

1 Authentication of feeding fats: classification of animal fats, fish
2 oils and recycled cooking oils
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16 **Abstract**

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18 Classification of fats and oils involves the recognition of one/several markers typical
19 of the product. The ideal marker(s) should be specific to the fat or oil. Not many
20 chemical markers fulfill these criteria. In fact, the natural variability of chemical
21 composition prevents having one discriminative marker for each type of oil. In
22 addition, oil refining and fat modifications may greatly influence the chemical
23 composition. Authenticity assessment is a difficult task which in most cases requires
24 the measurement of several markers and must take into account natural and
25 technology-induced variation. The present study focuses on the identity prediction of
26 three by-products of the fat industry (animal fats, fish oils, recycled cooking oils), the
27 first two of which may be used for animal feeding. Their identities were predicted by
28 their triacylglycerol fingerprints, their fatty acid fingerprints and their profiles of
29 volatile organic compounds. Partial Least Square Discriminant Analysis allowed
30 samples to be assigned successfully into their identity classes. Most successful were
31 triacylglycerol and fatty acid fingerprints (both 96%). Proton Transfer Reaction Mass
32 Spectra of the volatile compounds predicted the identity of the fats in 92% of the
33 samples.

1. Introduction

Co- and by-products of the food industry can have significant nutritional value and be therefore interesting for animal feeding. For integration of the food and feed chain a transparent classification of fats is imperative. On the one hand, safe fats with high nutritional significance should be allowed to use for feeding purposes, on the other hand a transparent classification will allow better controls and eventually protection of the consumer (Gasperini et al., 2007). In the EU research project 'Feeding Fats Safety' (FOOD-CT2004-007020) an attempt was made to classify feeding fats into ten defined classes. Classes included acid oils from chemical or physical refining, lecithins, recycled cooking oils, animal fats, oils from exhausted bleaching earth, fish oils, hydrogenated by-products, fatty acid calcium soaps, and a group miscellaneous products.

Fats and oils are complex mixtures comprising of a wide range of compounds. The main components are triacylglycerols (TAGs), diacylglycerols (DGs), free fatty acids (FFAs), phospholipids, and other minor components. The most important group of compounds is the TAGs, which are in chemical terms trihydric alcohols esterified with fatty acids (FAs) (Buchgraber et al., 2004). TAGs vary in their total carbon number, their degree of unsaturation and the position and configuration of the double bonds in each FA. The exact position of the 3 FAs on the glycerol backbone determine the region-specificity/stereo-specificity of the TAG molecule. In each oil or fat numerous TAGs are possible due to the large number of possible FA combinations on the glycerol backbone. Animal fats are rather complex and may consist of 10-40 different FAs. In ruminant fats, additional FAs are present due to the ruminal

58 microbial metabolism. For instance, over 400 different FAs have been identified in
59 milk fat (Buchgraber et al., 2004).

60 It is important to be able to check the real identity of a feeding fat for a variety
61 of reasons: legal compliance, economic reasons, use of safe ingredients, guarantee of
62 a constant well-defined quality, etc.. Traditional analytical strategies to determine the
63 identity of a feed or food macro-component, or to uncover adulteration and guarantee
64 quality have relied on wet chemistry determining the quantity of a marker compound
65 or compounds and subsequent comparison with those established in reference material
66 (Karoui & Baerdemaker, 2007). Fats used for feedings purposes have so far been
67 mostly characterized by a few simple parameters relating to caloric value: total fat
68 content, moisture, impurities, unsaponifiables (Gasperini et al., 2007). Traditional
69 methods for evaluating the quality of vegetable oils have relied on the measurement of
70 physico-chemical properties such as density, refractive index, saponification value,
71 iodine and acid numbers (Zhang et al., 2006). However, in order to discriminate
72 between fat classes, and to allow the prediction of the identity of unknown samples
73 for authentication purposes, a multivariate data evaluation seems the more promising
74 approach.

75 In the present study the identities of three fat classes (animal fats, fish oils,
76 recycled cooking oils) were predicted by their multivariate TAG profiles, their FA
77 profiles, and their volatile profiles with application of chemometrics. TAG and FA
78 profiling are rather relatively simple, commonly used techniques. However, the non-
79 targeted, non-biased chemometric approach is an interesting new aspect. Volatile
80 analysis was evaluated for screening purposes. Proton Transfer Reaction Mass
81 Spectrometry (PTR-MS) allows very rapid non-destructive measurements of volatile

organic compounds (VOCs; full mass spectrum < 30s), and may therefore be an interesting additional technique.

2. Materials and methods

2.1. Materials

Fifty-three samples, classified as animal fats (36), fish oils (9), and recycled cooking oils (8) were collected in the EU Research Project ‘Feeding Fats Safety’ (FOOD-CT2004-007020). The samples originated from a variety of European countries and some had a non-European origin. The samples were selected taking into account as much as possible both natural and technology-induced variation. Animal fats concerned products from the rendering process (sterilization, cooking and melting of animal tissues). Most animal fat samples originated from poultry (10), the remainder was classified as bovine, pork, sheep, ruminant, and some samples were mixtures of different species. Fish oils comprised oils obtained by rendering whole low-value fish or fish waste from the food industry (e.g. canned tuna, smoked salmon, salted sardines, etc.). The recycled cooking oils involved products from the collection of exhausted oils, leftover from the deep-frying industry or catering. It is not permitted to use the latter for feed applications, they are normally applied for technical/industrial use (Gasperini et al., 2007). Sample material was stored at -20°C in absence of light until analysis was carried out.

2.2. Methods

2.2.1. TAG analysis (update Maikel/Henk)

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108 The TAG analysis was carried out according to the Draft International
109 Standard ISO/DIS 17678|IDF 202 (Milk fat – Detection of foreign fats by gas
110 chromatographic analysis of triglycerides). Tricaprion (C18) was added to each
111 sample as internal standard. The reference material CRM 519 (IRMM, Geel,
112 Belgium) was used for determining the calibration factor of each triglyceride. Relative
113 concentrations (the total TAG measured was normalized to 100%) were calculated.
114 All fats and oils were analysed in triplicate.

