Authentication of feeding fats: classification of animal fats, fish oils and recycled cooking oils

S.M. van Ruth[a*], M. Rozijn[a], A. Koot[a], R. Perez Garcia[a], H. van der Kamp[a], R. Codony[b]

[a] *RIKILT – Institute of Food Safety, Wageningen University and Research Centre, P.O. Box 230, 6700 AE Wageningen, The Netherlands*

[b] *Nutrition and Food Science Department- CeRTA, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain*

* Corresponding author.

Email: saskia.vanruth@wur.nl (Saskia van Ruth).

Tel: +31-317-480250.

Fax: +31-317-417717.

Abstract

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

82 organic compounds (VOCs; full mass spectrum < 30s), and may therefore be an
83 interesting additional technique.
84
85 **2. Materials and methods**
86 *2.1. Materials*
87
88 Fifty-three samples, classified as animal fats (36), fish oils (9), and recycled
89 cooking oils (8) were collected in the EU Research Project 'Feeding Fats Safety'
90 (FOOD-CT2004-007020). The samples originated from a variety of European
91 countries and some had a non-European origin. The samples were selected taking into
92 account as much as possible both natural and technology-induced variation. Animal
93 fats concerned products from the rendering process (sterilization, cooking and melting
94 of animal tissues). Most animal fat samples originated from poultry (10), the
95 remainder was classified as bovine, pork, sheep, ruminant, and some samples were
96 mixtures of different species. Fish oils comprised oils obtained by rendering whole
97 low-value fish or fish waste from the food industry (e.g. canned tuna, smoked salmon,
98 salted sardines, etc.). The recycled cooking oils involved products from the collection
99 of exhausted oils, leftover from the deep-frying industry or catering. It is not
100 permitted to use the latter for feed applications, they are normally applied for
101 technical/industrial use (Gasperini et al., 2007). Sample material was stored at -20ºC
102 in absence of light until analysis was carried out.
103
104 *2.2. Methods*
105
106 *2.2.1. TAG analysis (update Maikel/Henk)*

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

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

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

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 30ºC at a rate of 55 ml/min which was led through a heated transfer line (60ºC) 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 (1Td=10^{-17} cm^2 V molecule^{-1}). Inlet

157 and drift chamber temperatures were 60°C. Each sample was analysed for at least 5
158 full mass scans. The headspace concentrations of the compounds during the cycles #3,
159 #4 and #5 were calculated as described by Hansel, Jordan, Holzinger, Prazeller,
160 Vogel, & Lindinger (1995) and background and mass discrimination corrections were
161 applied. Headspace concentrations were subsequently averaged over the three mass
162 scans for further statistical analysis. Previously in preliminary experiments some fat
163 samples had been analysed for seven cycles: the results did not show consistent
164 changes in headspace concentrations (especially no decrease) after the first cycle.
165 Therefore, cycles #3, #4 and #5 were selected for calculations.
166
167 *2.2.4. Statistical analysis*
168
169 The data were explored by Principal Component Analysis (PCA).
170 Subsequently, they were subjected to Aglomerative Hierarchial Cluster Analysis
171 (AHC) for non-supervised clustering of the data. In the study the identity of the
172 samples was known, which allowed use of Partial Least Square Discriminant Analysis
173 (PLS-DA) models (Barker and Rayens, 2003), a supervised clustering technique.
174 These models were estimated to predict the identity of the samples using either the
175 TAG data, FA data or VOC data. PLS-DA performs a dimension reduction on the
176 predictor variables. The dimensions (components) extracted are composed such that
177 they exhibit maximal correlation with Y (class membership, e.g. animal fat, fish oil,
178 recycled cooking oil). After estimation of the classification model, its performance
179 was evaluated by means of leave-1-out cross-validation: one of the samples was
180 randomly removed from the data set, and a model built with the remaining samples
181 was used to classify this left out sample. The procedure was repeated with the

182 remaining samples one by one to obtain predictions for all samples. For model
183 optimization data pre-treatment was carried out and various numbers of components
184 were explored before the final model was selected. All statistical analyses were
185 carried out using Pirouette 4.0 (Infometrix, Bothell, WA, USA).
186
187 **3. Results and discussion**
188
189 *3.1. TAG, FA and VOC data*
190
191 *3.1.1. TAG data*
192 The TAG profiles of the animal fats, fish oils, and recycled cooking oils were
193 analysed by GC. Twenty-one triacylglycerols and cholesterol were quantified in the
194 fats. Relative concentrations varied between and within fat groups (Table 1). In
195 animal fats the C52 (44%), C54 (26%), and C50 (17%) were the predominant TAGs,
196 whereas in fish oils predominant TAGs comprised a larger group: C54 (17%), C56
197 (17%), C52 (15%), C58 (14%). For the recycled cooking oils highest TAG contents
198 were observed for C54 (54%), C52 (22%), and C50 (7%). The composition of the
199 animal fats is similar to the compositions published for lard and beef tallow (Precht,
200 1992). TAGs make up the major part of naturally occurring fats and oils. Analysis of
201 intact TAGs is, as in the present study, usually performed by chromatographic
202 methods. Apart from the GC technique used here, high performance liquid
203 chromatography in normal and reversed phase mode, thin-layer chromatography, and
204 supercritical fluid chromatography are employed (Buchgraber et al., 2004).
205 Some exploratory statistics were applied to the TAG data set: data were
206 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.

