ASSAY FOR IDENTIFYING ANIMAL SPECIES IN RAW AND COOKED CONDITIONS

FIELD OF THE INVENTION

The present invention relates to a method for identifying animal species in raw and cooked conditions based on animal fats detection.

BACKGROUND OF THE INVENTION

Mixing undeclared species in meat products is illegal under Food Labeling Regulations. Apart from regulatory reasons, the prevention of adulteration of meat products with less desirable meat species is important to the consumers for economic, health, food safety and religious reasons.

A conventional PCR technology for halal authentication by extracting DNA from animal species has been well known. However, this thermal processing limits the use of DNA technologies as heat treatment degraded the protein due to heat denaturation.

Several methods have been developed to identify meat species including electrophoresis, chromatography, DNA hybridization, and immunoassays. Immunological techniques, including agar-gel immunodiffusion (AGID) and enzyme-linked immunosorbent assay (ELISA) are most commonly applied for meat species identification.

There are several disadvantages to the official method AGID. Concentrated antiserum preparations are required to obtain visible precipitin lines in AGID. Obtaining the antiserum is expensive in large-scale testing. Furthermore, the sensitivity of AGID is variable. Usually ten percent or more contamination must be present to detect adulteration with this method. Lastly, AGID cannot be used for species identification in cooked meat because of the shortage of commercial antiserum specific to cooked meats.

The ELISA method has emerged as a sensitive, rapid, and specific method for meat speciation. This method can detect levels as low as one percent or less of meat contamination. Furthermore, it requires only simple sample preparation and no expensive equipment or highly skilled operator. In addition, the ELISA technique can be used for both

qualitative and quantitative analysis of meat proteins. Specific antibodies, either polyclonal antibodies or monoclonal antibodies (MAbs), are required as capture reagents in ELISA.

Monoclonal antibodies as disclosed in ***WO02065126*** and ***US2003022248*** documents which bind to Troponin I, a thermostable muscle protein for raw and cooked meat species identification, however, adulteration with fat and oil of different/other animal species limit the use of monoclonal antibodies (the antibodies are only binded to muscle protein).

Most of the ELISA methods currently employed for meat species identification use polyclonal antibodies as a capture reagent. However, polyclonal antibodies have disadvantages such as limited production, variable affinity and a requirement for further purification to eliminate crossreaction.

The present invention overcomes these and other deficiencies of the above-mentioned drawbacks by providing a method for meat species identification by analysing the animal fat triacylglycerols (TAGs). The present invention provides a greater efficiency and economically during operation.

SUMMARY OF THE INVENTION

The present invention provides a method for analysis of triacylglycerols from animal fats comprising providing a preparation of a sample comprising triacylglycerols; applying the preparation of sample as determined by chromatography having a separation material; separating and detecting the triacylglycerols content of said preparation of the sample; collating the detection data into a data analysable form; and analysing the data using chemometrics for identifying and determining animal fats belonged to different animal species.

In one of the preferred embodiment, the sample is prepared using an accelerated solvent extraction and the chromatography used is a reverse phase high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (RPHPLC/MS-APCI).

In another preferred embodiment, a mobile phase gradient for separation of triacylglycerols is a combination of acetonitrile, dichloromethane and ammonium acetate in methanol.

In another preferred embodiment, the atmospheric pressure chemical ionization is operated in a positive ion mode.

In yet another embodiment, the chemometrics is a principal component analysis (PCA) and the animal species are cow, pig, chicken and lamb in raw condition and cooked condition.

A method for identifying animal species in raw and cooked conditions comprising providing a preparation of a sample comprising triacylglycerols; applying the preparation of sample as determined by chromatography having a separation material; separating and detecting the triacylglycerols content of said preparation of the sample; collating the detection data into a data analysable form; and analysing the data using chemometrics for identifying and determining animal fats belonged to different animal species.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate embodiments of the invention and, together with the description, serve to explain the principles of the invention.

Figure 1(A) illustrates a RP HPLC-APCI MS Chromatogram of TAGs from raw and cooked meat products of beef in accordance of the present invention.

Figure 1(B) illustrates a RP HPLC-APCI MS Chromatogram of TAGs from raw and cooked meat products of lamb in accordance of the present invention.

Figure 1(C) illustrates a RP HPLC-APCI MS Chromatogram of TAGs from raw and cooked meat products of chicken in accordance of the present invention.

Figure 1(D) illustrates a RP HPLC-APCI MS Chromatogram of TAGs from raw and cooked meat products of pork in accordance of the present invention.

