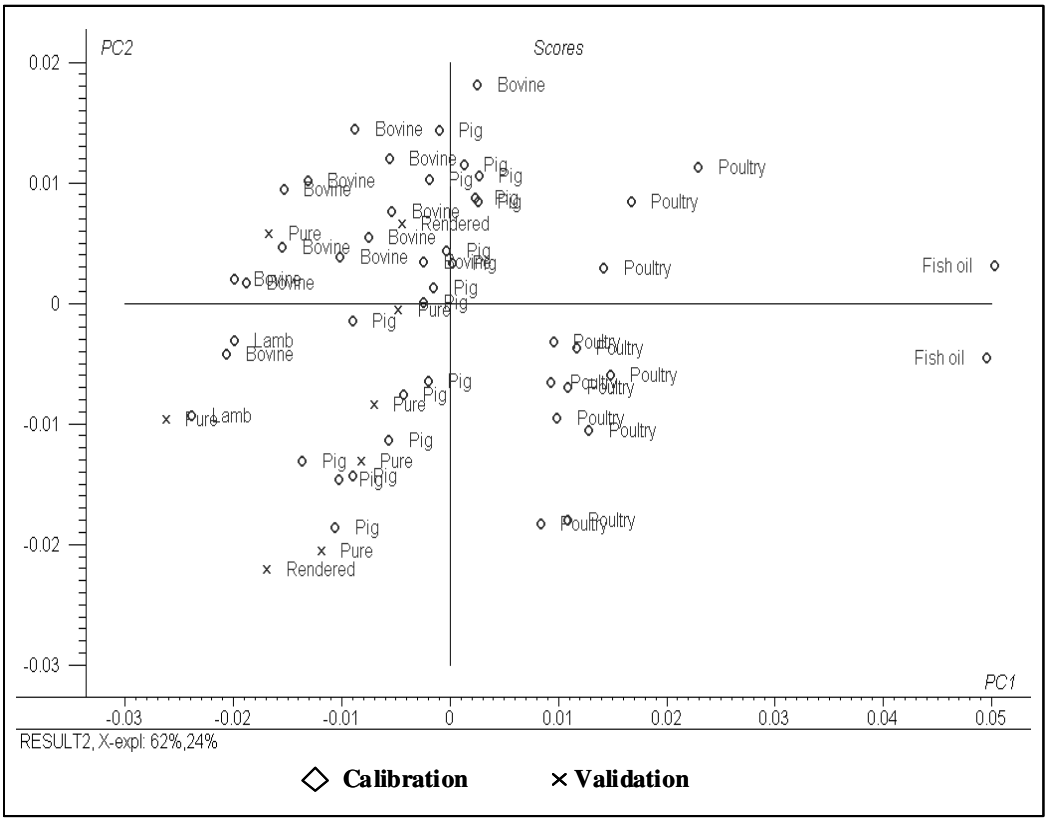
PC2
Scores
0.02
0.01
0
-0.01
-0.02
-0.03
PC1
-0.03
-0.02
-0.01
0
0.01
0.02
0.03
0.04
0.05
Bovine
Pig
Poultry
Rendered
Pure
Lamb
Fish oil
RESULT2, X-expl: 62%,24%
Calibration
Validation

Figure 5

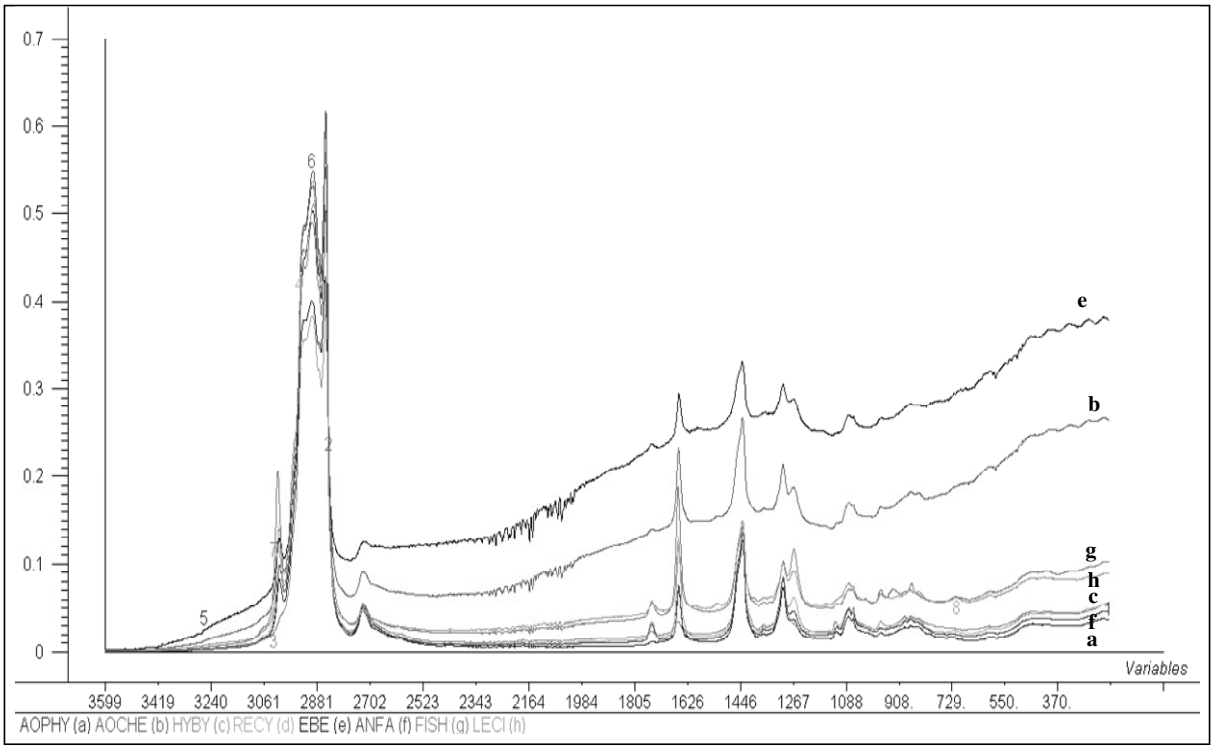
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
b
g
h
c
f
a
Variables
3599 3419 3240 3061 2881 2702 2523 2343 2164 1984 1805 1626 1446 1267 1088 908. 729. 550. 370.
AOPHY (a) AOCHE (b) HYBY (c) RECY (d) EBE (e) ANFA (f) FISH (g) LECI (h)

