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Identification of the Species of Origin for Meat Products by Rapid Evaporative Ionization Mass Spectrometry

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ABSTRACT. The increasingly abundant food fraud cases have brought food authenticity and safety into major focus. In this study, we present a fast and effective way to identify meat products using rapid evaporative ionization mass spectrometry (REIMS). The experimental setup was demonstrated to be able to record a mass spectrometric profile of meat specimens in a time frame of less than 5 seconds. A multivariate statistical algorithm was developed and successfully tested for the identification of animal tissue with different anatomical origin, breed and species with 100% accuracy at species and 97% accuracy at breed level. Detection of the presence of meat originating from a different species (horse, cattle, and venison) has also been demonstrated with high accuracy using mixed patties with a 5% detection limit. REIMS technology was found to be a promising tool in food safety applications providing a reliable and simple method for the rapid characterization of food products.

KEYWORDS. Food fraud, mass spectrometry, real-time tissue identification, horse meat

INTRODUCTION

Recently developed ambient ionization mass spectrometric methods removed a number of intrinsic constraints of traditional mass spectrometric analysis schemes, allowing in-situ, real-time analysis of a wide variety of samples. Although this feature of ambient MS methods has been demonstrated successfully, the early enthusiasm was curbed by problems regarding the interpretation of the resulting data. Since ambient sampling of unmodified objects does not allow reproducible analysis conditions (for instance in the case of the proposed luggage screening application at airports), the sensitivity of the method changes not only from sample type to sample type, but even in course of the analysis of a single object.[1]

Ambient profiling has been gradually introduced in the last few years, mostly for the analysis of biological material including tissues and unicellular organisms.[2] While Desorption Electrospray Ionization (DESI) has offered an excellent solution for the metabolic/lipidomic profiling of cells and biological tissues, DESI analysis requires frozen section samples for optimal performance.[3] This requirement is easily fulfilled in the case of tissue imaging analysis, however it is not suitable for rapid analysis. As a response to this problem, a number of alternative solutions were developed, following two fundamentally distinct strategies. In the case of probe electrospray (PESI) and related techniques a solid probe is immersed into the tissue specimen and the liquid phase residues are directly ionized from the sharp tip of the probe by direct electrospray ionization.[4] The alternative approach is based on the mechanical or thermal ablation of tissue material that leads to the formation of an aerosol containing charged particles. The underlying ionization method, i.e. the thermal ablation of largely aqueous samples by electric

current, was termed Rapid Evaporative Ionization Mass Spectrometry (REIMS).[5] REIMS-based tissue analysis generally takes a few seconds and can provide histological tissue identification with 90-98 % correct classification performance.[6] It has recently also been demonstrated that the REIMS spectrum of bacterial colonies, obtained by using standard bipolar electrosurgical tools as an ion source, shows excellent taxonomical specificity, allowing also the differentiation of strains at sub-species (e.g. serotype) level.[7] Based on these observations, REIMS technology is expected to find its application niche in the field of food security, with special emphasis on food authenticity and food microbiology applications.

Mass spectrometric techniques have been widely used for food security/authenticity applications, mostly in the form of LC-MS, GC-MS and isotope ratio MS (IRMS) assays.[8-9] GC-MS and LC-MS are largely used for the detection of xenobiotics (environmental pollutants, drug residues, illegal food additives, pesticides, etc.) in various foods or food ingredients.[10-13] More recently, biomarker-driven food authenticity testing has been gaining more attention, especially due to the high cost and time demand of the more traditional IRMS assays. Certain food types were found to contain chemically well-defined marker compounds (e.g. leptosin in Manuka honey), which can easily be detected using regular HPLC-MS/MS approaches, however the gold standard still remains IRMS where sub-ppm shifts in the $^{13}C/^{12}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$ ratios of certain organic constituents are associated with the biological and geographical origin of food components.[14-15] The most serious disadvantage of these methods is that the associated analytical schemes all require field sampling, transport of samples to the analytical laboratory, storage, extensive sample preparation, chromatographic separation and eventually mass spectrometric analysis of the individual molecular constituents of interest. As a result, the reporting times vary

in the range of a few days to a few weeks, while the associated costs can reach several hundreds of dollars per individual sample. REIMS technology may solve some of these issues, as no sample preparation is needed, and the analysis can be done within a two seconds with a simple monopolar handpiece and the mass spectrometer with no extra cost, leaving the only issue of transporting the samples to a laboratory containing a suitable mass spectrometer.

Since food counterfeiting (including mislabeling) is an emerging problem worldwide, the field of food authenticity testing requires new, cost-efficient, preferably on-site and real-time analytical approaches. This challenge was partially responded to by the widespread introduction of infrared profiling techniques, which can also be performed by handheld infrared probes.[16-17] While infrared spectroscopic profiles show excellent sensitivity to a broad range of variability including origin, presence of macroscopic constituents or method of preparation, the link between a detected anomaly and its chemical origin cannot be easily linked. For example if a new spectroscopic feature is detected, the spectroscopic information does not give sufficient information for the identification of its molecular origin. In principle, REIMS profiling can solve this and the previously mentioned problems by providing in-situ, real-time molecularly resolved information. The main purpose of the current study was to explore the capabilities of REIMS profiling with regard to the species- and breed-level differentiation of raw meat products. Due to the scandal associated with horse meat found in various meat-containing food products in the UK in 2013, one of the specific aims was to demonstrate the capabilities of REIMS with regard to the identification of horse meat.

