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Authentication of Animal Fats Using Direct Analysis in Real Time (DART) Ionization−Mass Spectrometry and Chemometric Tools

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ABSTRACT: A combination of direct analysis in real time (DART) ionization coupled to time-of-flight mass spectrometry (TOFMS) and chemometrics was used for animal fat (lard and beef tallow) authentication. This novel instrumentation was employed for rapid profiling of triacylglycerols (TAGs) and polar compounds present in fat samples and their mixtures. Additionally, fat isolated from pork, beef, and pork/beef admixtures was analyzed. Mass spectral records were processed by principal component analysis (PCA) and stepwise linear discriminant analysis (LDA). DART-TOFMS profiles of TAGs were found to be more suitable for the purpose of discrimination among the examined fat types as compared to profiles of polar compounds. The LDA model developed using TAG data enabled not only reliable classification of samples representing neat fats but also detection of admixed lard and tallow at adulteration levels of 5 and 10% (w/w), respectively. The presented approach was also successfully applied to minced meat prepared from pork and beef with comparable fat content. Using the DART-TOFMS TAG profiles of fat isolated from meat mixtures, detection of 10% pork added to beef and vice versa was possible.

KEYWORDS: animal fat, meat, adulteration, direct analysis in real time, mass spectrometry, multivariate analysis

INTRODUCTION

Authenticity represents an important food quality parameter, which is of high concern not only for food producers, regulatory bodies, or scientific professionals but also for consumers.[1] Considering the global nature of the food trade and the complexity and length of current food chains as well as recent food scandals, the growing sensitivity of all stakeholder groups with regard to various categories of potential food fraud is not surprising.

Like other commercially important food commodities, such as fruit juices, wine, alcoholic beverages, honey, and plant oils/fats, also animal fats have been frequent subjects to fraud.[2] Besides various methods of milk fat falsification, the most common procedure applied by fraudulent producers employs the addition of beef tallow to pork lard. This type of adulteration can be categorized as profit-driven, due to the relatively higher price of pork lard compared to beef tallow. It should be also noted that the undeclared presence of components derived from pork in some food products is not acceptable for certain groups of consumers following the Kosher or Halal diet, because of religious restrictions. Attention has also been focused on the safety of tallow derivatives from cattle tallow with regard to potential human health risks associated with bovine spongiform encephalopathy (BSE).[3]

To prevent food commodity adulteration, rapid, reliable, and cost-effective analytical methods are required for laboratory control. Currently, approaches routinely employed for authentication of lard and tallow are most often based on the analysis of fatty acid (FA) composition, which is, as shown in the scientific literature,[4] inherently species-dependent. Following the release from fat by saponification, and after their conversion to methyl esters, the FA pattern is typically determined by gas chromatography (GC) coupled to flame ionization detection (FID).[5,6] However, this procedure is rather labor- and time-demanding and can be successfully applied only when a higher amount of adulterant is present in the sample. As an alternative with a higher reporting value, high-performance liquid chromatography (HPLC) interfaced to mass spectrometry (MS) employing atmospheric pressure chemical ionization (APCI) might be used for the analysis of complex triacylglycerol (TAG) mixtures.[7] A HPLC-MS technique, using multidimensional off-line chromatographic separation, was used in a study focused on the proof of beef tallow in adulterated lard.[8] On the basis of the assessment of regioisomeric TAG ratio, detection of the adulterant at a level as low as 5% (w/w) was possible. In another study, comparative characterization of lard, beef tallow, and milk fat TAGs was performed by a matrix-assisted laser desorption/ionization−time-of-flight mass spectrometry (MALDI-TOFMS) technique, allowing the separation step to be omitted.[9] Also, spectroscopic techniques, such as Fourier transform infrared (FTIR) spectroscopy,[10] near-infrared (NIR),[11] and Fourier transform Raman spectroscopy,[12] were applied for the authentication/discrimination of animal fats and fat-containing foods.

For processing of large data sets generated during food authenticity/quality assessments by the above instrumental techniques, pattern recognition chemometric techniques were proven to be powerful tools. Whereas principal component analysis (PCA) enables inspection of data internal structure, linear discriminant analysis (LDA), partial least-squares discriminant analysis (PLS-DA), and/or artificial neural networks (ANNs) are suitable for the construction of mathematical models for the sample classification.[13]

In this study, an ambient desorption ionization mass spectrometry (AMS) technique is presented as an attractive alternative

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to already established authentication approaches. Thanks to the minimal requirements for sample preparation and the omission of chromatographic separation, AMS has been often applied in various high-throughput food analyses.[14] As shown in our previous study,[15] a direct analysis in real time (DART) technique coupled to a high-resolution TOFMS enables effective ionization of plant oil components and provides mass spectral fingerprints suitable for authenticity assessment/adulteration detection. Taking into account the above encouraging results, we decided to use this analytical approach also for the authentication of animal fats, particularly for the discrimination of pork lard and beef tallow and their admixtures. Additionally, fat extracted from minced beef and pork meat mixtures was examined by DART-TOFMS to explore the feasibility of the above strategy to control meat authenticity. The obtained mass spectral fingerprints were subjected to PCA and LDA.