232

233 *3.1.2. FA and VOC data*

234

235 The TAG composition is directly related to the FAME composition, which is
236 presented in Table 2. Thirty different FAs were determined. Animal fats were rich in
237 C18:1 (40%), C16:0 (23%), C18:0 (13%), and C18:2 (11%). Fish oils were composed
238 mainly of C18:1 (17%), C16:0 (16%), C22:6 (15%), C20:5 (9%), and recycled
239 cooking oils of C18:1 (39%), C18:2 (37%), C16:0 (13%).
240 Volatile profiles of the fat samples were analyzed by PTR-MS, and mass
241 resolved fingerprints of headspace volatiles were obtained. All of the fat samples
242 produced signals on most masses in the measurement range 20-130 amu indicating the
243 complex VOC composition of the three types of fat. Ions of volatiles in the mass
244 range > 130 amu were more common in fish oils and recycled cooking oils than in
245 animal fats. Mean sample mass spectra for each fat group are displayed in Fig. 3. The
246 VOCs with higher volatility (lower mass) dominate the spectrum in terms of signal
247 intensity, although lower masses may also result from fragmentation of larger
248 compounds. Generally, fish oils and recycled oils showed considerably higher
249 intensities of volatiles than the animal fats. Although PTR-MS is a one dimensional
250 technique, mass resolved fingerprints allow tentative assignment of ions to origins in
251 volatiles with fragmentations patterns typical of PTR-MS (Buhr et al., 2002). Some of
252 the protonated masses showing large signals can be tentatively assigned to volatiles
253 based on reports of their presence in fats and oils and their known fragmentation
254 patterns: e.g. m/z 39 (hexenyl acetate, fragment), 41 (hexanol (fragment), 43 (acetic
255 acid, hexanol), 45 (acetaldehyde), 47 (ethanol), 57 (hexanal, hexenal, hexanol), 59
256 (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

282 hyperspace it is assigned to the corresponding class. On the other hand, class-
283 modeling tools build a separate model for each category: samples fitting the model are
284 accepted by that category, while samples falling outside the model are considered as
285 outliers for the specific class. In the present study all classes (fat types) were known,
286 which means that a pure classification method could be used. Furthermore, in PTR-
287 MS analysis we deal with more variables than samples, which implies that
288 discriminant analysis is not appropriate and a PCA-like reduction of the variables is
289 required before samples can be classified. PLS-DA combines both aspects.
290 The statistical analyses in this study used the TAG, FA and VOC data as
291 'fingerprints', i.e. the compounds/masses and their corresponding signal intensities in
292 each sample mass spectrum act as a pattern for inter-comparison of the samples.
293 PLS-DA was applied to the TAG data to classify the samples into fat types
294 (animal fat, fish oil, recycled cooking oil). A five-component model (data auto-scaled)
295 was fitted to estimate the identity of the samples. Rates of successful classification in
296 cross-validation are listed in the leftmost part of Table 3. Of all samples, 96% were
297 successfully classified into their fat type classes: 100% of the animal fats, 89% of the
298 fish oil and 88% of the recycled cooking oils. The scores of the samples on the first
299 two PLS-dimensions are presented in Fig. 4. Samples FISH-6 and RECI-6 were the
300 only samples that were misclassified. Both were more or less on the demarcation line
301 between two classes. The fish oil originated from France, and the recycled cooking oil
302 from Italy. Considering the wide range of sample material (origin, species,
303 technology, etc.) it is surprising that the samples could be so successfully classified.
304 The animal fat group was the largest subset and consisted of 10 poultry fat samples
305 and 26 fat samples of other species. PLS-DA classification of the animal fat samples
306 into poultry and non-poultry groups resulted in a two component model (no data pre-

307 treatment). Cross-validation resulted in 97% correct classification. The single
308 misclassification concerned a chicken fat sample from Spain that was classified as
309 'other fats'. A scores plot of the first two dimensions of the PLS-DA on the animal fat
310 data is displayed in Fig. 5. ANFA-23 is the incorrectly classified fat sample.
311
312 *3.2.2. FA and VOC data*
313
314 FA data were subjected to the same statistical treatment as the TAG data,
315 results are listed in the center of Table 3 (data pre-treatment: autoscaling). Overall,
316 96% of the samples were successfully classified. There were two misclassifications
317 only: samples FISH-9 and RECI-6. Since RECI-6 was also misclassified in the TAG
318 analysis, this sample had probably unusual compositional characteristics compared to
319 the other recycled cooking oils. The raw data showed that RECI-6 was high in FA
320 C16:0 and low in C18:2 compared to the other samples. Its TAG composition
321 revealed correspondingly higher concentrations of C50 and C52, and lower levels of
322 C54.
323 PLS-DA classification of the VOC data resulted in 92% of the samples in
324 successful classifications: 100% of the animal fats, 88% of the fish oils and 63% of
325 the recycled cooking oils (Table 3, rightmost; data pre-treatment: autoscaling). Four
326 samples were misclassified, one fish oil (FISH-8) and three recycled cooking oils
327 (RECI-1, -4, -5). The fish oil originated from Norway, the cooking oils from Italy (1)
328 and Spain (2). The PLS-DA plot in Fig. 6 shows that the misclassified samples are at
329 the demarcation line of the classes.
330
331 **4. Conclusions**

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

Acknowledgement

Authors wish to thank for feeding fat samples which they obtained through the EU research Project Food-CT2004-007020 "Quality and Safety of Feeding Fats Obtained from Co- or By-products from the Food Chain – (Feeding Fats Safety)" which was supported by the European Commission under Research Programme FP6, Quality and Safety. The analytical part of the study was financially supported by the Dutch Government, Department of Agriculture, Nature and Food Quality. Additionally, we would like to thank Ionicon Analytik GmbH for PTR-MS support.