MATERIALS AND METHODS

The proof of concept study was based on venison, Wagyu beef, grain-fed beef and horse minced meat samples obtained from Kezie Foods ltd. (Duns, Scotland) in 2x0.5 kg packs. Horse meat and horse liver samples were obtained from the University of Veterinary Sciences (Budapest, Hungary). The reproducibility and authenticity study was performed on five equine (Equine Centre, Johnstown, Ireland), five bovine samples supplied by an Irish abattoir (ABP Waterford, Ferrybank, Ireland), including two Hereford Cross, two Limousin Cross and one Blonde Cross breed, and ten Scottish bovine (Aberdeen Angus) samples supplied by a Scottish abattoir, all samples were from different animals. All measurements were carried out using a modified Xevo G2-S Quadrupole Time-of-Flight mass spectrometer (Waters Corporation, Wilmslow, UK). The experimental setup was identical to that reported earlier, [18] originally developed for the intraoperative identification of biological tissues. Briefly, REIMS analysis of tissue specimens was performed by electrosurgical evaporation and on-line mass spectrometric analysis of the aerosol produced. The monopolar electrosurgical handpiece was connected to an Erbotom ICC 300 (Erbe Elektromedizin GmbH, Tübingen, Germany) electrosurgical generator providing power-controlled sinusoidal 330 kHz alternating current. The generator was used in 'cut' mode at a 40 W power setting. All specimens were deposited on the 'neutral' or return electrode of the electrosurgical setup and incisions were made using a custom-built monopolar handpiece, that was equipped with a smoke evacuation line. The 4 m x 3.97 mm o.d., 2.38 mm i.d. Tygon PVC smoke evacuation line was connected to an air driven Venturi pump, which was mounted on the atmospheric interface of the mass spectrometer. The Venturi pump was driven by 2 bar nominal inlet pressure of zero grade pressurized air or nitrogen. The exhaust of the Venturi

pump device was sampled orthogonally by the inlet capillary of the mass spectrometer. In order to avoid the contamination of the instrument and enhance the sensitivity of the analytical setup, the atmospheric interface was equipped with a heated jet disruptor surface as it is shown in Figure 1. The jet disruptor was a model S coil (Kanthal, Hallstahammar, Sweden) and was kept at 800 °C. The depicted setup efficiently stops larger aerosol particles entering the Stepwave ion guide and all material deposited on the Kanthal surface undergoes subsequent carbonization and combustion into carbon dioxide and water. An additional 2ng/μL leucine-enkephalin solution in isopropyl-alcohol (0.2mL/min) was introduced directly into the atmospheric interface to provide internal reference peak for lock-mass calibration of data. Negative ion mass spectra were acquired in the mass range of *m/z* 150-1500 at a mass spectrometric resolution of 15,000 full width at half maximum (FWHM) at *m/z* 600. The chemical identity of detected ionic species was determined by accurate mass measurement, MS/MS fragmentation of molecular ions and comparison of this data to data obtained by the analysis of authentic standards under identical conditions. Phospholipid standards were obtained from Cayman Chemical (Ann Arbor, MI) and Avanti Polar Lipids (Alabaster, AL). All acquired data files were pre-processed using a custom-built software package (Waters Research Centre, Budapest, Hungary) containing standard Masslynx pre-processing algorithms (Waters). First the recorded scans were combined into average spectra containing 3-5 scans, resulting in 5-10 replicate spectra of each sample. Each meat sample was divided into 4 pieces in order to obtain replicates for reproducibility, while burger samples were recorded on 6 separate days, 4 batches each day. Frying was done in a Teflon coated frying pan with or without vegetable (Venus sunflower) oil and two different seasoning was studied, a simple with salt and pepper and a complex using seasoning mix

(containing: salt, dried vegetables (tomatoes, garlic with sulfite, onion), sugar, seasoning (paprika, black pepper, celery seed, nutmeg, cumin, coriander, chili), flavor intensifiers (sodium-glutamate, sodium-guanylate, sodium-inosinate), yeast extract, vegetable oil and silicone-dioxide). The spectra were background subtracted, and lockmass corrected using lockmass *m/z* 699.4970 in case of burgers or *m/z* 554.2516 in case of meat pieces. After lockmass correction, spectra were TIC normalized and rebinned to 0.1 Da bin.