MATERIALS AND METHODS

Chemicals and Reagents. Toluene and methanol (both of HPLC grade) were supplied by Merck (Darmstadt, Germany), and petroleum ether was from Lach-Ner (Neratovice, Czech Republic). Water was purified with the use of a Milli-Q system (Millipore, Bedford, MA). Polyethylene glycol (PEG, average relative molecular weight of 600 Da) was from Sigma-Aldrich (Steinheim, Germany), and an aqueous ammonia solution (25%, w/w) and sodium sulfate (p.a.) were purchased from Penta (Chrudim, Czech Republic).

Samples. To work on a representative sample set reflecting the variation within each group of fat samples, pork lard (n = 16) and beef tallow (n = 13) were both obtained by rendering the adipose tissue (procedure described below) of various parts of slaughtered animals and purchased from the retail market (various Czech producers). Admixtures of pork lard and beef tallow were prepared from randomly selected samples in the weight ratios of 2:98, 5:95, 10:90, 25:75, 50:50, 75:25, 90:10, 95:5, and 98:2. Each mixture was prepared in duplicate. Prior to further processing, samples were stored in the refrigerator at 4 °C.

Pork and beef meats were purchased from reliable retail market sources. The contents of fat (determined as described below) in pork and beef were 21.4 and 26.2% (w/w), respectively. After homogenization (mincing) of meats with the use of a Moulinette meat-mincer (Moulinex, Madrid, Spain), beef−pork mixtures with components weight ratios of 5:95, 10:90, 25:75, 50:50, 75:25, 90:10, and 90:5 were prepared in duplicate.

Animal fats were separated from homogenized animal tissues (adipose tissues and minced meats) by rendering in a laboratory thermostat held for 30 min at 130 °C. After sedimentation, released fat was transferred into a 50 mL polypropylene (PP) tube and further processed prior to DART-TOFMS examination. In addition to rendering, a more straightforward procedure was employed to isolate fat from meat and meat mixtures as described under Sample Preparation for DART-TOFMS Analysis.

Determination of Fat Content in Meat. Homogenized meat sample was dried at a temperature of 103 ± 2 °C[16] and subsequently extracted with petroleum ether in a Soxtec 2043 fat extraction system (Foss, Höganäs, Sweden) according to ISO 1443-1973 standard.[17]

Sample Preparation for DART-TOFMS Analysis. For TAG fingerprinting, 0.2 g of fat was weighed into a 15 mL PP centrifuge tube and dissolved in 10 mL of toluene. After sonication (1 min), approximately 700 μL of the sample was taken for instrumental analysis. Rapid extraction of the lipid fraction from minced meat was carried out as follows: 2 g of homogenized sample mixed with 6 g of anhydrous sodium sulfate (desiccant) was weighed into a 50 mL PP centrifuge tube. Subsequently, 6 mL of toluene was added, and the suspension was vigorously shaken by hand for 2 min. After removal of solid components by centrifugation, the supernatant extract was analyzed by DART-TOFMS.

Polar compounds were extracted from fat using 1 g of the sample and 6 mL of a methanol−water mixture (50:50, v/v). Due to a solid state of examined fats at laboratory temperature, a 15 mL PP tube containing the sample was placed into a hot water bath (approximately 70 °C, 1 min), and the sample was allowed to melt. Addition of the extraction mixture was followed by 2 min of intense shaking and centrifugation (11000 rpm, 1 min, 20 °C). The upper layer (methanol−water) was used for subsequent analysis.

DART-TOFMS. For instrumental analyses, the DART-TOFMS system consisting of a DART ion source (IonSense, Danvers, MA), an AccuTOF LP TOF mass spectrometer (JEOL (Europe) SAS, Croissy sur Seine, France), and an HTC PAL autosampler AutoDART-96 (Leap Technologies, Carrboro, NC) was used. Optimal settings for the DART-TOFMS system, which differed for analysis of TAGs (measured in positive ionization mode) and polar compounds (measured both in positive and negative ionization modes), were as follows: (i, TAG analysis) helium flow:, 3.0 L min^{-1}; gas temperature, 350 °C; discharge needle voltage, −3000 V; perforated electrode, +150 V; grid electrode, +250 V; ion guide voltage, 1100 V; (ii, polar compound analysis) helium flow, 3.0 L min^{-1}; gas temperature, 220 °C; discharge needle voltage, ±3000 V; perforated electrode, ±150 V; grid electrode, ±250 V; ion guide voltage, 800 V. Mass spectra were recorded in the range m/z 50−1000 at an acquisition rate of 2 spectra s^{-1}. Samples were automatically transferred in front of the DART gun exit on the glass rod of a Dip-it sampler (IonSense, Danvers, MA) and desorbed from its surface in hot helium gas for 30 s. For each sample, a minimum of three repeated measurements were carried out. To support the ionization of TAGs, ammonia was used as a dopant. For this purpose, a 2 mL autosampler vial containing aqueous ammonia was placed beneath the ion source exit. At the end of each analysis, mass spectra of PEG 600 (dissolved in methanol, 200 μg mL^{-1}) were acquired to enable mass drift compensation and, thus, accurate mass measurements.