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116 2.2.2. *FA methyl ester (FAME) analysis* (update Maikel/Henk)

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118 The fats were methylated and the fatty acid methyl esters analysed according
119 to the international standard ISO 15885:2002. Nonanoic acid (C9:0) was added as
120 internal standard (Sigma Aldrich Chemie, Zwijndrecht, the Netherlands). As reference
121 material a home-made standard FAME mixture composed of C4:0, methyl butyrate
122 (Fluka, 19358, Sigma Aldrich Chemie, Zwijndrecht, the Netherlands); C6:0, methyl
123 capraote (Fluka, 21599); C8:0, methyl caprylate (Fluka, 21719); C10:0, methyl
124 decanoate (Fluka, 21479); C12:0, methyl laurate (Fluka, 61689); C14:0, methyl
125 myristate (Fluka, 70129); C16:0, methyl palmitate (Fluka, 76159); C18:0, methyl
126 stearate (Fluka, 85769); C18:1, methyl oleate (Fluka, 75160); C9:0, methyl nonanoate
127 (Sigma, 245895) was used to calculate calibration factors of the various FAMEs. FA
128 concentrations were expressed as relative concentrations (the total FAME measured
129 was normalized to 100%). All fats and oils were analysed in triplicate.

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131 2.2.3. *VOC analysis*

PTR-MS is a technique for analysis of volatile compounds. Proton transfer reactions are used to induce chemical ionization of the vapors to be analyzed. The sample gas is continuously introduced into a drift tube, where it is mixed with H_3O^+ ions formed in a hollow cathode ion source. Volatile compounds that have proton affinities higher than water (>166.5 kcal/mol) are ionized by proton transfer from H_3O^+ , mass analyzed in a quadrupole mass spectrometer and eventually detected as ion counts/s (cps) by a secondary electron multiplier. The outcome is a mass resolved fingerprint of the total volatile profile of a samples. PTR-MS is interesting for this fingerprinting approach as (1) it requires no pre-treatment of the sample, (2) it allows rapid measurements (typically < 1 min for a complete mass spectrum) and (3) the technique is extremely sensitive (ppt level). In the present study, the volatiles were measured in the headspace of the butters after equilibration (van Ruth et al., 2007).

For headspace analysis, 5 ml of fat or oil was placed in a glass flask (250 ml) at 30°C for 30 min to allow equilibration. Preliminary experiments showed that 30 min was sufficient for equilibration. Three replicates of each sample were analysed. The volatile organic compounds (VOCs) in the headspace of the samples were analysed at 30°C by PTR-MS according to the method described by Lindinger, Hirber, & Paretzke (1993). A constant drift voltage of 600 V and a pressure of 2.09 ± 0.01 mbar were maintained in the reaction chamber. The headspace was drawn from the sample flask at 30°C at a rate of 55 ml/min which was led through a heated transfer line (60°C) into the high sensitivity PTR-MS for on-line analysis. Data were collected for the mass range m/z 20-165 using a dwell time of 0.2 s.mass^{-1} . The instrument was operated at a standard E/N (ratio of electric field strength across the drift tube, E, to buffer gas density, N) of 138 Td ($1\text{Td}=10^{-17} \text{ cm}^2 \text{ V molecule}^{-1}$). Inlet

and drift chamber temperatures were 60°C. Each sample was analysed for at least 5 full mass scans. The headspace concentrations of the compounds during the cycles #3, #4 and #5 were calculated as described by Hansel, Jordan, Holzinger, Prazeller, Vogel, & Lindinger (1995) and background and mass discrimination corrections were applied. Headspace concentrations were subsequently averaged over the three mass scans for further statistical analysis. Previously in preliminary experiments some fat samples had been analysed for seven cycles: the results did not show consistent changes in headspace concentrations (especially no decrease) after the first cycle. Therefore, cycles #3, #4 and #5 were selected for calculations.

2.2.4. Statistical analysis

The data were explored by Principal Component Analysis (PCA). Subsequently, they were subjected to Agglomerative Hierarchical Cluster Analysis (AHC) for non-supervised clustering of the data. In the study the identity of the samples was known, which allowed use of Partial Least Square Discriminant Analysis (PLS-DA) models (Barker and Rayens, 2003), a supervised clustering technique. These models were estimated to predict the identity of the samples using either the TAG data, FA data or VOC data. PLS-DA performs a dimension reduction on the predictor variables. The dimensions (components) extracted are composed such that they exhibit maximal correlation with Y (class membership, e.g. animal fat, fish oil, recycled cooking oil). After estimation of the classification model, its performance was evaluated by means of leave-1-out cross-validation: one of the samples was randomly removed from the data set, and a model built with the remaining samples was used to classify this left out sample. The procedure was repeated with the

remaining samples one by one to obtain predictions for all samples. For model optimization data pre-treatment was carried out and various numbers of components were explored before the final model was selected. All statistical analyses were carried out using Pirouette 4.0 (Infometrix, Bothell, WA, USA).

3. Results and discussion

3.1. TAG, FA and VOC data

3.1.1. TAG data

The TAG profiles of the animal fats, fish oils, and recycled cooking oils were analysed by GC. Twenty-one triacylglycerols and cholesterol were quantified in the fats. Relative concentrations varied between and within fat groups (Table 1). In animal fats the C52 (44%), C54 (26%), and C50 (17%) were the predominant TAGs, whereas in fish oils predominant TAGs comprised a larger group: C54 (17%), C56 (17%), C52 (15%), C58 (14%). For the recycled cooking oils highest TAG contents were observed for C54 (54%), C52 (22%), and C50 (7%). The composition of the animal fats is similar to the compositions published for lard and beef tallow (Precht, 1992). TAGs make up the major part of naturally occurring fats and oils. Analysis of intact TAGs is, as in the present study, usually performed by chromatographic methods. Apart from the GC technique used here, high performance liquid chromatography in normal and reversed phase mode, thin-layer chromatography, and supercritical fluid chromatography are employed (Buchgraber et al., 2004).

Some exploratory statistics were applied to the TAG data set: data were subjected to PCA. PCA is used abundantly in all forms of analysis – from

neuroscience to computer graphics – because it is a simple, non-parametric method of extracting relevant information from confusing data sets. PCA provides a roadmap for how to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structure that often underlie it. It is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. A scores (sample) plot and loadings (TAGs) plot of the first two dimensions of the PCA are displayed in Fig. 1. Fish oils formed a group and were separated from animal fats and recycled cooking in the first dimension (upper plot, horizontal axis). The animal fats and the recycled cooking oils were separated in the second dimension (upper plot, vertical axis), although some overlap existed. The fish oil samples correlated with relatively high intensities of the TAGs in the lower right quadrant of the loadings plot (lower plot), i.e. C46, C54-C58, C60-C64.