The resulting data vectors were subjected to multivariate statistics in order to obtain a classifier for the identification of the species of origin. Principal component analysis (PCA) was used to eliminate chemical noise and reduce the dimensionality of the dataset. Following PCA, the first 25 principal components were subjected to linear discriminant analysis (LDA). The LDA classifiers were tested with leave 20%-out cross validation in case of minced meat patties and leave one animal out cross-validation in case of authentic meat samples, i.e. each data file containing spectra acquired from one animal was left out of the training set, a model was built on all other datasets and the data files left out were classified using the training model. Each animal was left out and classified exactly once. Each spectrum was classified to the closest class in the LDA space, however if the spectrum was farther from the closest class average than 5*standard deviation of the class, the spectrum was marked ‘outlier’ and excluded from analysis. The correct classification rate was calculated based on number of spectra classified correctly, compared to all spectra of the full dataset. An LDA classifier was built containing 0, 25, 50, 75, 100% of pure and mixed minced samples (venison, Wagyu beef, grain fed beef and equine) acquired on 6 different days, then used for on-line meat detection on samples containing 0, 1.25, 2.5, 5, 10, 25 and 33% of Wagyu beef and horse meat mixed into venison and grain fed beef meat. The acquired

spectrum was transformed by the PCA-LDA projection matrix to the LDA space of the training set, where Mahalanobis squared distance was calculated to each class average. The test specimen was classified to the tissue type for which the Mahalanobis distance is minimal, or marked 'outlier' as previously described and excluded from data analysis. The studies on specific phospholipid species were done in MATLAB (Mathworks, Natick, MA).

RESULTS AND DISCUSSION

The REIMS analysis of various animal tissues was found to yield spectra dominated by fatty acids and complex glycerophospholipids as it is shown in Figures 2 A, B and C. Although most spectrometric features are identical in case of the two different types of beef, a few characteristic differences can also be observed. In contrast, there is a clear difference between horse and beef spectra. The delay between sampling and appearance of the signal is less than two seconds, while a 2-3 second analysis time provides sufficient signal-to-noise ratio for the subsequent data analysis and identification of the sample. Carryover effects were studied by investigating the attenuation of the signal after evaporation was ceased and the effect of alternating the sample (e.g. beef-horse-beef) on the identification performance. Signal was observed for less than 2 seconds following the end of tissue evaporation and no carryover effects were observed when the samples were alternated without cleaning the setup in between the individual analyses, although visible contamination was observed on the inner wall of the ion transfer tube. The seemingly contradictory results of visible contamination with no signal carryover were explained by the nature of the ionization phenomenon. As described earlier, the charged aerosol particles are

165 formed upon the thermal evaporation of tissues and they are subsequently dissociated to produce
166 the observed molecular ions. Since the ionic species used for tissue identification (i.e.
167 glycerophospholipids) are non-volatile and cannot undergo desorption ionization from the inner
168 wall of the transfer tube, the observed contamination does not interfere with the recorded signal.
169 The only surface where phospholipid desorption can occur is the jet disruptor (Figure 1), however
170 the jet disruptor surface is kept at 800 °C and exposed to oxygen, hence the long-term survival of
171 organic species is unlikely there.

172 Regarding to the age and diet of the animals, previously we have shown that although age and
173 diet does not have an effect on the phospholipid profile, it does not affect the classification
174 accuracy. The deviation due to age and diet differences is significantly lower than the distance
175 between different classes.[18]

176 **Comparison of equine and bovine meat.** The first series of experiments were aimed at
177 establishing the difference between the spectroscopic data obtained from the REIMS analysis of
178 beef (*Bos Taurus*) and horse (*Equus caballus*) meat, respectively. In order to assess the real-life
179 applicability of the method, 50 g burgers were fried in a pan with or without the use of oil, and
180 different seasonings were also tested. The cooked samples were analyzed alongside the original
181 raw specimens. The resulting spectroscopic data was pre-processed and subjected to PCA. The
182 results are shown in Figure 3A, clearly demonstrating that data points referring to different
183 species of origin separate along the first principal component, with data points showing partial
184 separation along the second principal component as a result of processing (cooking). The PCA
185 analysis establishes that processing of the meat does not interfere with the identification of the

186 species of origin. A PCA-LDA model was built from a training set consisting of pure horse and
187 Angus beef spectra acquired from both raw and fried mince patties. The cross-validation of this
188 PCA-LDA model resulted in a 99.5% correct classification of horse and beef, nevertheless the
189 raw and cooked samples could be separated with an 80.1-97.8% accuracy. The preparation of
190 sample has clearly no effect on the differentiation of the species. Most well-established tests
191 available for the identification of horse meat in food items employ either immunochemical or
192 molecular genetic methods and both of these methods suffer from the hydrolysis of protein or
193 DNA targets associated with heat treatment,[19-21] however a number of ELISA-based and LC-
194 based methods provide excellent results for heat-treated samples.[22-24] In this aspect, the REIMS-
195 based identification methods are also largely insensitive to heat treatment.

196 Following the proof of concept study, fifteen authentic bovine and five equine samples were
197 obtained. All samples were divided into 4 pieces and a total of 30 sampling points were taken in 4
198 separate experiments. A multivariate model was built from all sampling points (e.g. a total of 600
199 spectra including 300 Aberdeen Angus beef, 30 Blonde cross beef, 60 Hereford cross, 60
200 Limousin cross and 150 equine) and the classification performance was evaluated using leave-
201 one animal out cross validation resulting in 100% separation between equine and bovine samples.
202 A second, leave 20% out cross validation was calculated for different breed types, as there was
203 only one Blonde cross animal sample, thus leave-one animal out was not applicable. There was a
204 97.48% correct classification rate. The pseudo 3-dimensional LDA plot is shown on Figure 4A. A
205 small portion of samples were ground and four 10 g patties were created containing 2.5, 5, 10%
206 of equine meat in Hereford cross bovine meat and 25% equine in Limousin cross meat. The
207 patties were tested subsequently by sampling each of them 10 times and classified immediately

208 using the multivariate model built from all 600 sampling points. The breed of the bovine meat
209 was correctly classified in all four cases, while the horse meat was detected in three cases out of
210 four (Figure 4B). In the case of 5% equine – 95% bovine, the horse meat was not detected.