Data Processing and Chemometric Analysis. Mass spectral data, recorded during DART ionization of examined samples, were processed with the Mass Center software (version 1.3, 2006; JEOL, Tokyo, Japan); that is, background subtraction and a mass drift compensation were carried out. Prior to the chemometric analysis, constant row sum transformation[13] of the data was carried out (the intensity of each ion was divided by the sum of intensities of all selected ions of each sample), and results of repeated measurements were averaged. PCA and LDA were performed employing Statistica (version 8.0; StatSoft, Tulsa, OK) and statistiXL (version 1.8, 2008; statistiXL, Broadway, Nedlands, Australia) software packages, respectively.

Classification results of LDA were presented in terms of recognition and prediction abilities. Recognition ability represents a percentage of successfully classified samples in the training set. Prediction ability is a percentage of correctly classified samples in the test set by using the model developed during the training step. For the prediction ability, a leave-one-out cross-validation procedure was used.

RESULTS AND DISCUSSION

The first part of this study was aimed at optimization of the sample preparation procedure and instrument settings to acquire mass spectra (spectral fingerprints) containing the most comprehensive information applicable for discrimination of the animal fat samples. The experimental strategy was based on experience obtained in our previous study concerned with the authentication of olive oil. Satisfactory results, that is, rich mass spectra and acceptable repeatability of observed ion intensities, were obtained when the procedures and measurement conditions used in our previous work were employed.[15] After careful examination of

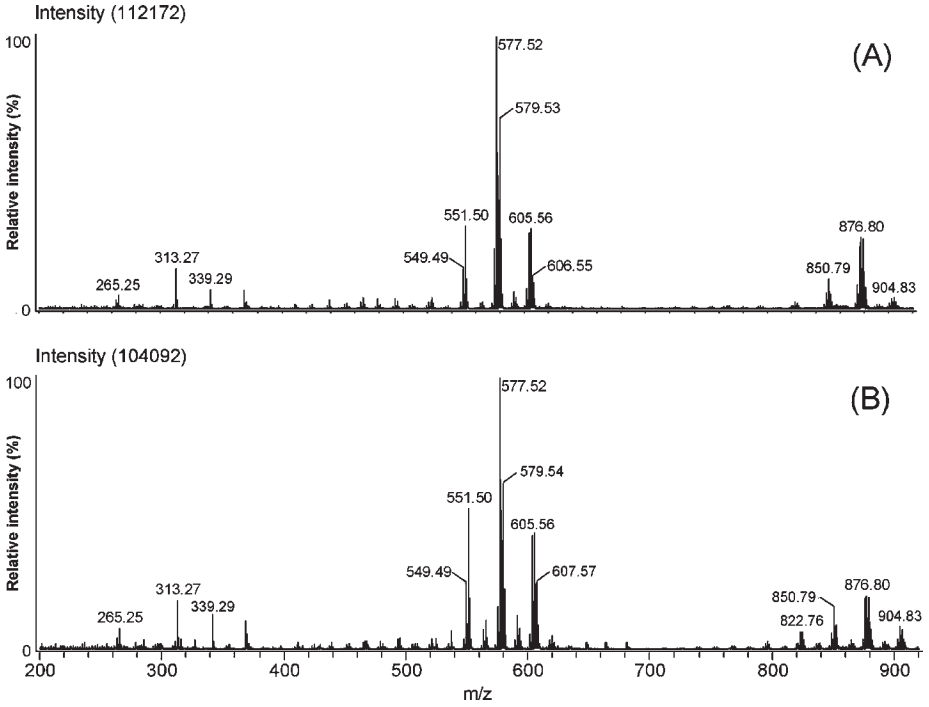

Figure 1. DART-TOFMS mass spectra (positive ionization mode, ammonia dopant) of experimental fats dissolved in toluene, 350 °C: (A) pork lard; (B) beef tallow.

mass spectral records obtained by an automated DART-TOFMS analysis of samples (dissolved fats and methanol−water extracts), tentative identification of detected compounds and chemometric treatment of the data were carried out. The results demonstrating feasibility of this approach for animal fat/meat (pork lard/pork and beef tallow/beef in this particular case) authentication are presented below.