Cluster analysis was carried out on the TAG data in order to find (unsupervised) an optimum tree (dendrogram) or set of clusters. A hierarchical classification proceeds by grouping together the most similar samples, and subsequently groups into progressively larger and more heterogeneous units. At each stage the groups or samples linked are those giving the minimum increase in group heterogeneity. A dendrogram is presented in Fig. 2. It reveals that initially two groups are formed: the fish samples and the other samples. A division in the other samples group perfectly divides the animal fats and the recycled cooking oils.

3.1.2. FA and VOC data

The TAG composition is directly related to the FAME composition, which is presented in Table 2. Thirty different FAs were determined. Animal fats were rich in C18:1 (40%), C16:0 (23%), C18:0 (13%), and C18:2 (11%). Fish oils were composed mainly of C18:1 (17%), C16:0 (16%), C22:6 (15%), C20:5 (9%), and recycled cooking oils of C18:1 (39%), C18:2 (37%), C16:0 (13%).

Volatile profiles of the fat samples were analyzed by PTR-MS, and mass resolved fingerprints of headspace volatiles were obtained. All of the fat samples produced signals on most masses in the measurement range 20-130 amu indicating the complex VOC composition of the three types of fat. Ions of volatiles in the mass range > 130 amu were more common in fish oils and recycled cooking oils than in animal fats. Mean sample mass spectra for each fat group are displayed in Fig. 3. The VOCs with higher volatility (lower mass) dominate the spectrum in terms of signal intensity, although lower masses may also result from fragmentation of larger compounds. Generally, fish oils and recycled oils showed considerably higher intensities of volatiles than the animal fats. Although PTR-MS is a one dimensional technique, mass resolved fingerprints allow tentative assignment of ions to origins in volatiles with fragmentations patterns typical of PTR-MS (Buhr et al., 2002). Some of the protonated masses showing large signals can be tentatively assigned to volatiles based on reports of their presence in fats and oils and their known fragmentation patterns: e.g. m/z 39 (hexenyl acetate, fragment), 41 (hexanol (fragment), 43 (acetic acid, hexanol), 45 (acetaldehyde), 47 (ethanol), 57 (hexanal, hexenal, hexanol), 59 (acetone, propanal, hexenol (fragment)), 61 (acetic acid, variety of esters), 63

(dimethyl sulfide, acetaldehyde (hydrate)), 69 (pentanal), 73 (butanal, 2-butanone), 75 (methyl acetate), 81 (hexanal (fragment), 87 (hexanol), 89 (butyrate esters, butanoic acid). Hai and Wang (2006) reported an oil authentication study with use of an electronic nose. The electronic nose was used for the detection of maize oil adulteration in camellia seed oil and sesame oil. Based on artificial neural network models, the electronic nose could not predict the percentage of adulteration in camellia seed oil, but could be used successfully in the quantitative determination of adulteration in sesame oil. Gonzalez Martin et al. (2001) reported the successful classification of virgin olive oil, non-virgin olive oil and seed oils by their electronic nose fingerprints. The concept of an electronic nose was proposed in 1982 by Persaud and Dodd. It is an electronic system with a dynamic headspace sampler which detects volatiles with a variety of sensors. Its sensor output is usually processed with a statistical pattern recognition technique. Major drawback is that no information on the identity of the compounds is obtained.

3.2. Classifications

3.2.1. TAG data

Due to the large variation within fat groups it is difficult to evaluate the data using a univariate approach. Therefore, a multivariate approach was adopted. When considering the available pattern recognition methods, a distinction can be made between pure classification and class-modeling techniques (Vandeginste et al., 1998). The former divide the sample space in as many regions as the number of classes under investigation, so that if a sample falls in a specific region of the

hyperspace it is assigned to the corresponding class. On the other hand, class-modeling tools build a separate model for each category: samples fitting the model are accepted by that category, while samples falling outside the model are considered as outliers for the specific class. In the present study all classes (fat types) were known, which means that a pure classification method could be used. Furthermore, in PTR-MS analysis we deal with more variables than samples, which implies that discriminant analysis is not appropriate and a PCA-like reduction of the variables is required before samples can be classified. PLS-DA combines both aspects.

The statistical analyses in this study used the TAG, FA and VOC data as ‘fingerprints’, i.e. the compounds/masses and their corresponding signal intensities in each sample mass spectrum act as a pattern for inter-comparison of the samples.

PLS-DA was applied to the TAG data to classify the samples into fat types (animal fat, fish oil, recycled cooking oil). A five-component model (data auto-scaled) was fitted to estimate the identity of the samples. Rates of successful classification in cross-validation are listed in the leftmost part of Table 3. Of all samples, 96% were successfully classified into their fat type classes: 100% of the animal fats, 89% of the fish oil and 88% of the recycled cooking oils. The scores of the samples on the first two PLS-dimensions are presented in Fig. 4. Samples FISH-6 and RECI-6 were the only samples that were misclassified. Both were more or less on the demarcation line between two classes. The fish oil originated from France, and the recycled cooking oil from Italy. Considering the wide range of sample material (origin, species, technology, etc.) it is surprising that the samples could be so successfully classified. The animal fat group was the largest subset and consisted of 10 poultry fat samples and 26 fat samples of other species. PLS-DA classification of the animal fat samples into poultry and non-poultry groups resulted in a two component model (no data pre-

treatment). Cross-validation resulted in 97% correct classification. The single misclassification concerned a chicken fat sample from Spain that was classified as 'other fats'. A scores plot of the first two dimensions of the PLS-DA on the animal fat data is displayed in Fig. 5. ANFA-23 is the incorrectly classified fat sample.

3.2.2. FA and VOC data

FA data were subjected to the same statistical treatment as the TAG data, results are listed in the center of Table 3 (data pre-treatment: autoscaling). Overall, 96% of the samples were successfully classified. There were two misclassifications only: samples FISH-9 and RECI-6. Since RECI-6 was also misclassified in the TAG analysis, this sample had probably unusual compositional characteristics compared to the other recycled cooking oils. The raw data showed that RECI-6 was high in FA C16:0 and low in C18:2 compared to the other samples. Its TAG composition revealed correspondingly higher concentrations of C50 and C52, and lower levels of C54.

PLS-DA classification of the VOC data resulted in 92% of the samples in successful classifications: 100% of the animal fats, 88% of the fish oils and 63% of the recycled cooking oils (Table 3, rightmost; data pre-treatment: autoscaling). Four samples were misclassified, one fish oil (FISH-8) and three recycled cooking oils (RECI-1, -4, -5). The fish oil originated from Norway, the cooking oils from Italy (1) and Spain (2). The PLS-DA plot in Fig. 6 shows that the misclassified samples are at the demarcation line of the classes.