211 Principal component analysis not only allowed us to look at the similarities and differences in
212 large multivariate datasets, but also shed light on the background of the separation of data groups
213 in the form of loading functions explaining the composition of individual principal components.
214 For mass spectrometric data, the loading functions show what the contribution of individual mass
215 spectrometric peaks is to the given principal component, as it's shown in Figure 4C and D. The
216 loading function responsible for the separation of the REIMS lipidomic profiles of horse and
217 cattle skeletal muscle shows clear biochemical information. The base peaks in both directions
218 (i.e. positive and negative) correspond to phosphatidyl-ethanolamine species including
219 plasmalogens with 34, 36, 38 carbon atom cumulative acyl chain length, however horse tends to
220 produce stearoyl-linoleyl-phosphatidyl-ethanolamines (PE(36:2), PE(38:2)) and plasmalogens
221 with even number of double bonds PE(P-36:4), while cattle produces species with odd number of
222 double bonds, i.e. PE(34:1), PE(36:1) and plasmalogens PE(P-34:1) in larger amounts, as
223 revealed by the loading plot and MS/MS fragmentation of the corresponding ions (Figure 4C).
224 Similar saturation/desaturation patterns were observed for other PE molecular ions and their [M-
225 $NH_4]^-$ counterparts and phosphatidyl-inositol ions (PI) produced by the REIMS process. The six
226 most abundant peaks of PC1 and PC2 were selected for further "marker ratio" analysis. All of
227 them were coupled and the four combinations with the highest discriminating power were
228 selected namely PE(P-34:1)/PE(P-36:4) and PE(P-34:1)/PE(38:2) and PE(P-36:4)/PE(38:2) and
229 PE(P-34:1)/PI(36:1) and tested (Figure 5). The cross-validation based uniquely on these peaks

between equine and bovine resulted in 100% separation. Figure 5A and B both show the combination of one bovine and one equine specific peak, thus the ratio of the peaks could be used for the separation of the two species as shown on the insert box plot. Even using one measure as the ratio of two significant phospholipid species can be used for the separation of equine and bovine. Figure 5C shows a combination of two peaks more abundant in equines, while Figure 5D shows the combination of two peaks more abundant in bovines. Both combinations have the power to differentiate between the species, however the ratio would not be suitable for separation.

Comparison of different tissue types within the same species. Two different tissue types of the same species and a given tissue type of two species were analyzed in an experiment and the results were subjected to PCA analysis in order to demonstrate the relative magnitudes of the difference between species and tissue types. The results are shown in Figure 3B, demonstrating that there are comparable differences at both species and organ level, however these do not interfere with each other. There was a 100% correct classification of the three different products based on the cross-validation underlying the clear separation observed on the PCA plot. Results imply that the REIMS technique, given that sufficient amount of reference data is available, is able to identify not only the species of origin, but also the type of tissues. This is not surprising in the light of earlier human studies, where REIMS was used for the identification of various healthy and cancerous human histological tissue types.[6]

Comparison of different type of cattle, horse, venison and their mixtures. In food counterfeiting cases where ground meat is involved, the actual product is rarely pure with regard

to its species of origin. In most of the cases the mixtures contain variable amount of the inappropriate component, hence food testing assays are expected to detect the 'contaminant' down to 1% concentration. While the multivariate model is not ideal for the trace detection of a tissue with a certain taxonomical origin in the matrix of the other, we tested the capabilities of the method in this direction by constructing a PCA/LDA supervised model featuring horse (*E. caballus*), venison (roe deer) (*Capreolus capreolus*) and two types of cattle (Black angus, Wagyu) (*B. taurus*) skeletal muscle and the 1:1 binary mixture of all pairs. The model featuring complete separation among the individual groups in the demonstrated three dimensions is shown in Figure 6. Leave-20% out cross-validation of all pure and 50-50% mixed minced samples resulted in 97.79% correct classification. Although the localization of binary mixtures in between their components is reassuring regarding the linearity of the model, the relative dispersion of the homogeneous data groups indicates the lack of feasibility of proper quantitation using the model. The model is likely to allow the detection of 10-20% contribution of tissue produced by another species in the sample, however these numbers are not yet satisfactory from a legal/regulatory aspect.

Detection limit. In order to test the feasibility of the detection of various amounts of different meat present, the various types of tissues were finely ground and thoroughly mixed together at different ratios (1.25, 2.5, 5, 10% Wagyu beef and horse meat mixed into venison and grain fed beef meat; 33% each of three different meat types mixed together and 25% each of all four different meat mixed into one patty). Mince patties were prepared using 25 g (~6 oz) of sample and were sampled 11 times (in an approximate timeframe of 3 s/sampling point). Spectra were classified using a model containing pure horse/beef/venison/Wagyu and different mixtures (e.g.