DART-TOFMS Mass Spectra. Figure 1 shows typical DART-TOFMS spectra of pork lard and beef tallow (toluene solutions). The mass spectra are characterized by molecular adduct ions $[M + NH_4]^+$ and fragment ions of TAGs, which are the main components in fats. The formation of TAG ammonium adducts is induced by ammonia vapors present in the sampling area. It should be noted that signals of $[M + NH_4]^+$ are of significantly higher intensities (approximately by 1 order of magnitude) as compared to the intensities of protonated molecules $[M + H]^+$, which are observed when the dopant solution (aqueous ammonia) is excluded from the experimental setup. Additionally, as the result of the fragmentation processes occurring during DART ionization of parent TAGs, diacylglycerol fragment ions $[M + H - R_iCO_2H]^+$, monoacylglycerol fragment ions $[M + H - R_iCO_2 - R_iCO]^+$, and acylium ions $[RCO]^+$ were formed. More detailed inspection of a higher m/z region of lard and tallow mass spectra (Figure 2) shows apparent differences in TAG composition of the tested sample types. For example, the relative intensities of ions at m/z 820.74, 822.76, 848.77, 850.79, 904.83, and 906.85 were significantly higher in lard than in beef tallow. On the other hand, signals with m/z 874.79 and 878.82 were more abundant in tallow (Table 1). Lard and beef tallow records also significantly differed in relative abundance of fragment ions. The repeatability of the relative intensities seen for the observed ions calculated as relative standard deviation (RSD) from five repeated measurements was dependent on the abundance of the respective signal (the higher the abundance, the lower calculated RSDs) and ranged from 0.4 to 24.4%. Unequivocal identification of TAGs detected in the samples and/or resolution among their stereoisomers was not possible with the DART-TOFMS technique due to the simultaneous desorption/ionization of all present compounds. As shown in Table 1, in many cases more than one TAG could be attributed to an elemental composition estimated on the basis of the accurate mass of measured signals (TAGs with stearic, palmitic, palmitoleic, oleic, myristic, and linoleic fatty acids bound to the glycerol backbone were considered). It is worth noting that for sample discrimination using the chemometric processing of recorded spectral fingerprints, the identification of respective ions is not indispensable. Nevertheless, as far as more information is required, then in particular cases, the unequivocal identification of TAGs in examined fats can be carried out by employing HPLC separation coupled to APCI-MS.[7,8]

In the next step, mass spectra of fat samples isolated from pork and beef minced meat (obtained by both rendering and extraction with toluene) were compared with those obtained by DART-TOFMS analysis of dissolved neat fats. No statistically significant differences were observed in terms of TAG adduct and fragment ion relative intensities (variations were within the repeatability of the method). In our previous study,[15] the setting of the helium gas temperature and the degree of sample dilution with solvent were identified as the most important parameters affecting both absolute and relative intensities of ions yielded by TAGs during DART ionization. As the temperature of the ionization gas was kept constant (350 °C) during the analyses of all samples and the degree of dilution was the same for neat fats and samples isolated from meat by rendering, the fat-to-solvent ratio in the samples extracted with toluene was the unknown parameter, which could have caused the diversity of TAG mass spectra of the samples. When the fat content in the samples and the volume of toluene used for the extraction are taken into consideration, the theoretical fat-to-solvent ratios in pork and beef extracts should be 1:14 (w/v) and

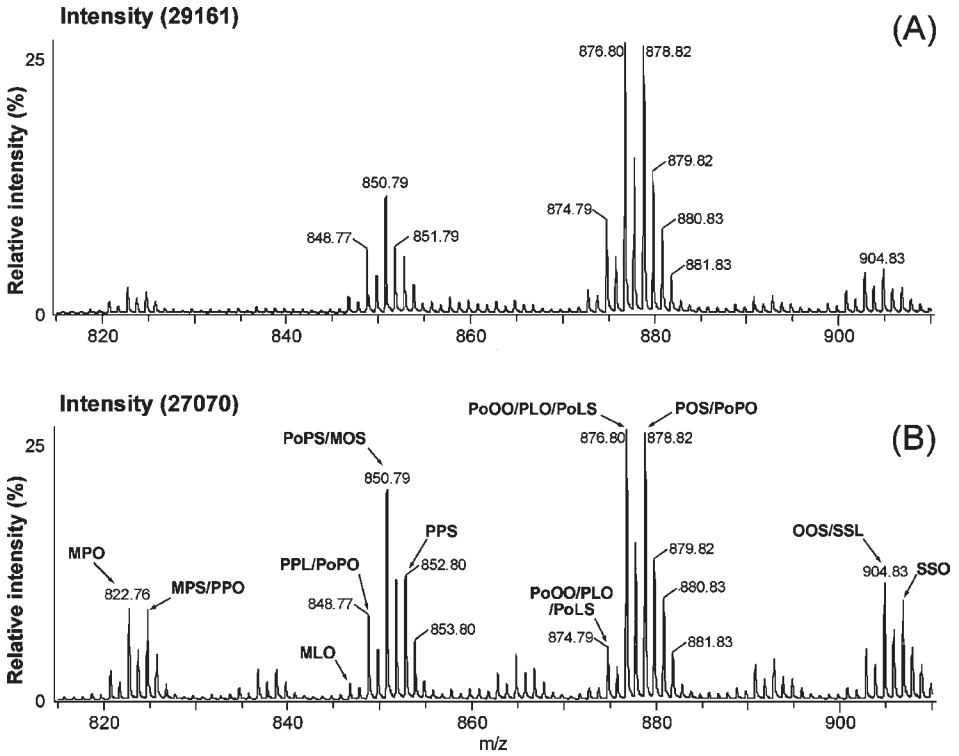

Figure 2. DART-TOFMS profiles of TAGs: (A) pork lard; (B) beef tallow (detailed zoom of Figure 1).