References

357 Barker, M., Rayens, W., 2003. Partial Least squares for discrimination. J. Chemometr.
358 17, 166-173.
359 Buchgraber, M., Ulberth, F., Emons, H., Anklam, E., 2004. Triacylglycerol profiling
360 by using chromatographic techniques. Eur. J. Lipid Sci. Technol. 106, 621-
361 648.
362 Buhr, K., van Ruth, S., Delahunty, C., 2002. Analysis of volatile flavour compounds
363 by proton transfer reaction-mass spectrometry: fragmentation patterns and
364 discrimination between isobaric and isomeric compounds. Int. J. Mass
365 Spectrom. 221, 1-7.
366 Gasperini, G., Fusari, E., Della Bella, L., Bondioli, P., 2007. Classification of feeding
367 fats by FT-IR spectroscopy. Eur. J. Lipid Sci. Technol. 109, 673-681.
368 Gonzalez Martin, Y., Cerrato Oliveros, M.C., Perez Pavon, J.L., Garcia Pinto, C.,
369 Moreno Cordero, B., 2001. Electronic nose based on metal oxide
370 semiconductor sensors and pattern recognition techniques: characterisation of
371 vegetable oil. Anal. Chim. Acta 449, 69-80.
372 Hai, Z., Wang, J., 2006. Detection of adulteration in camellia seed oil and sesame oil
373 using an electronic nose. Eur. J. Lipid Sci. Technol. 108, 116-124.
374 Hansel, A., Jordan, A., Holzinger, R., Prazeller, P., Vogel, W., Lindinger, W., 1995.
375 Proton transfer reaction mass spectrometry: on-line trace gas analysis at the
376 ppb level. Int. J. Mass Spectrom. Ion Proc. 149/150, 609-619.
377 Karoui, R., De Baerdemaker, J., 2007. A review of the analytical methods coupled
378 with chemometric tools for the determination of the quality and identity of
379 dairy products. Food Chem. 102, 621-640.

Lindinger, W., Hirber, J., Paretzke, H., 1993. An ion/molecule-reaction mass spectrometer used for on-line trace gas analysis. J. Mass Spectrom. Ion Proc. 129, 79-88.

Precht, D., 1992. Detection of foreign fat in milk fat. I. Qualitative detection by triacylglycerol formulae, Z. Lebenm. Unters. Forsch. 194, 1-8.

Vandeginste, B.G.M., Massart, D.L., Buydens, L.M.C., de Jong, S., Lewi, P.J., Smeyers-Verbeke, J., 1998. Handbook of Chemometrics and Qualimetrics: Part B. Elsevier, Amsterdam.

van Ruth, S.M., Koot, A., Akkermans, W., Araghipour, N., Rozijn, M., Baltussen, M., Wisthaler, A., Märk, T.D., Frankhuizen, R. 2008. Butter and butter oil classification by PTR-MS, Eur. Food Res. Technol. 227, 307-317.

Zhang, G., Ni, Y., Churchill, J., Kokot, S., 2006. Authentication of vegetable oils on the basis of their physico-chemical properties with the aid of Chemometrics. Talanta 70, 293-300.

397

Table 1
Relative triacylglycerol composition of animal fats, fish oils, and recycled cooking oils (mean ± SD[a])

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

398 [a] Standard deviations were calculated over sample means, not over replicate

399 measurements.

400

401

Table 2
Relative fatty acid composition of animal fats, fish oils and recycled cooking oils (mean ± SD[a])

Fatty acid	Animal fats (n=36)	Fish oils (n=9)	Recycled cooking oils (n=8)
C4:0	0.00±0.00	0.00±0.00	0.01±0.01
C6:0	0.00±0.00	0.00±0.00	0.01±0.01
C8:0	0.02±0.01	0.10±0.01	0.11±0.02
C10:0	0.06±0.03	0.00±0.00	0.02±0.02
C12:0	0.14±0.07	0.08±0.02	0.10±0.10
C14:0	1.89±0.95	4.78±1.32	0.35±0.19
C14:1	0.25±0.17	0.05±0.01	0.01±0.01
C15:0	0.22±0.15	0.57±0.19	0.03±0.01
C16:0	23.29±1.97	15.94±2.30	12.77±4.68
C16:1	3.05±0.78	5.44±1.10	0.37±0.19
C18:0	13.32±5.46	3.81±1.06	4.33±0.50
C18:1 trans1	0.30±0.18	0.13±0.04	0.64±0.79
C18:1 trans 2	1.00±1.02	0.31±0.28	0.08±0.19
C18:1	36.32±3.95	12.94±3.08	36.69±3.80
C18:1 cis 1	1.88±0.58	3.21±0.80	1.27±0.58
C18:1 cis 2	0.35±0.23	0.22±0.07	0.14±0.09
C18:1 total[b]	39.85±3.56	16.80±3.06	38.83±4.92
C18:2	10.81±7.90	2.21±1.39	37.33±11.18
C18:2 conj	0.18±0.13	0.04±0.03	0.04±0.01
C18:3	1.05±0.77	0.89±0.47	0.92±0.94
C20:1	0.00±0.00	3.72±1.77	0.00±0.00
C20:2	0.00±0.00	0.38±0.10	0.00±0.00
C20:3 (n-6)	0.00±0.00	0.15±0.03	0.00±0.00
C20:5 (n-3)	0.00±0.00	9.02±3.99	0.00±0.00
C22:0	0.00±0.00	0.17±0.06	0.00±0.00
C22:1	0.00±0.00	0.53±0.18	0.00±0.00
C22:2	0.00±0.00	0.07±0.02	0.00±0.00
C22:5	0.00±0.00	1.98±0.59	0.00±0.00
C22:6	0.00±0.00	14.58±5.53	0.00±0.00
C24:0	0.00±0.00	0.11±0.06	0.00±0.00
C24:1	0.00±0.00	0.68±0.11	0.00±0.00
Rest	5.86±1.19	17.89±3.56	4.77±0.85