25, 50, 75% of each). The results of this study are shown in Table 1. below. In the case of 1.25% and 2.5% patties, in only 1 out of 4 cases was the meat detected, however in all other cases all meat types mixed in the patty were detected. The results show that detection limit down to 5% concentration level is feasible using the REIMS technology. In real life examples, most of the food fraud cases will contain more than 5% of illegal mixture within the product, in those cases where the whole species is not replaced by a less expensive alternative. In order to assess the feasibility of the detection of even lower concentration levels (preferably down to 0.1%) of tissue of another species, acquisition of considerably more data is necessary. Furthermore, trace detection will require a different bioinformatics approach, most likely by the discovery of species-specific biomarkers which can be used as presence/absence marker for a given species.

Application of the REIMS technique for the rapid lipidomic profiling of food-grade meat products was successfully demonstrated for the first time. The described method does not require any sample preparation or high resolution mass spectrometric analysis, thus it may be performed on small portable mass spectrometers in the future. The results clearly demonstrate that the lipidomic profiles can be recorded in a few seconds timeframe and the profiles show good species- and breed-level specificity.

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FIGURE CAPTIONS

Figure 1. REIMS experimental setup used for sampling. A. The setup using a custom built monopolar handpiece for sampling the different meat types. The handpiece was equipped with a smoke evacuator connected to the REIMS source of the mass spectrometer on the distal end; B. The REIMS source without the Venturi chamber. The heated collision surface or jet disruptor element stops larger particles from entering the mass spectrometer and facilitates ion formation with the impact of large droplets on its surface.

Figure 2. Mass spectra acquired from A. and B. two different breed bovine meats and C. equine meat with REIMS. The spectra mainly feature fatty acids in the *m/z* range 100-500 and glycerophospholipids in the range *m/z* 600-900.

Figure 3. Identification of different products and effect of cooking on mass spectra. A. Three-dimensional PCA plot of raw and cooked Angus beef and horse meat; B. Three-dimensional PCA plot of two different horse organ products and beef.

Figure 4. A. Pseudo 3-dimensional plot of data obtained by the analysis of 4 different bovine breed and equine samples; B. Real-time analysis of meat with home-build classification software; C. First principal component loading plot with an insert showing the first two principal components; D. Third principal component loading plot with an insert containing the first and third principal component. PC3 was chosen because the separation between different cattle breed was more specific to PC3, while the separation between equine and bovine was more specific to PC1 and PC2.

Figure 5. Comparison of level of unique phosphatidyl-ethanolamine and phosphatidyl-inositol species in bovine and equine. A. Relative intensity of PE(P-34:1) and PE(P-36:4). The insert contains the 25:75 percentile – median - box plot showing the ratio of the two species; B. Relative intensity of PE(P-34:1) and PE(38:2). The insert contains the 25:75 percentile – median - box plot showing the ratio of the two species; C. Relative intensity of PE(P-36:4) and PE(38:2) and D Relative intensity of PE(P-34:1) and PI(36:1).

Figure 6. Pseudo 3-dimensional plot of products from 4 different species or breed and the 1:1 mixture. The axes represent the projected coordinates of linear discriminant 1, LD1 (red), LD2 (green) and LD3 (blue).

TABLES

Table 1. Classification of Patties Containing One or More Different Meat Type.

% of Different Meat Types in the Patty	Horse (HO) Meat Detected	Wagyu (WA) Meat Detected	Venison (VE) Meat Detected	Grain Beef (GR) Meat Detected	False Positive/ Negative
1.25HO98.75GR	-	-	-	+	FN
1.25HO98.75VE	-	-	+	-	FN
1.25WA98.75GR	-	-	-	+	FN
1.25WA98.75VE	-	-	+	+	
2.5HO97.5GR	-	-	-	+	FN
2.5HO97.5VE	-	-	+	-	FN
2.5WA97.5GR	-	-	-	+	FN
2.5WA97.5VE	+	+	+	-	FP
5HO95GR	+	+	-	+	
5HO95VE	+	-	+	+	FP
5WA95GR	-	+	-	+	
5WA95VE	-	+	-	+	
10H090GR	+	-	-	+	
10HO90VE	+	-	+	+	FP
10WA90GR	-	+	-	+	
10WA90VE	-	+	+	-	
GR	-	-	-	+	
33GR33HO33WA	+	+	-	+	
HO	+	-	-	-	
33HO33VE33GR	+	-	+	+	
VE	-	-	+	+	FP
33VE33HO33WA	+	+	+	+	
25VE25HO25WA25GR	+	+	+	+	
WA	-	+	-	+	
33WA33VE33GR	-	+	+	+	

FIGURE GRAPHICS

Figure 1.