4. Conclusions

332
333 TAG, FA and VOC ‘fingerprints’ were made for a range of animal fats, fish oils, and
334 recycled cooking oils. Multivariate statistical analysis of these data allowed samples
335 to be separated successfully into classes of identity. Most successful in terms of
336 prediction rate were the TAG and FA fingerprints (both 96%). VOC mass spectral
337 fingerprints acquired by PTR-MS resulted in a success rate of 92% but its advantage
338 of the other two methods is its simplicity, rapidity, efficiency, and reproducibility.
339 Direct PTR-MS headspace analyses were made without prior sample preparation and
340 mass spectra were obtained in just over 2 min. Such a method could, combined with
341 auto-sampling procedures, a future screening technique that is both fast and accurate.
342 The TAG and FA methods are more time-consuming, robust, and can be used for
343 identity confirmation assessments.

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346
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Table 1

Relative triacylglycerol composition of animal fats, fish oils, and recycled cooking oils (mean \pm SD^a)

Triacylglycerol	Animal fats (n=36)	Fish oils (n=9)	Recycled cooking oils (n=8)
Cholesterol	0.28 \pm 0.21	0.77 \pm 0.37	0.02 \pm 0.01
C24	0.01 \pm 0.01	0.25 \pm 0.52	0.03 \pm 0.01
C26	0.01 \pm 0.01	0.00 \pm 0.01	0.08 \pm 0.03
C28	0.00 \pm 0.01	0.02 \pm 0.04	0.02 \pm 0.01
C30	0.02 \pm 0.01	0.11 \pm 0.14	0.00 \pm 0.01
C32	0.06 \pm 0.07	0.22 \pm 0.27	0.14 \pm 0.28
C34	0.41 \pm 0.36	0.51 \pm 0.35	0.28 \pm 0.20
C36	1.49 \pm 1.29	0.79 \pm 0.55	1.45 \pm 0.87
C38	1.34 \pm 1.21	0.83 \pm 0.51	3.26 \pm 2.10
C40	0.08 \pm 0.06	0.81 \pm 0.44	0.36 \pm 0.09
C42	0.10 \pm 0.06	0.78 \pm 0.32	0.26 \pm 0.04
C44	0.38 \pm 0.16	0.67 \pm 0.15	0.51 \pm 0.06
C46	1.14 \pm 0.74	1.62 \pm 0.58	0.95 \pm 0.21
C48	5.04 \pm 2.44	4.74 \pm 1.32	1.32 \pm 0.78
C50	17.22 \pm 2.90	9.55 \pm 1.85	6.81 \pm 3.57
C52	43.55 \pm 6.13	14.51 \pm 1.80	21.59 \pm 3.63
C54	26.04 \pm 5.14	17.30 \pm 0.71	53.51 \pm 8.92
C56	2.39 \pm 0.65	17.03 \pm 1.04	5.68 \pm 0.44
C58	0.43 \pm 0.12	14.06 \pm 1.34	2.34 \pm 0.38
C60	0.00 \pm 0.00	9.46 \pm 1.71	1.06 \pm 0.16
C62	0.00 \pm 0.00	5.01 \pm 0.96	0.30 \pm 0.04
C64	0.00 \pm 0.00	0.97 \pm 0.23	0.00 \pm 0.00

^a Standard deviations were calculated over sample means, not over replicate

measurements.

Table 2

Relative fatty acid composition of animal fats, fish oils and recycled cooking oils
(mean \pm SD^a)

Fatty acid	Animal fats (n=36)	Fish oils (n=9)	Recycled cooking oils (n=8)
C4:0	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.01
C6:0	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.01
C8:0	0.02 \pm 0.01	0.10 \pm 0.01	0.11 \pm 0.02
C10:0	0.06 \pm 0.03	0.00 \pm 0.00	0.02 \pm 0.02
C12:0	0.14 \pm 0.07	0.08 \pm 0.02	0.10 \pm 0.10
C14:0	1.89 \pm 0.95	4.78 \pm 1.32	0.35 \pm 0.19
C14:1	0.25 \pm 0.17	0.05 \pm 0.01	0.01 \pm 0.01
C15:0	0.22 \pm 0.15	0.57 \pm 0.19	0.03 \pm 0.01
C16:0	23.29 \pm 1.97	15.94 \pm 2.30	12.77 \pm 4.68
C16:1	3.05 \pm 0.78	5.44 \pm 1.10	0.37 \pm 0.19
C18:0	13.32 \pm 5.46	3.81 \pm 1.06	4.33 \pm 0.50
C18:1 trans 1	0.30 \pm 0.18	0.13 \pm 0.04	0.64 \pm 0.79
C18:1 trans 2	1.00 \pm 1.02	0.31 \pm 0.28	0.08 \pm 0.19
C18:1	36.32 \pm 3.95	12.94 \pm 3.08	36.69 \pm 3.80
C18:1 cis 1	1.88 \pm 0.58	3.21 \pm 0.80	1.27 \pm 0.58
C18:1 cis 2	0.35 \pm 0.23	0.22 \pm 0.07	0.14 \pm 0.09
C18:1 total ^b	39.85 \pm 3.56	16.80 \pm 3.06	38.83 \pm 4.92
C18:2	10.81 \pm 7.90	2.21 \pm 1.39	37.33 \pm 11.18
C18:2 conj	0.18 \pm 0.13	0.04 \pm 0.03	0.04 \pm 0.01
C18:3	1.05 \pm 0.77	0.89 \pm 0.47	0.92 \pm 0.94
C20:1	0.00 \pm 0.00	3.72 \pm 1.77	0.00 \pm 0.00
C20:2	0.00 \pm 0.00	0.38 \pm 0.10	0.00 \pm 0.00
C20:3 (n-6)	0.00 \pm 0.00	0.15 \pm 0.03	0.00 \pm 0.00
C20:5 (n-3)	0.00 \pm 0.00	9.02 \pm 3.99	0.00 \pm 0.00
C22:0	0.00 \pm 0.00	0.17 \pm 0.06	0.00 \pm 0.00
C22:1	0.00 \pm 0.00	0.53 \pm 0.18	0.00 \pm 0.00
C22:2	0.00 \pm 0.00	0.07 \pm 0.02	0.00 \pm 0.00
C22:5	0.00 \pm 0.00	1.98 \pm 0.59	0.00 \pm 0.00
C22:6	0.00 \pm 0.00	14.58 \pm 5.53	0.00 \pm 0.00
C24:0	0.00 \pm 0.00	0.11 \pm 0.06	0.00 \pm 0.00
C24:1	0.00 \pm 0.00	0.68 \pm 0.11	0.00 \pm 0.00
Rest	5.86 \pm 1.19	17.89 \pm 3.56	4.77 \pm 0.85