402 [a] Standard deviations were calculated over sample means, not over replicate

403 measurements.

404 [b] C18:1 total is the sum of C18:1, C18:1 trans 1 and 2, and C18:1 cis 1 and 2.

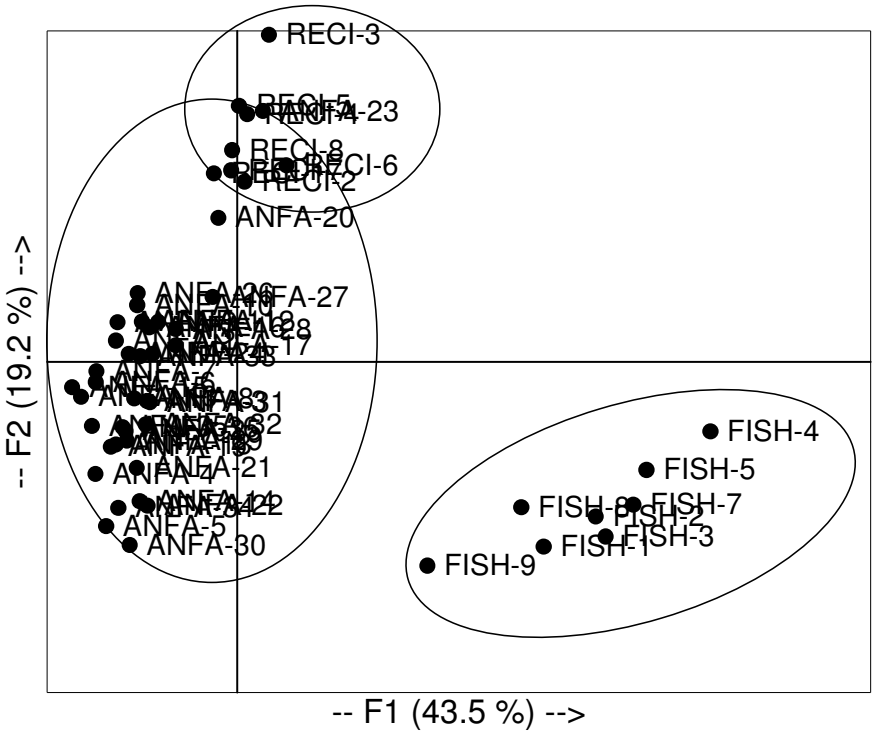
Table 3

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

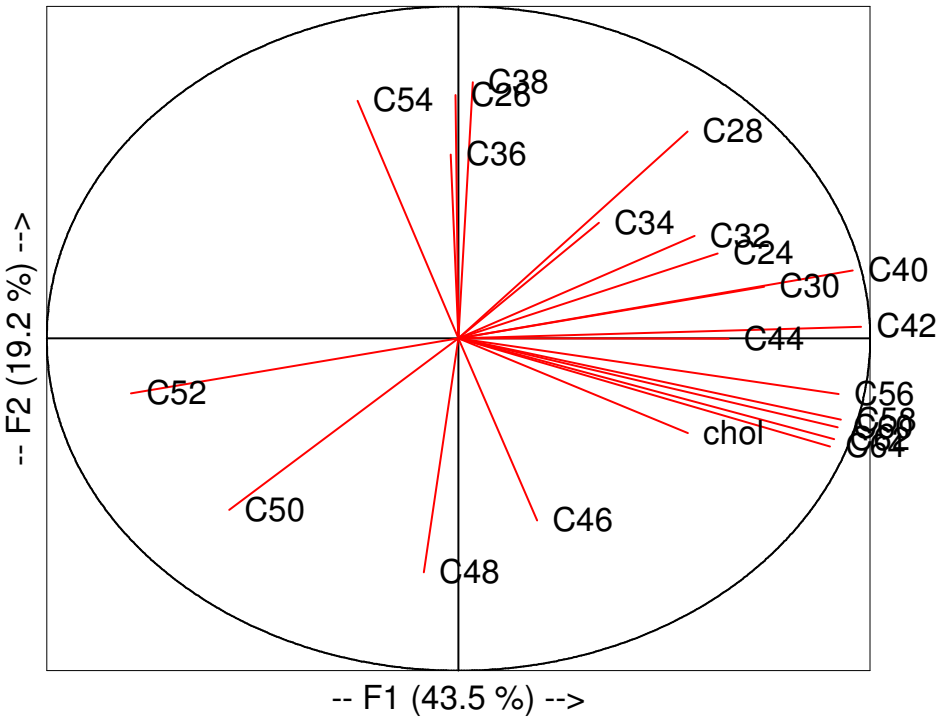
Sample	PLS-DA classification					
	Triacylglycerol composition		Fatty acid composition		Volatile profiles*	
	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
Animal fats	36 (100%)	0 (0%)	36 (100%)	0 (0%)	36 (100%)	0 (0%)
Fish oils	8 (89%)	1 (11%)	8 (89%)	1 (11%)	7 (88%)	1 (12%)
Recycled cooking 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%)