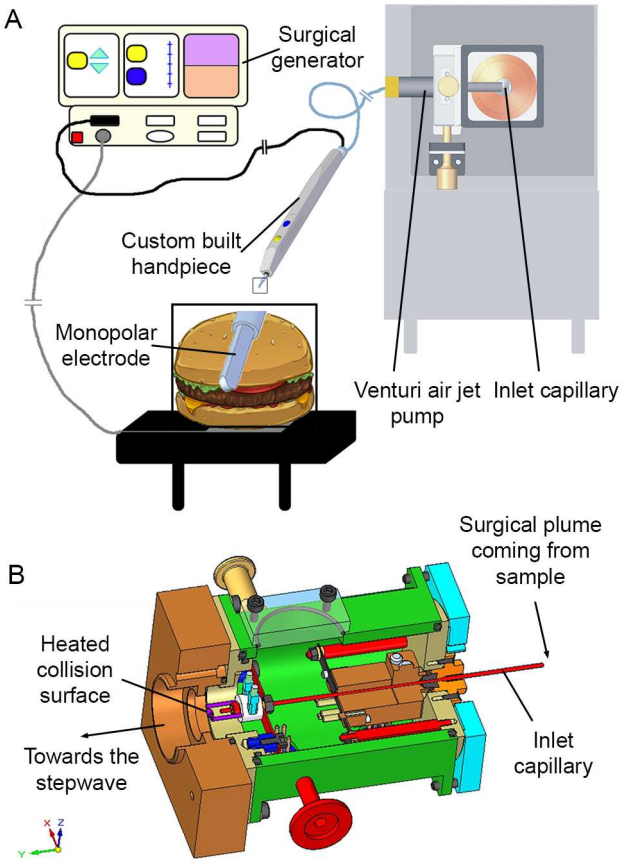


Figure 2

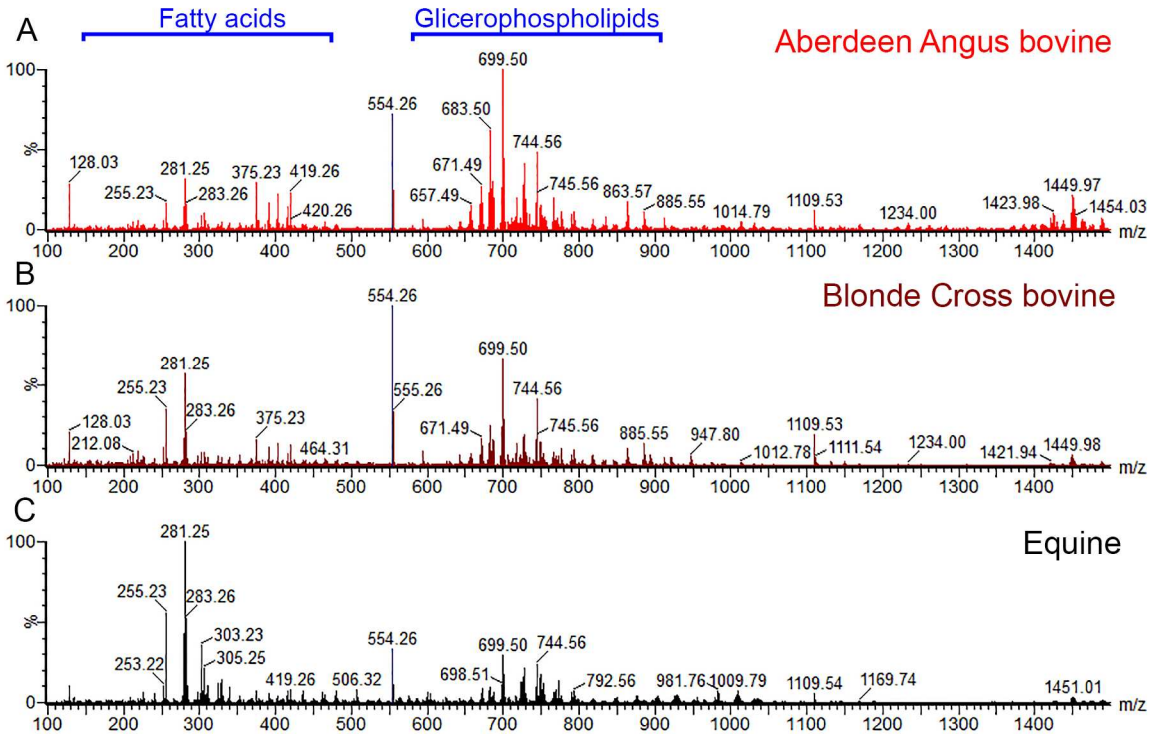

A
Fatty acids
Glicerophospholipids
Aberdeen Angus bovine
699.50
554.26
683.50
744.56
671.49
128.03
281.25
375.23
419.26
255.23
283.26
657.49
745.56
863.57
885.55
1014.79
1109.53
1234.00
1423.98
1449.97
1454.03
420.26
B
Blonde Cross bovine
554.26
699.50
281.25
255.23
555.26
744.56
283.26
128.03
375.23
671.49
745.56
885.55
947.80
1109.53
212.08
464.31
1012.78
1111.54
1234.00
1421.94
1449.98
C
Equine
281.25
255.23
283.26
303.23
554.26
699.50
744.56
253.22
305.25
698.51
419.26
506.32
792.56
981.76
1009.79
1109.54
1169.74
1451.01
%
m/z
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400