^a Standard deviations were calculated over sample means, not over replicate

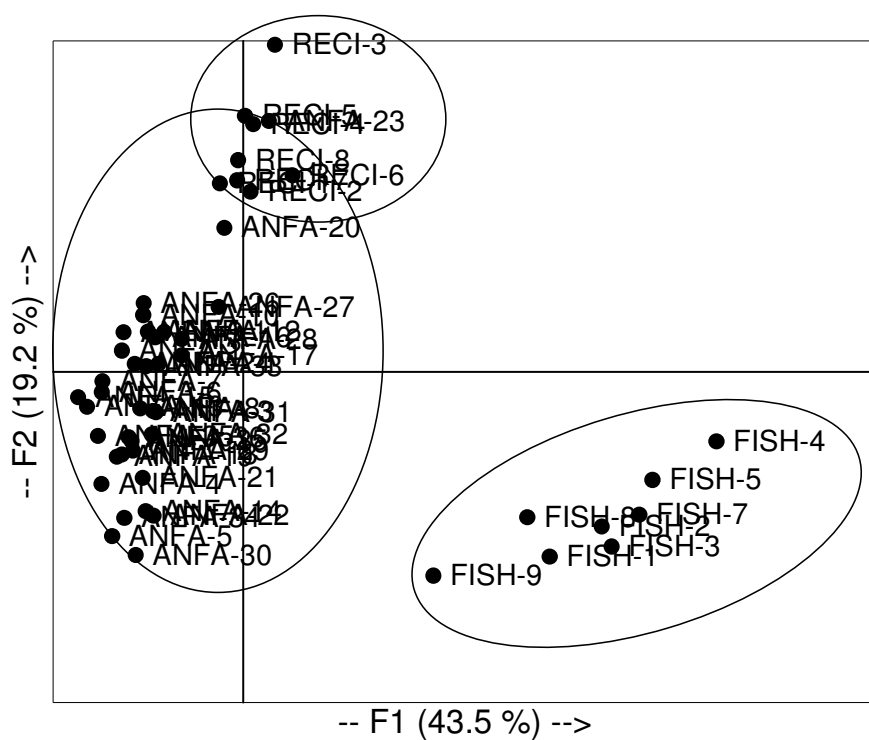
measurements.

^b C18:1 total is the sum of C18:1, C18:1 trans 1 and 2, and C18:1 cis 1 and 2.

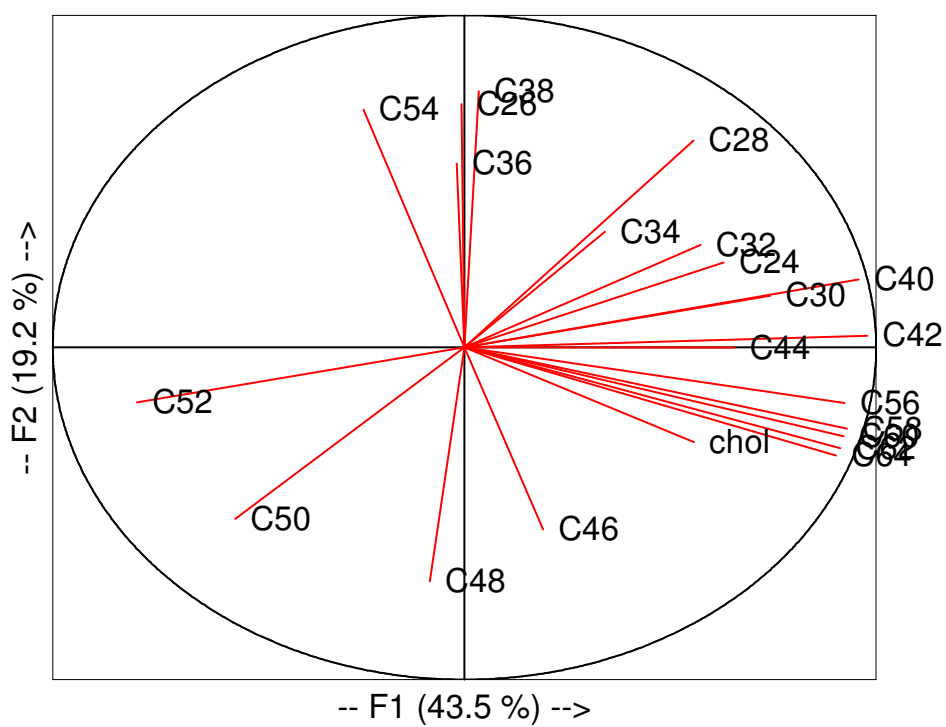
Table 3

Prediction of the identities of animal fats, fish oils and recycled cooking oils by their triacylglycerol profiles, their fatty acid profiles, and volatile profiles determined by PTR-MS: number of correctly and incorrectly predicted samples (percentages) per product class and analytical technique

Sample	PLS-DA classification					
	Triacylglycerol		Fatty acid		Volatile profiles*	
	composition		composition			
	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
Animal fats	36 (100%)	0 (0%)	36 (100%)	0 (0%)	36 (100%)	0 (0%)
Fish oils	8 (89%)	1 (11%)	8 (89%)	1 (11%)	7 (88%)	1 (12%)
Recycled cooking oils	7 (88%)	1 (12%)	7 (88%)	1 (12%)	5 (63%)	3 (37%)
Mean	51 (96%)	2 (4%)	51 (96%)	2 (4%)	48 (92%)	4 (8%)



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408

409 Fig. 1. First two dimensions of Principal Component Analysis on the triacylglycerol
 410 data of animal fats, fish oils, and recycled cooking oils: scores plot (upper) and
 411 loadings plot (lower).