405

406



407



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).

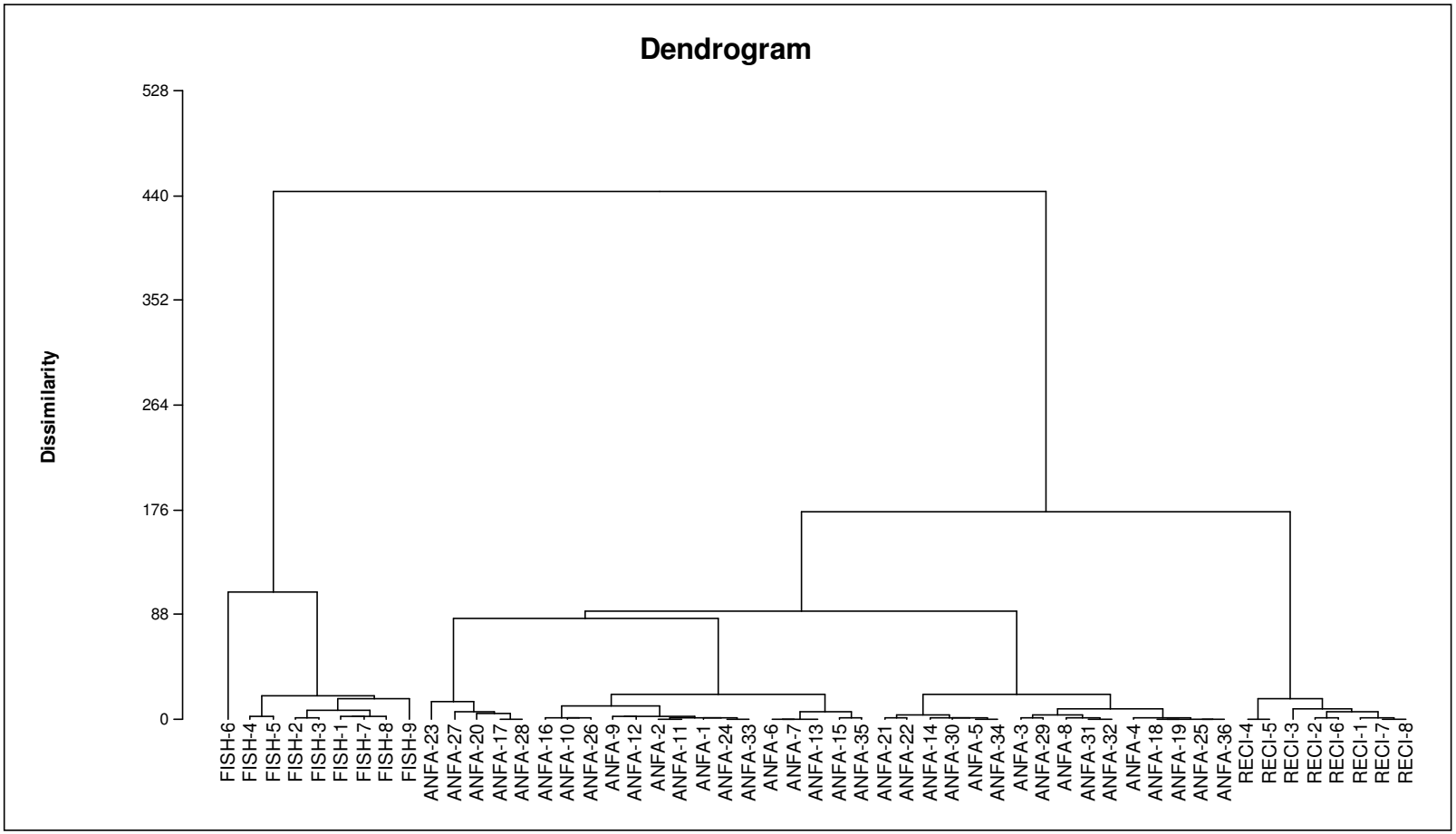