Figure 6

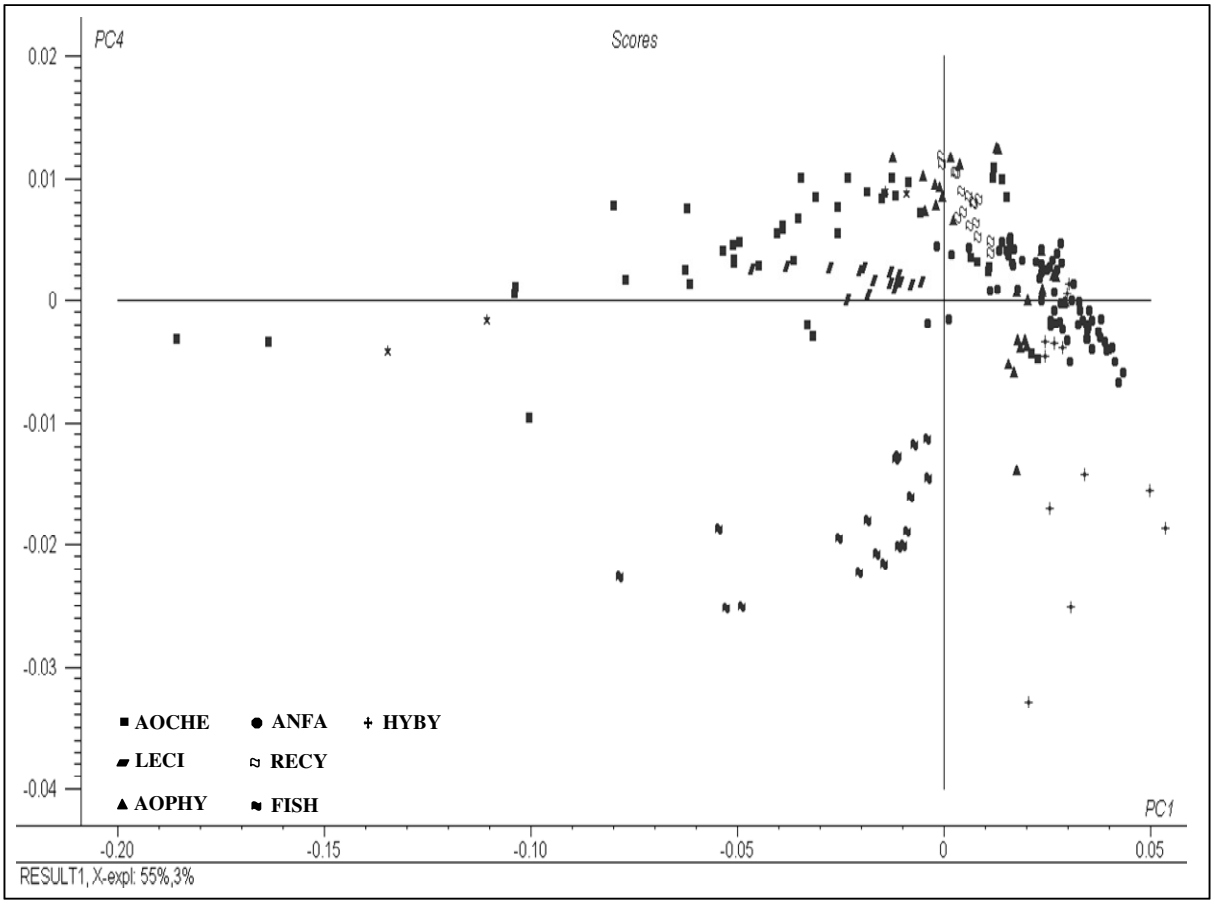
PC4
Scores
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
AOCHE
ANFA
HYBY
LECI
RECY
AOPHY
FISH
PC1
-0.20
-0.15
-0.10
-0.05
0
0.05
RESULT1, X-expl: 55%,3%

Table 1

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.
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.

Table 2

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

Table 3

Class	Poultry	Pig	Bovine	Lamb	Fish oil
Sensitivity (CV)	0.917	0.900	0.923	1.000	1.000
Specificity (CV)	1.000	0.897	0.944	1.000	1.000
Classification error (CV)	0.0416	0.101	0.066	0	0

CV: Cross-validation

Table 4

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*