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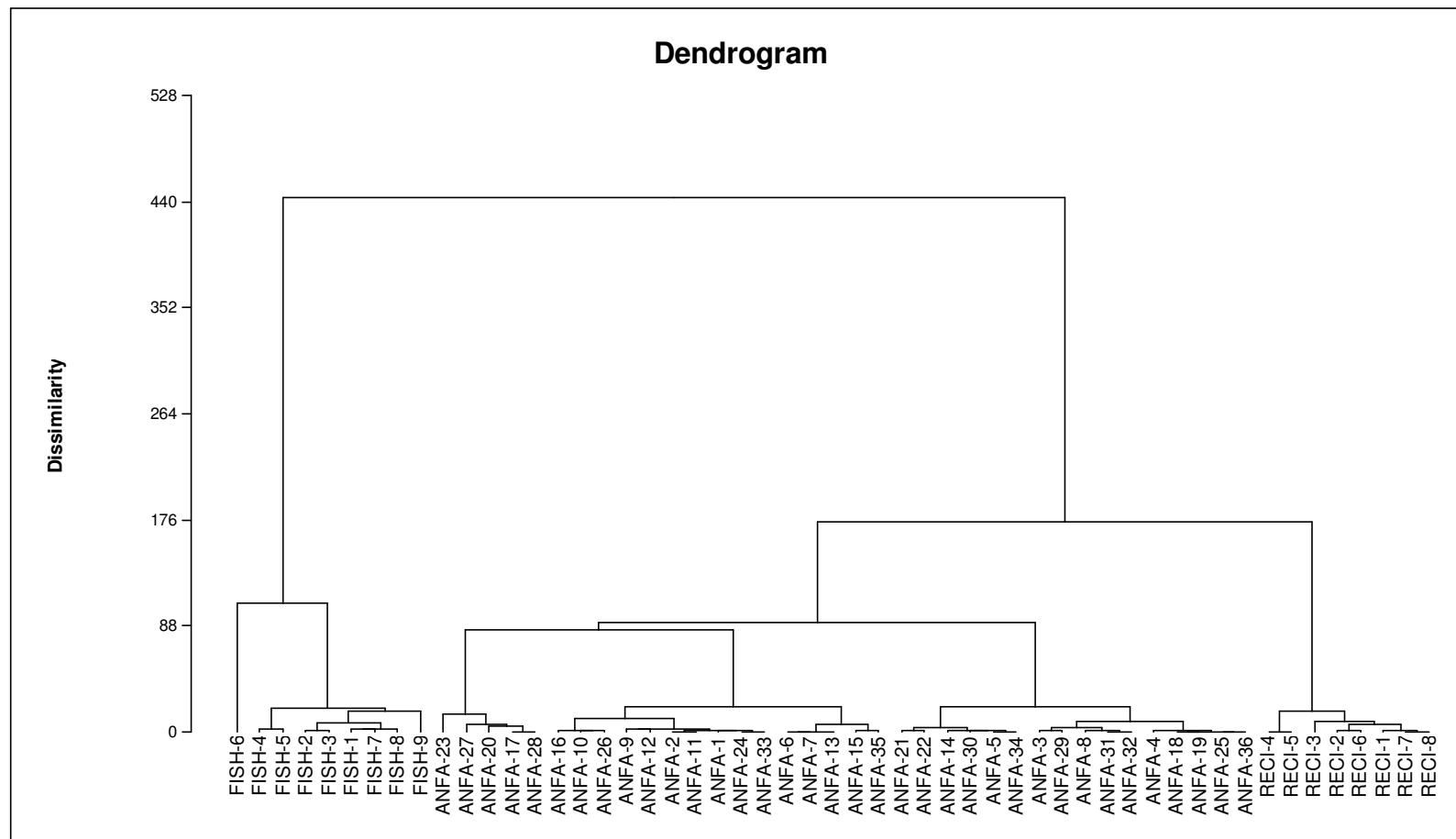
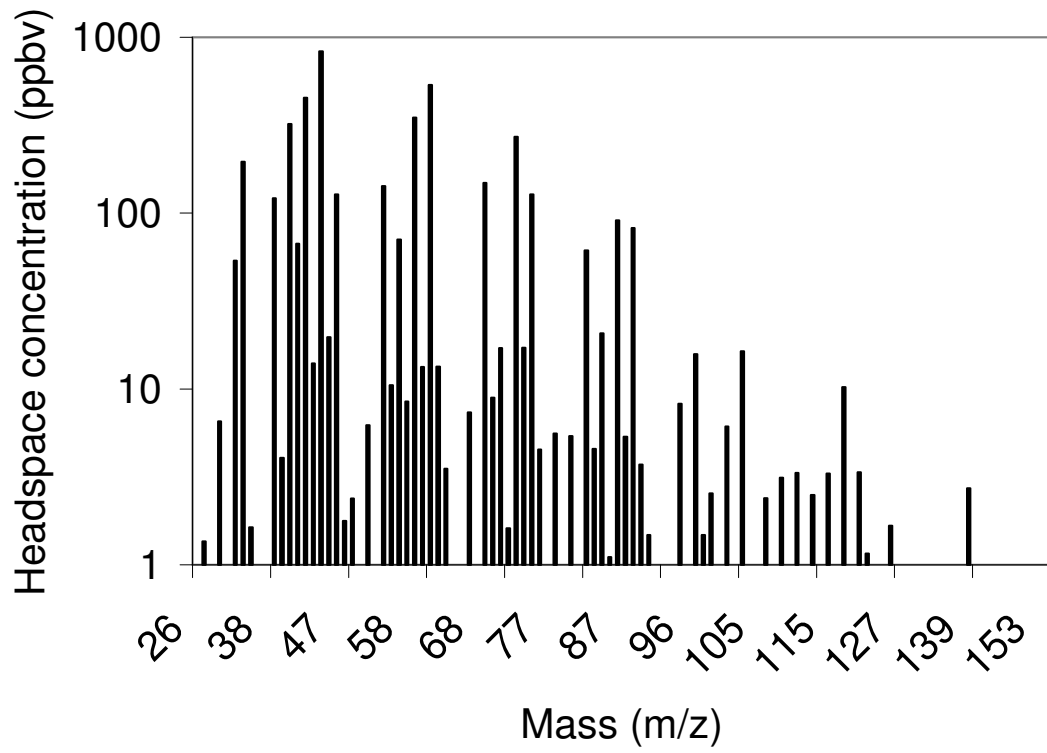