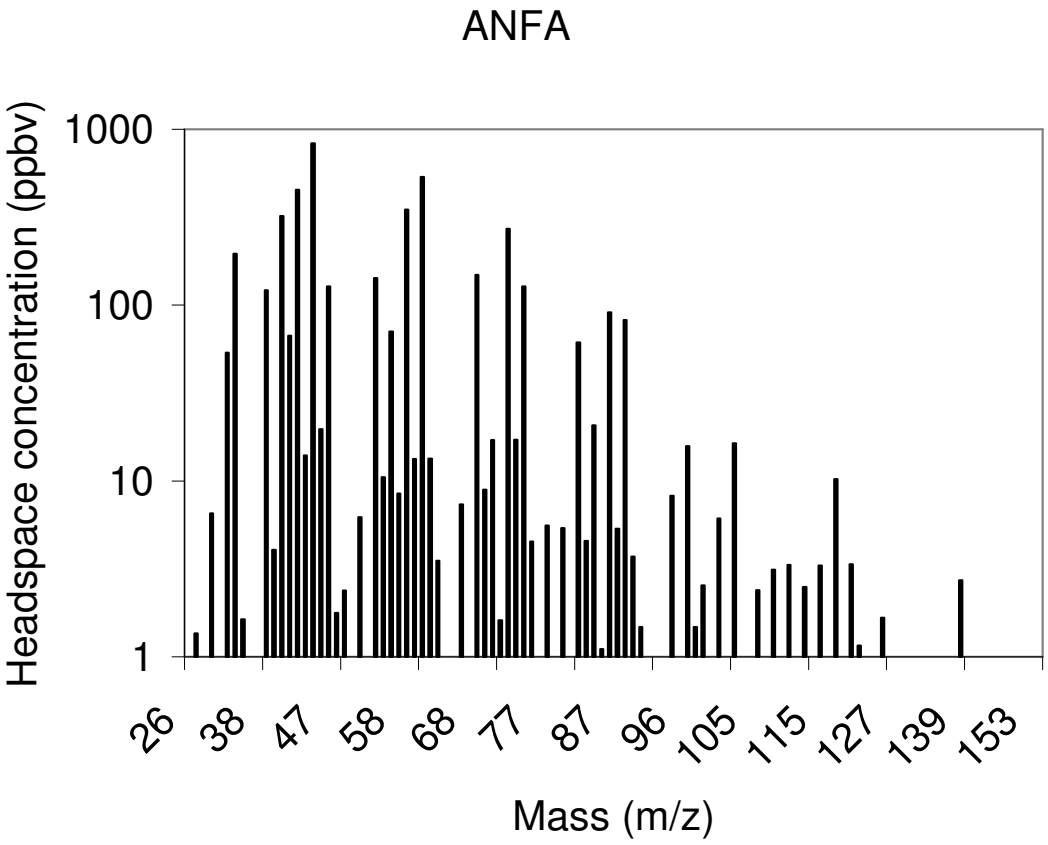
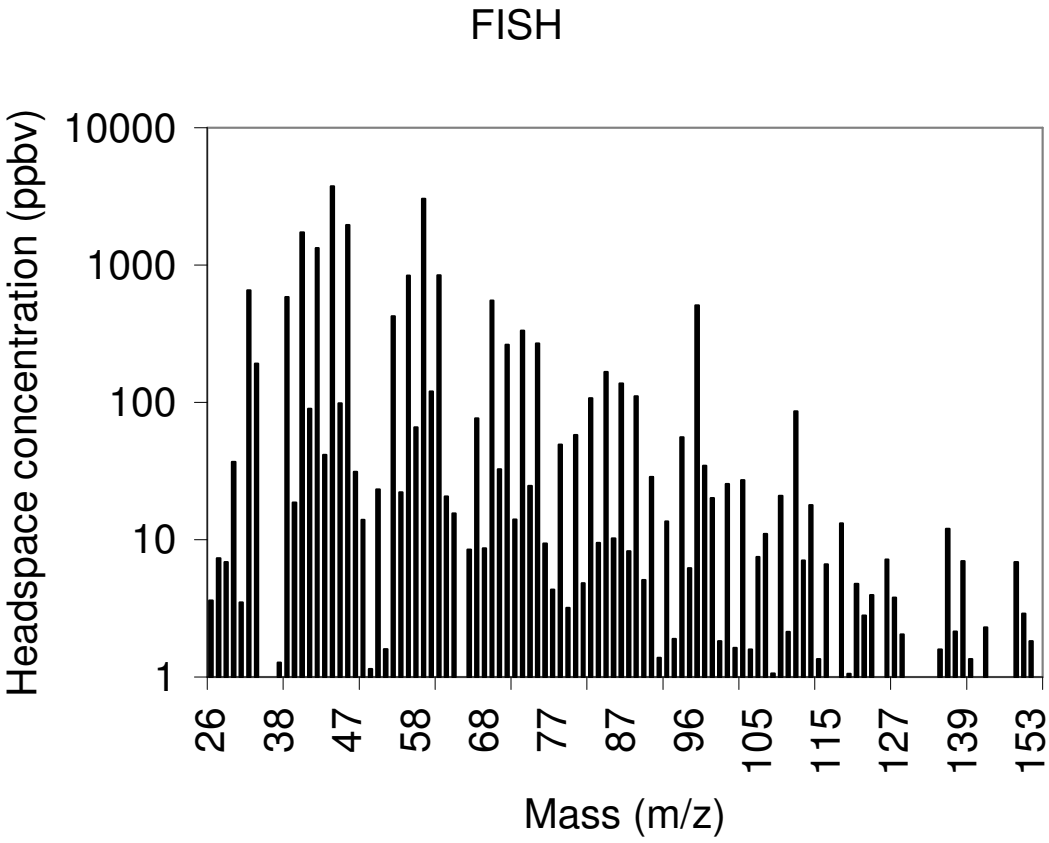