Figure 3

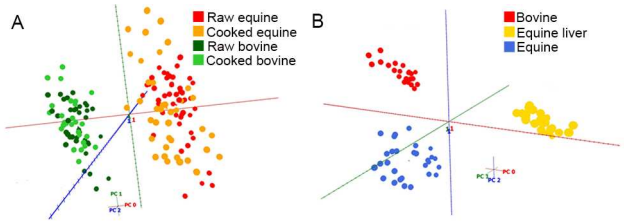

A
Raw equine
Cooked equine
Raw bovine
Cooked bovine
B
Bovine
Equine liver
Equine

Figure 4

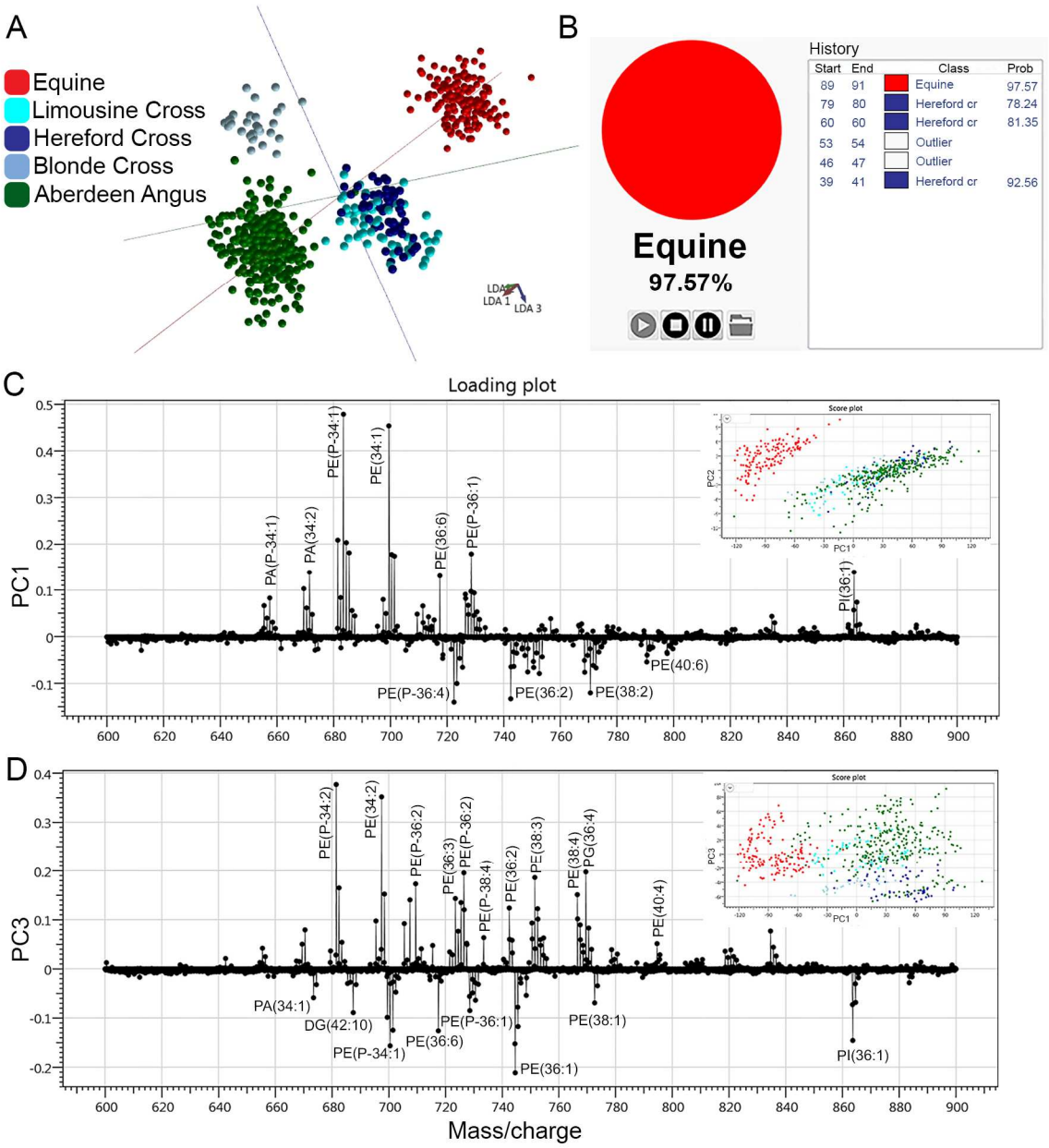

A
Equine
Limousine Cross
Hereford Cross
Blonde Cross
Aberdeen Angus
LDA 1
LDA 3
B
Equine
97.57%
History
Start End Class Prob
89 91 Equine 97.57
79 80 Hereford cr 78.24
60 60 Hereford cr 81.35
53 54 Outlier
46 47 Outlier
39 41 Hereford cr 92.56
C
Loading plot
PC1
PA(P-34:1)
PA(34:2)
PE(P-34:1)
PE(34:1)
PE(36:6)
PE(P-36:1)
PE(P-36:4)
PE(36:2)
PE(38:2)
PE(40:6)
PI(36:1)
Score plot
PC2
PC1
D
PC3
PE(P-34:2)
PE(34:2)
PE(P-36:2)
PE(36:3)
PE(P-36:2)
PE(P-38:4)
PE(36:2)
PE(38:3)
PE(38:4)
PG(36:4)
PE(40:4)
PA(34:1)
DG(42:10)
PE(P-34:1)
PE(36:6)
PE(P-36:1)
PE(36:1)
PE(38:1)
PI(36:1)
Score plot
PC3
PC1
Mass/charge