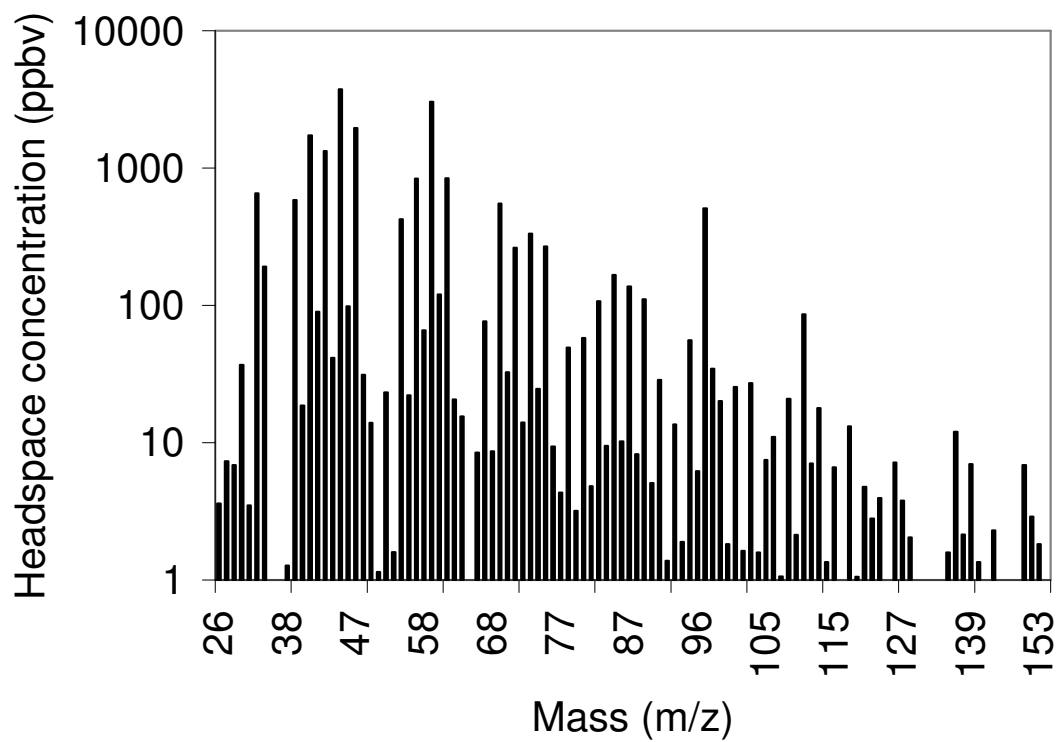
Fig. 2. Dendrogram of Agglomerative Hierarchial Cluster Analysis on the triacylglycerol data of animal fats, fish oils, and recycled cooking oils.