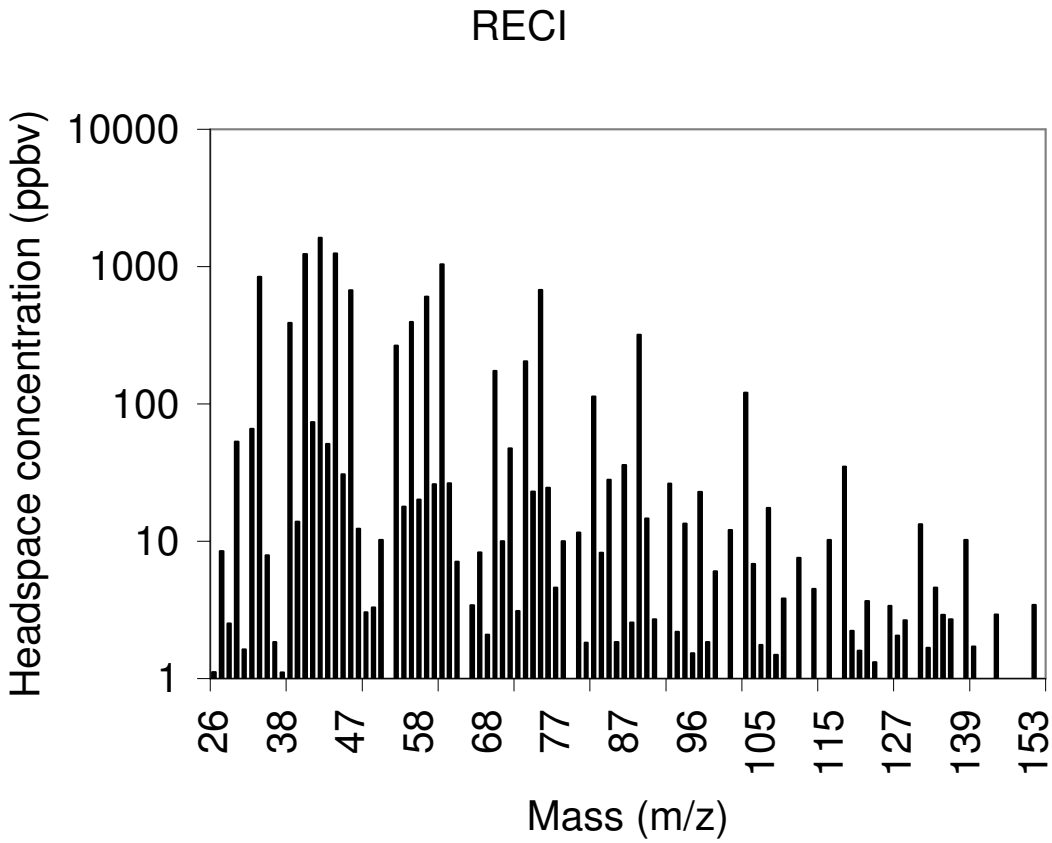
Fig. 2. Dendrogram of Aglomerative Hierarchial Cluster Analysis on the triacylglycerol data of animal fats, fish oils, and recycled cooking oils.


ANFA
Headspace concentration (ppbv)
1000
100
10
1
26
38
47
58
68
77
87
96
105
115
127
139
153
Mass (m/z)

441


FISH
Headspace concentration (ppbv)
10000
1000
100
10
1
26
38
47
58
68
77
87
96
105
115
127
139
153
Mass (m/z)

442



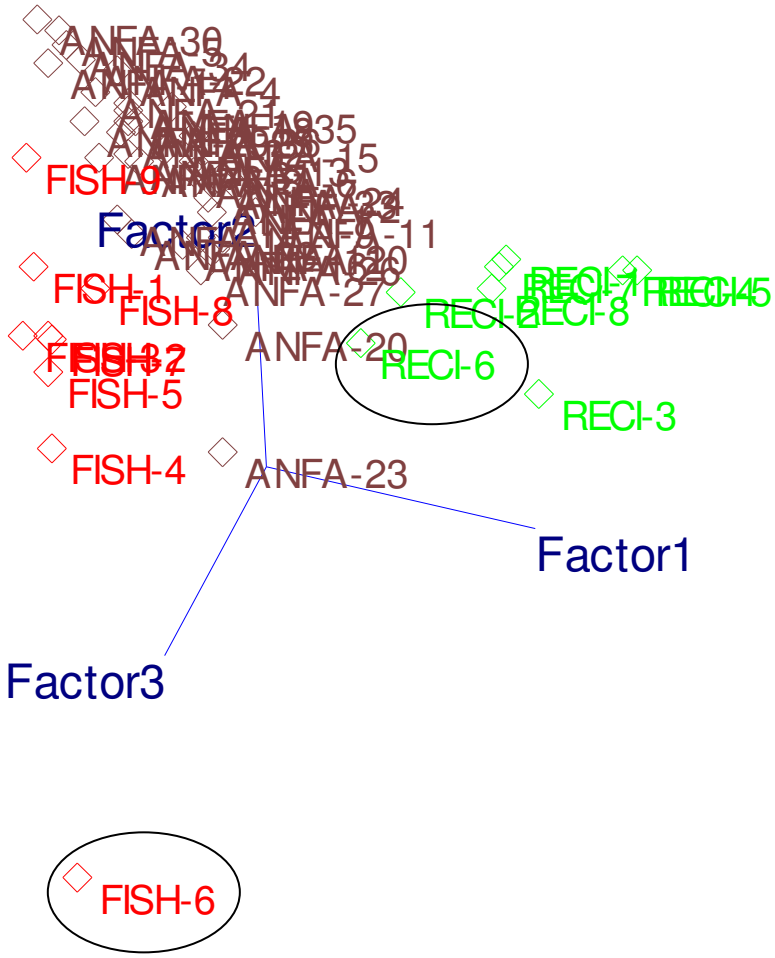
443

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.