1:11 (w/v), respectively. However, as expected, the ratios in the actual extracts were significantly lower due to the relatively poor recovery of the accelerated extraction procedure (approximately 30%). On the basis of the fat content in the filtered extracts determined gravimetrically after evaporation of toluene, the "experimental" fat-to-solvent ratios were 1:47 (w/v) for pork and 1:43 (w/v) for beef extract, which were almost identical to the optimal value used for dissolution of neat and rendered fats [1:50 (w/v)]. Additionally, similar ratios were obtained also for the samples representing toluene meat mixture extracts. It should be emphasized that to ensure comparability of mass spectra and to enable general use of the extraction procedure, it is essential to determine the fat content in the examined meat extract and to adjust the fat-to-solvent ratio to its optimal value. Unfortunately, this drawback lowers the overall throughput of the procedure. The fact that TAG mass spectrometric profiles of fat solutions obtained by different procedures were comparable to each other was further used in chemometric analysis of the data, as it enabled us to create common LDA models for classification of both neat fats and meat samples as discussed below.

In Figure 3, DART-TOFMS profiles of fat methanol−water extracts recorded in positive and negative ionization mode are shown. In this particular case, ions with rather lower mass-to-charge ratios were observed in both ionization modes; also, the number of detected signals is considerably lower as compared to the records of dissolved fats. Employing accurate mass measurement, elemental compositions of major ions present in the profiles were estimated, and tentative identification was performed. The overview of these ions is provided in Table 2. Ions with elemental formulas corresponding to the protonated and deprotonated molecules of myristic, palmitoleic, palmitic, linoleic oleic, and stearic acids, which represent the major fatty acids of lard and tallow, were predominant signals observed in positive and negative ionization mode, respectively. The results of experiments carried out with the use of pure triolein and tristearin standards showed that $[M + H]^+$ and $[M - H]^-$ fatty acid ions are not formed by fragmentation or thermal degradation processes from TAGs, which can be potentially transferred into the methanol−water layer during the extraction step (data not shown). The observed signals most probably originated from free fatty acids (FFAs) naturally present in fat samples. It is noteworthy that the levels of FFAs in fats can be increased as a result of fat hydrolysis, for example, during thermal processing. In addition to FFAs, a signal at m/z 369.35 was detected in a positive ionization DART-TOFMS mass spectra and tentatively identified as the $[M - H_2O + H]^+$ ion of cholesterol formed by a neutral loss of a water molecule from the protonated compound.

To maximally utilize discriminative information in the TAG and polar compound profiles, obtained mass spectra were processed by PCA and LDA.

Principal Component Analysis. PCA represents a widely used unsupervised pattern recognition technique, which allows visualization of the multidimensional information in the form of a few principal components retaining the maximum possible variability within the data set. In the first phase of chemometric analysis, PCA was applied to the data sets obtained by instrumental analysis of dissolved lard/tallow (TAG profiles recorded in positive ionization mode) and methanol−water extracts of lard/tallow (polar compounds profiles measured in both positive and negative ionization mode) to explore any clustering behavior related to the origin of the fat. Transformed abundances of all ions given in Tables 1 and 2 were employed as input variables; thus, data matrices of $\{29 \times 34\}$, $\{29 \times 7\}$, and $\{29 \times 6\}$ were obtained. The projection of the samples along the directions identified by the first two principal components calculated within the PCA of the above data sets is reported in Figure 4. Whereas the PCA plot

Table 1. Typical Average Relative Intensities ($n = 5$) of TAG Adduct and Fragment Ions Detected in DART-TOFMS Mass Spectra of Pork Lard and Beef Tallow Solutions (in Toluene)