ANFA

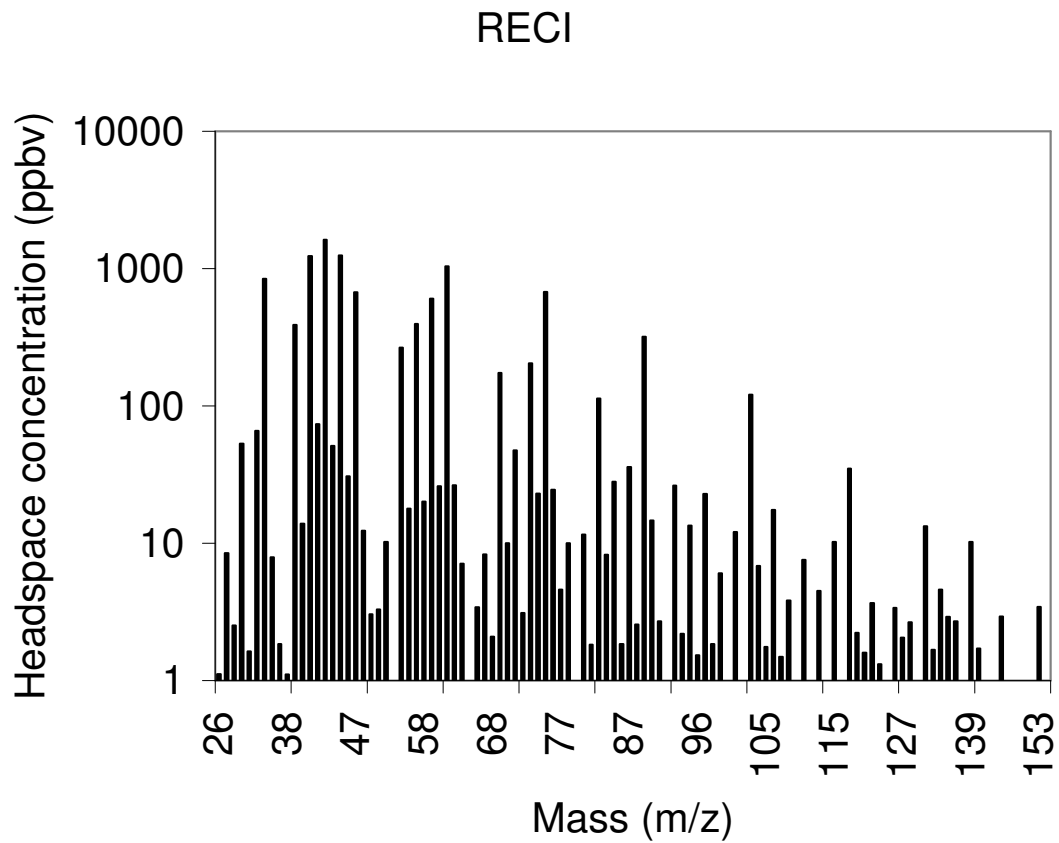


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FISH



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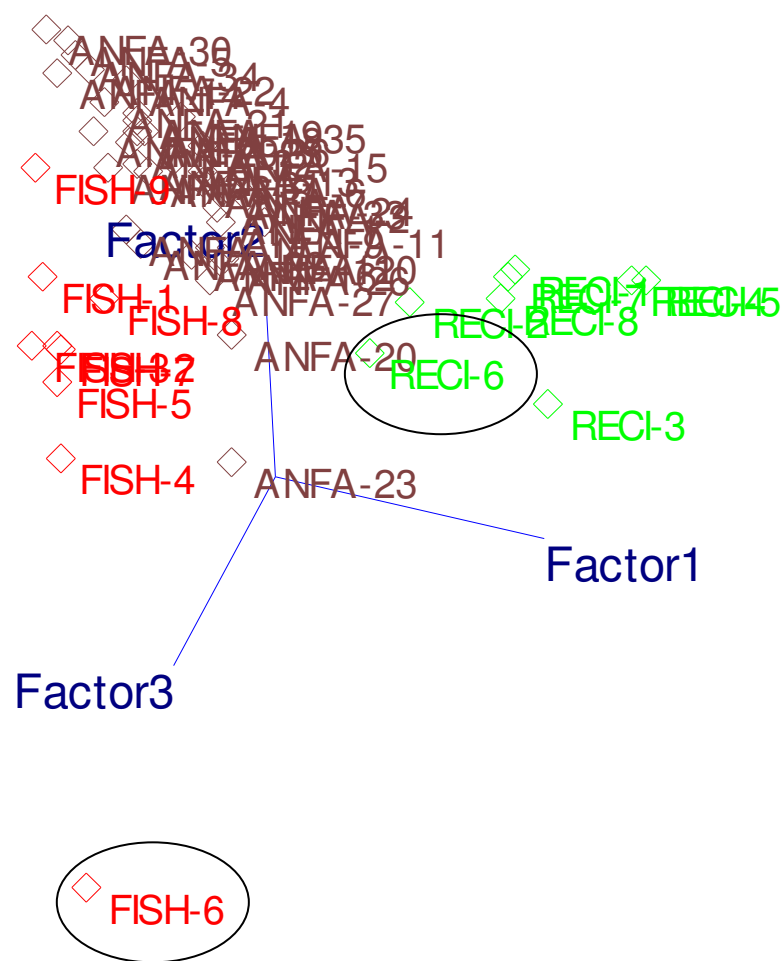
443

444 Fig. 3. Volatile profiles of animal fats (ANFA), fish oils (FISH), and recycled cooking

445 oils (RECI): mean fingerprint mass spectra of the volatile organic compounds in the

446 headspace of samples generated by Proton Transfer Reaction Mass Spectrometry.

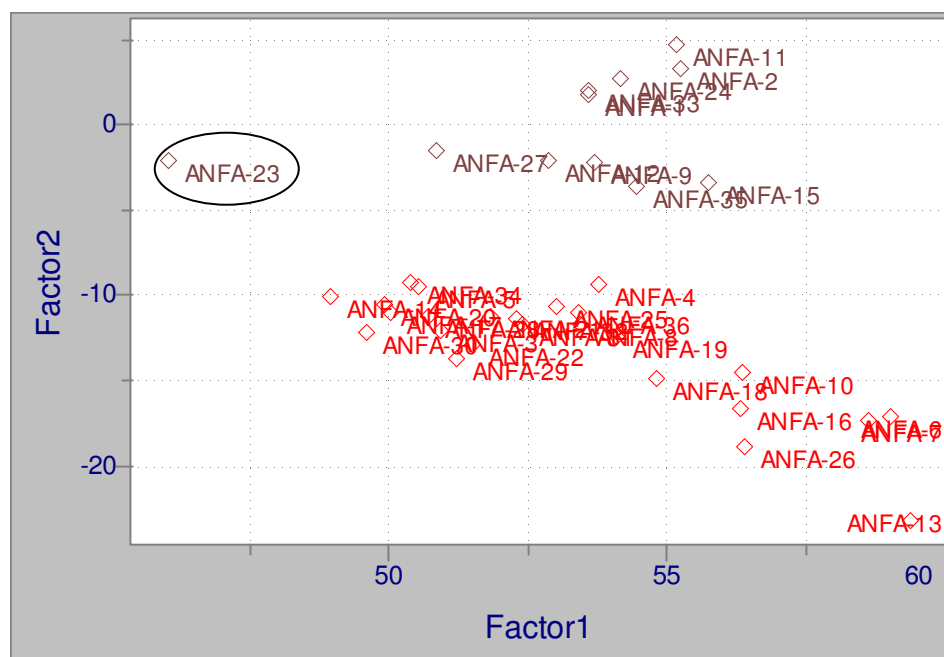
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Fig. 4. Scores plot of the first three dimensions of PLS-DA on the triacylglycerol data of animal fats (ANFA, brown), fish oils (FISH, red), and recycled cooking oils (RECI, green). Incorrectly classified samples are circled.

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458 Fig. 5. Scores plot of the first two dimensions of PLS-DA on the triacylglycerol data

459 of animal fats: poultry fat (brown) and others (red). Incorrectly classified sample is

460 circled.

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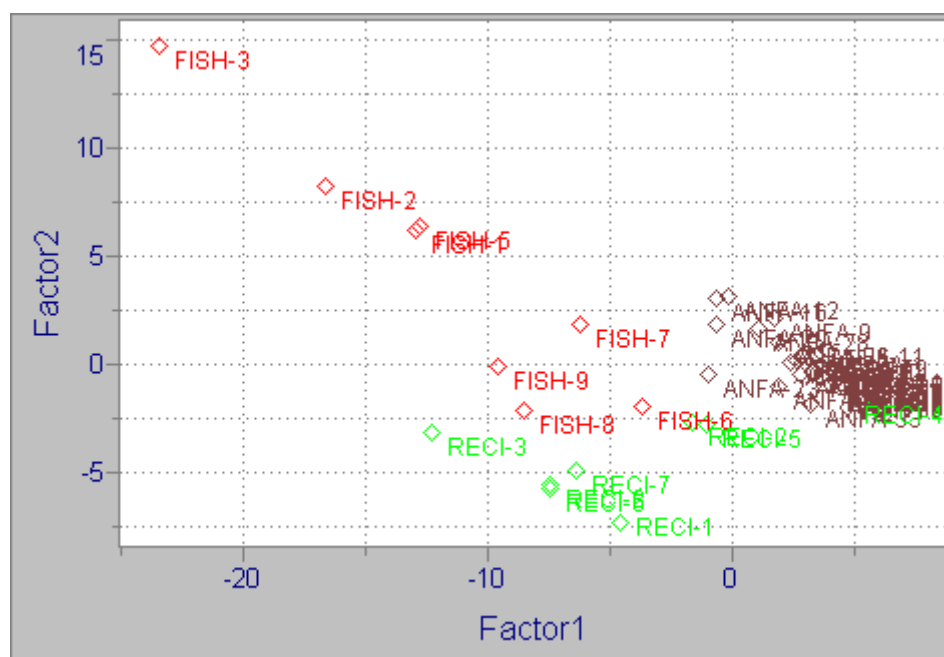


Fig. 6. Scores plot of the first two dimensions of PLS-DA on the volatile organic compounds data of animal fats (ANFA, brown), fish oils (FISH, red), and recycled cooking oils (RECI, green).