447



448
449
450
451 Fig. 4. Scores plot of the first three dimensions of PLS-DA on the triacylglycerol data
452 of animal fats (ANFA, brown), fish oils (FISH, red), and recycled cooking oils (RECI,
453 green). Incorrectly classified samples are circled.
454

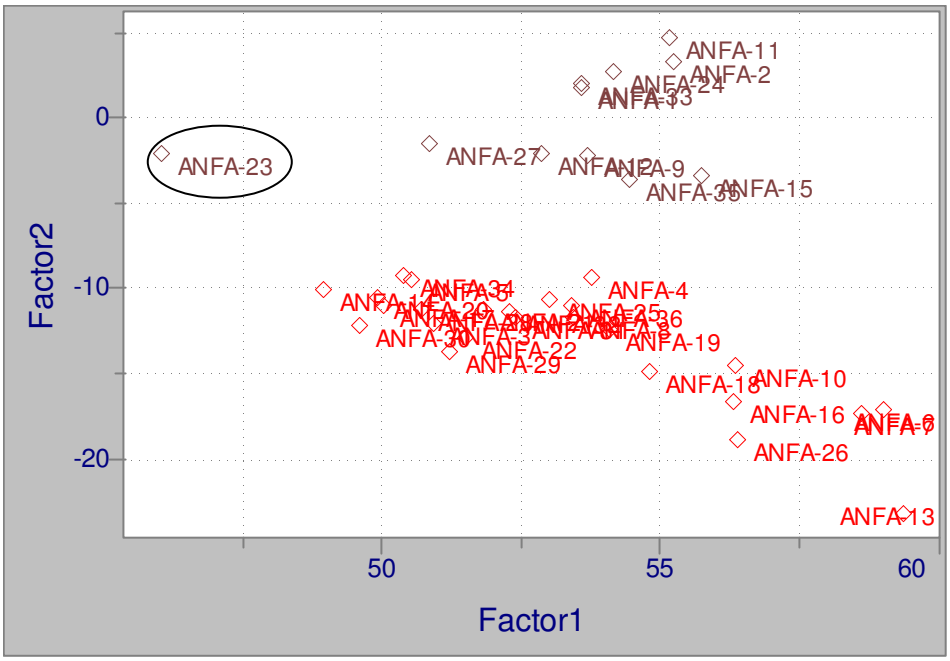
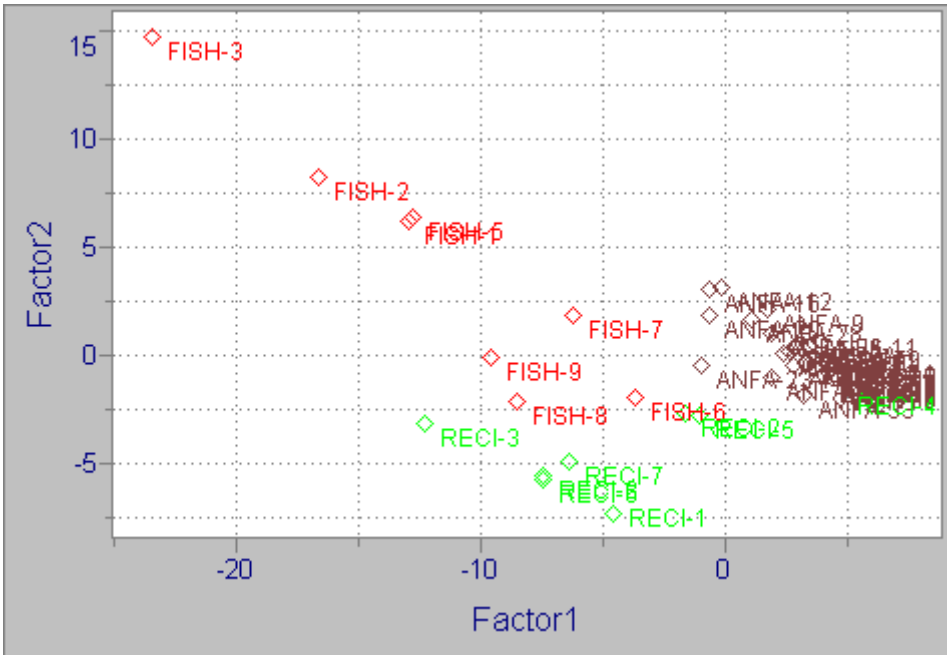


Fig. 5. Scores plot of the first two dimensions of PLS-DA on the triacylglycerol data of animal fats: poultry fat (brown) and others (red). Incorrectly classified sample is circled.



462

463 Fig. 6. Scores plot of the first two dimensions of PLS-DA on the volatile organic

464 compounds data of animal fats (ANFA, brown), fish oils (FISH, red), and recycled

465 cooking oils (RECI, green).