	lard		beef tallow		
m/z	relative intensity (%)	repeatability (RSD, %)	relative intensity (%)	repeatability (RSD, %)	composition of respective ion[a]
263.24	3.9	22.6	3.7	17.3	L[b]
265.25	6.3	11.1	7.5	8.2	O[b]
311.26	1.9	12.8	4.3	17.3	Po[c]
313.27	15.8	8.7	22.8	15.0	P[c]
337.27	2.4	11.3	2.7	24.4	L[c]
339.29	12.0	13.0	20.8	9.4	S[c]
341.31	7.6	12.4	13.6	15.8	O[c]
547.47	2.7	2.3	6.5	4.4	ML[d]
549.49	14.3	3.8	29.9	4.9	PPo/MO[d]
551.50	29.3	4.8	47.7	2.5	MS/PP[d]
573.49	2.7	10.8	2.3	12.8	PoO/PoL[d]
575.50	22.1	2.0	19.9	3.2	PL[d]
577.52	100.0		100.0		PO/PoS[d]
579.54	68.4	1.6	48.7	1.6	PS[d]
601.52	7.9	4.0	5.8	6.8	LO[d]
603.54	28.6	2.3	43.3	4.2	LS/OO[d]
605.56	29.8	1.2	46.6	1.5	SO[d]
607.57	9.6	0.4	16.7	5.0	SS[d]
820.74	0.0		4.2	4.1	MPoO[e]
822.76	2.4	4.9	9.2	7.8	MPO[e]
824.77	1.9	6.1	7.2	7.9	MPS/PPO[e]
846.76	1.8	6.9	2.2	6.3	MLO[e]
848.77	5.9	7.5	9.8	5.4	PPL/PoPO[e]
850.79	10.8	7.5	18.2	6.9	PoPS/MOS[e]
852.80	5.3	6.0	8.9	6.9	PPS[e]
872.77	2.2	7.5	1.3	8.8	LLP/PoOL[e]
874.79	9.0	7.2	5.4	4.1	PoOO/PLO/PoLS[e]
876.80	26.0	2.7	26.0	3.5	PoOS/PLS/OOP[e]
878.82	25.4	3.4	20.1	3.2	POS/PoSS[e]
880.83	7.9	5.2	6.7	4.6	SSP/PPA[e]
900.80	2.2	4.4	1.2	8.7	LLS/OOL[e]
902.82	4.0	4.8	5.3	4.7	LOS/OOO[e]
904.83	4.3	3.5	9.4	5.1	OOS/SSL[e]
906.85	2.5	5.9	6.6	4.9	SSO[e]

[a] Fatty acids in TAGs and fragment ions are indicated according to following legend: L, linoleic acid (C18:2); M, myristic acid (C14:0); O, oleic acid (C18:1); P, palmitic acid (C16:0); Po, palmitoleic acid (C16:1); S, stearic acid (C18:0). [b] Acylium ion $[RCO]^+$. [c] Monoacylglycerol fragment ion $[M - R_iCO_2 - R_iCO]^+$. [d] Diacylglycerol fragment ion $[M - R_iCO_2]^+$. [e] TAG adduct ion $[M + NH_4]^+$.

based on TAG profiles data demonstrates very good separation of samples representing lard and beef tallow, less separated and relatively dispersed clusters were obtained by PCA of the other two data sets (polar compounds profiles acquired in either positive or negative ion mode). These observations can be attributed to higher variability of FFA concentration levels across the sample set and relatively low number of input variables. With the PCA results taken into account, DART-TOFMS profiles of TAGs were identified as more suitable for pork lard/beef tallow differentiation compared to polar compound profiles.

Linear Discriminant Analysis. After PCA, stepwise forward LDA was applied to the data to establish a predictive model for sample classification. This frequently employed supervised pattern recognition technique is based on the calculation of linear discriminant functions, which maximize the ratio of variance between given classes and simultaneously minimize the ratio of within-class variance.[13] In total, six LDA models, summarized in Table 3, were developed. Recognition and prediction abilities representing the percentage of correctly classified samples during model training and cross-validation were calculated. To avoid model overfitting, the number of input variables was reduced during stepwise LDA algorithm.

From a comparison of the LDA models obtained from the DART-TOFMS profiles of neat fats (models 1−3), it is apparent that the maximal recognition and prediction abilities (both 100%), that is, no misclassification of the samples, was achieved for the model based on TAG data. The number of samples correctly classified into the assigned groups, observed for polar compound profile models, was considerably lower. In this case, recognition abilities of the models based on the data recorded in positive and