Figure 5

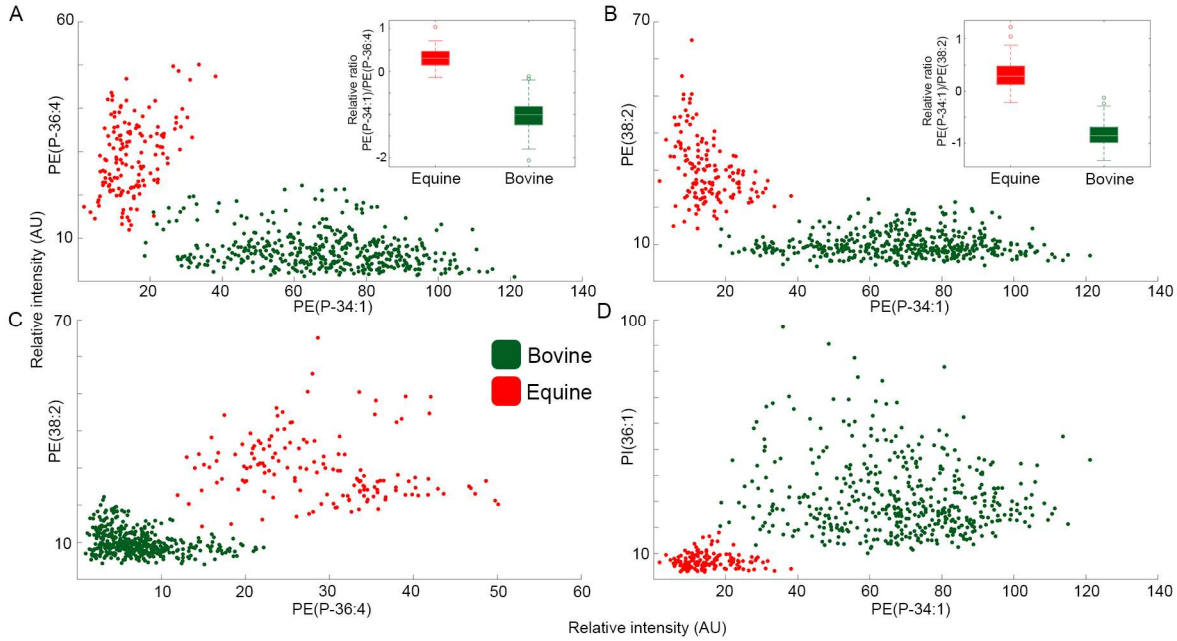
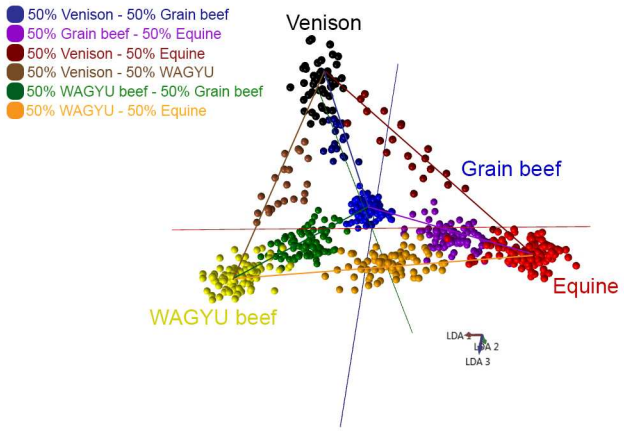
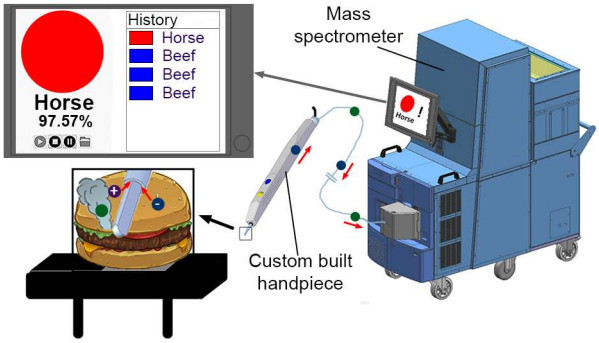

A
B
C
D
PE(P-36:4)
PE(P-34:1)
Relative intensity (AU)
Relative ratio PE(P-34:1)/PE(P-36:4)
Equine
Bovine
PE(38:2)
Relative ratio PE(P-34:1)/PE(38:2)
PI(36:1)
Bovine
Equine

Figure 6


50% Venison - 50% Grain beef
50% Grain beef - 50% Equine
50% Venison - 50% Equine
50% Venison - 50% WAGYU
50% WAGYU beef - 50% Grain beef
50% WAGYU - 50% Equine
Venison
Grain beef
Equine
WAGYU beef
LDA 1
LDA 2
LDA 3

TOC GRAPHIC


History
Horse
Beef
Beef
Beef
Horse
97.57%
Mass
spectrometer
Custom built
handpiece