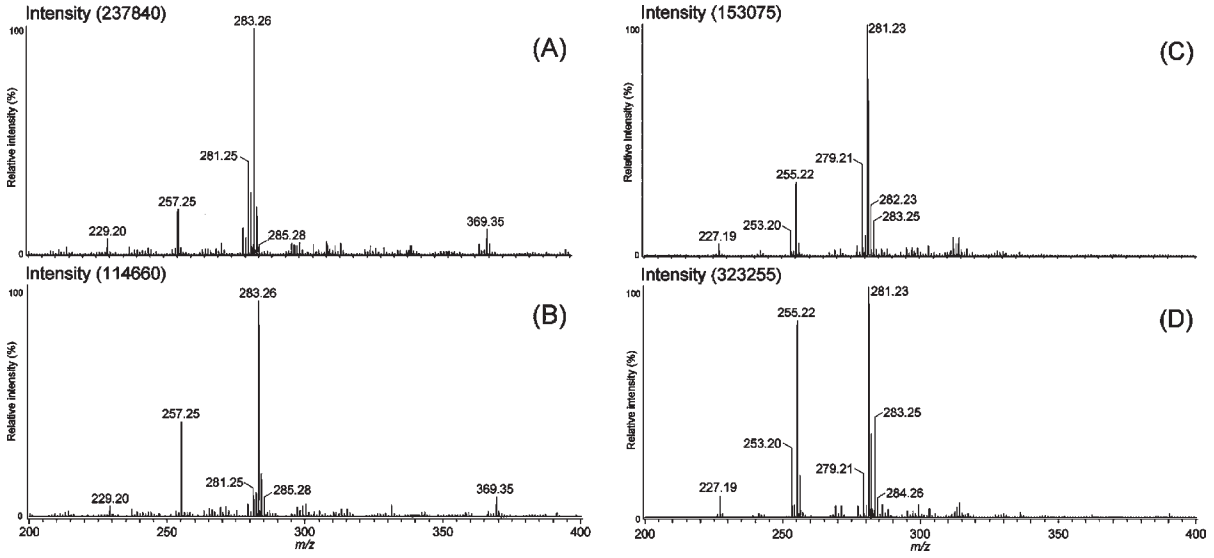

Figure 3. DART-TOFMS mass spectra of methanol−water extracts at 220 °C: (A) pork lard, positive ionization mode; (B) beef tallow, positive ionization mode; (C) pork lard, negative ionization mode; (D) beef tallow, negative ionization mode.

Table 2. Typical Average Relative Intensities ($n = 5$) of the Ions Detected in DART-TOFMS Mass Spectra of Pork Lard and Beef Tallow Methanol−Water Extracts

	lard		beef tallow		
m/z	relative intensity (%)	repeatability (RSD, %)	relative intensity (%)	repeatability (RSD, %)	identification
			Positive Ion Mode		
229.20	6.9	11.1	5.6	13.8	myristic acid[a]
255.23	2.8	10.8	3.0	9.9	palmitoleic acid[a]
257.25	25.3	8.1	24.1	6.3	palmitic acid[a]
281.25	45.7	5.7	10.1	16.9	linoleic acid[a]
283.26	100.0		100.0		oleic acid[a]
285.28	2.9	9.9	3.8	15.9	stearic acid[a]
369.35	12.5	6.6	9.8	7.8	cholesterol[b]
			Negative Ion Mode		
227.19	5.6	12.0	7.8	6.4	myristic acid[c]
253.20	10.9	5.7	29.6	4.9	palmitoleic acid[c]
255.22	35.2	6.1	92.1	4.3	palmitic acid[c]
279.21	45.1	4.0	18.5	15.8	linoleic acid[c]
281.23	100.0		100.0		oleic acid[c]
283.25	26.4	8.9	50.5	9.0	stearic acid[c]

[a] $[M + H]^+$. [b] $[M - H_2O + H]^+$. [c] $[M - H]^-$.

negative ionization mode were 93.1 and 89.7%, respectively. A prediction ability of 89.7%, which corresponds to the incorrect classification of three samples, was obtained for the models constructed with the use of either positive or negative ionization data. On the basis of these observations, which are also in line with the results of PCA, only TAG profile data were used in further chemometric analysis.

To explore the potential of the method for fat/meat adulteration detection, three additional LDA models were developed (models 4−6). In addition to the two original classes (pork lard and beef tallow), samples of neat fat admixtures (model 4) or meat admixtures (model 5 and 6) prepared in the given ratios were assigned the third sample class. It should be noted that samples obtained by rendering or toluene extraction of unadulterated pork and beef were added to the respective sample classes representing neat fats. As shown in Table 3, prediction abilities of 100% were obtained for all LDA models; thus, reliable discrimination among the respective classes was possible. Figure 5 displays score plots along two discriminant functions with well-resolved sample classes calculated in models 4 and 6. The described method enabled detection of 10% (w/w) beef tallow added to lard and 5% (w/w) of lard admixed to beef tallow. Using the TAG profile data of lard, tallow, and fat isolated from pork and beef admixtures, models capable of detection 10% (w/w) pork added to beef and vice versa were obtained. These admixed meat amounts corresponded to the approximate contents of 8 and 12% (w/w)

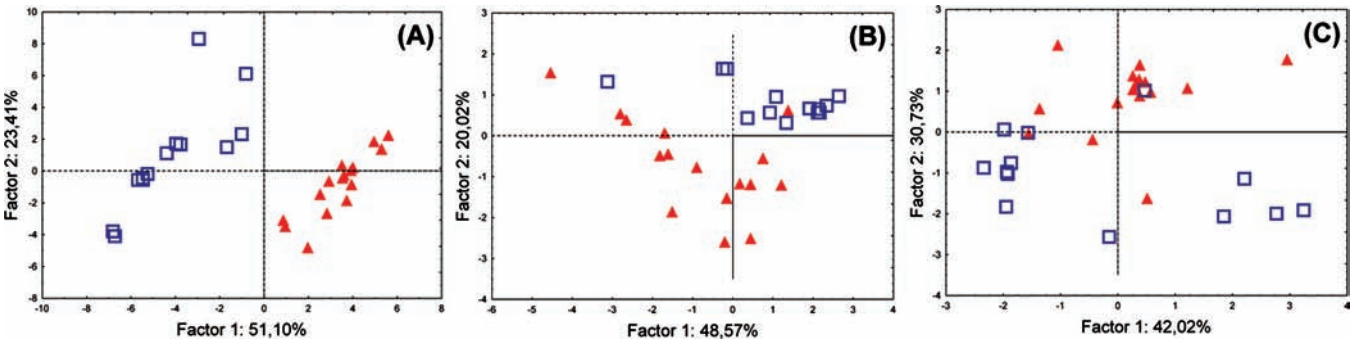

Figure 4. First and second PCA scores for (▲) pork lard and (□) beef tallow samples according to profiles of (A) triacylglycerols, (B) polar compounds, positive ionization mode, and (C) polar compounds, negative ionization mode.

Table 3. Overview of LDA Models Obtained within the Study

			no. of variables			
model	analyte	sample classes (no. of samples)	initial	stepwise reduced	recognition ability (%)	prediction ability (%)
1	triacylglycerols	lard (n = 16), tallow (n = 13)	34	8	100.0	100.0
2	polar compounds (positive ion mode)	lard (n = 16), tallow (n = 13)	7	5	93.1	89.7
3	polar compounds (negative ion mode)	lard (n = 16), tallow (n = 13)	6	4	89.7	89.7
4	triacylglycerols	lard (n = 17), tallow (n = 14), fat mixes (n = 12)[a]	34	11	100.0	100.0
5	triacylglycerols	lard (n = 17), tallow (n = 14), meat mixes (n = 10)[b]	34	11	100.0	100.0
6	triacylglycerols	lard (n = 17), tallow (n = 14), meat mixes (n = 10)[c]	34	13	100.0	100.0

[a] Mixes of neat lard and beef tallow in the ratios 5:95, 10:90, 25:75, 50:50, 75:25, and 90:10 (w/w). Each mixture in duplicate. [b] Fat isolated from pork and beef mixes [ratios 10:90, 25:75, 50:50, 75:25, and 90:10 (w/w)] by rendering. Each mixture in duplicate. [c] Fat isolated from pork and beef mixes [ratios 10:90, 25:75, 50:50, 75:25, and 90:10 (w/w)] by extraction with toluene. Each mixture in duplicate.

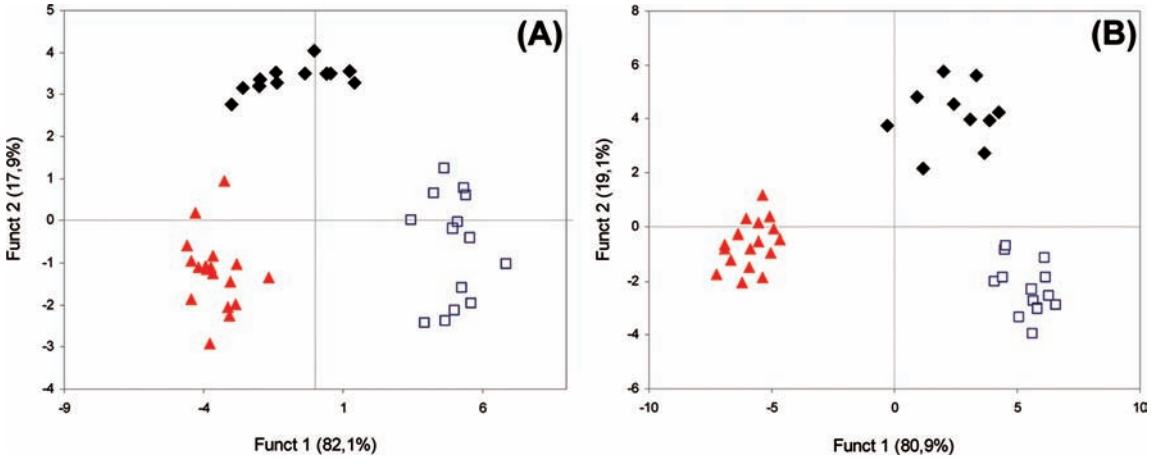

Figure 5. First and second LDA scores for (▲) pork lard, (□) beef tallow, and (◆) lard/tallow or pork/beef mixtures based on triacylglycerols: (A) model 4; (B) model 6.

of lard and tallow, respectively. The use of an accelerated procedure for fat isolation from meat did not increase the distinguishable amounts of admixed meats; however, the limits of adulteration detection were obviously strongly dependent on the fat content in both adulterated and adulterant meat.

The DART-TOFMS instrument was successfully used for a rapid differentiation and classification of lard and tallow samples in terms of the fat type by subjecting mass spectrometric data (profiles of TAGs and polar compounds) to PCA and LDA. With the LDA model constructed with the use of TAG profiles, the

detection of lard added to tallow and tallow admixed to lard at adulteration levels of 5 and 10% (w/w), respectively, was possible. By applying this approach to fat samples isolated by a simple procedure from pork/beef mixtures, the potential of DART-TOFMS for meat authenticity assessment was demonstrated.

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