Figure 2 (A) illustrates PCA scores plot of TAGs for raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

Figure 2 (B) illustrates PCA scores plot of TAGs for raw and cooked meat products of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

Figure 3 (A) illustrates a loadings plot for i) PC1 and ii) PC2 of TAGs from raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

Figure 3 (B) illustrates plots of PC1 vs. PC2 loadings values from PCA scores plot of TAGs from raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

Figure 4 (A) illustrates a loadings plot for i) PC1 and ii) PC2 of TAGs from raw and cooked meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

Figure 4 (B) illustrates plots of PC1 vs. PC2 loadings values from PCA scores plot of TAGs from raw and cooked meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention.

DETAILED DESCRIPTIONS OF THE INVENTION

The present invention will now be described in detail in connection with specific embodiments with reference to the accompanying drawings. Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense that is as "including, but not limited to". Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring

to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

HPLC is a very well known method for the separation and analysis of solute molecules. The separation process is effected through liquid chromatography and relies on the fact that a number of component solute molecules in a sample stream of fluid (mobile phase) flowing through a packed bed of particles (stationary phase) can be efficiently separated from one another with a high degree of resolution. This is based on the fact that individual components in the mobile phase have a different affinity for the stationary phase and therefore a different rate of migration and exit through the column. The efficiency of separation is determined by the amount of spreading through the column which is determined by the column composition.

Triacylglycerols (TAGs) or triglycerides are the main components of fats and oils and are comprised of three fatty acids esterified at the stereo-specific number (sn) positions of n-1, sn-2 and sn-3 of a glycerol molecule. Triacylglycerols in fats and oils have many properties which depend upon their fatty acid composition and especially the stereo-specific distribution of the fatty acids on the glycerol moiety. The three letter code of TAGs in the present invention includes the suffix "where more than one regioisomer can exist in a form that can, potentially, be separated chromatographically" **(Is this a limitation of this method? If the sample does not have more than one regioisomer can this method still validly detect the components in raw and cooked conditions? Please comment).**

By meat type or class is intended a broader class of meats including several meat species. Thus, type or class refers to mammalian, avian, poultry, and the like. By meat or meat species is generally intended meats which are commonly used for consumption, including

poultry such as chicken and mammalian meats, such as pork, beef and lamb. Thus, particular meat species include but are not limited to chicken, pork, beef, lamb and the like.

The present invention relates to a separation of animal fats, TAGs on a single stage reversed phase high performance liquid chromatography (RP HPLC) coupling with atmospheric pressure chemical ionization tandem mass spectrometry (APCI) and ion trap mass spectrometer has been developed for a potential method for discriminating different animal species (beef, pork, chicken and lamb) cooked by microwave, roasting, hotpot and currying. The TAG profiles are analysed by the application of principal component analysis (PCA) to explore the extent of variation occurring with different types of cooking and different animal species. Several studies have reported separation of TAGs from animal fats by RPHPLC coupled with an APCI ionization source as the interface to a mass spectrometry (MS) analyzer (Fauconnot et al., 2004; Dugo et al., 2006; Kallio et al., 2001; Mottram et al., 2001 and Leskinen et al., 2007). There is, however, evidence of co-elution in the chromatograms produced. In the present invention, a method is developed for complete separation of TAGs from animal fats having a developed mobile phase gradients (combination of acetonitrile, dichloromethane and 10 mM ammonium acetate in methanol) are in **Table 1**.

Table 1. The mobile phase gradient for separation of TAGs

Time (min)	Flow rate (mL/min)	% acetonitrile	% dichloromethane	% ammonium acetate (10 mM)
0	1	76	20	4
82	1	75	21	4
100	1	69	27	4
145	1	57	39	4

In APCI, TAGs are identified from the ammoniated molecules and their diacylglycerols (DAG) ions in MS/MS or tandem mass spectrometry (MS^2). The fatty acid (FA) positions are

identified from the presence and relative intensities of the DAG product ions in MS^2. The relative intensities of DAG product ions in the MS^2 spectra of the ammoniated TAG ions depend on FA position along the glycerol backbone (Byrdwell, 2005). The TAGs exhibiting no co-elution give clean spectra, which is important in obtaining the correct DAG ion ratios for compound identification.

To overcome the difficulties in attempting to detect adulterants that have approximately the same chemical composition as the original food product, the instrumental techniques of RP HPLC APCI MS used are associated with the use of chemometrics which is principal component analysis (PCA) in the present invention.

The improved RP HPLC separation of TAGs developed in the present invention has shown to give more reliable discrimination of different animal species than conventional methods. An improved separation of important TAGs has lead to improved PCA scores for different animal species. All animal species separated well in the principal component analysis (PCA) plot of TAG profiles.

In one preferred method for analysis of triacylglycerols from animal fats of the present invention comprising by providing a preparation of a sample comprising triacylglycerols at the beginning. The preparation sample is determined by chromatography having a separation material. A process of separation and detection of triacylglycerols content of the preparation of the sample is occurred. The detection data collected is then collated into a data analysable form and finally chemometrics is used to analyse the data to identify and determine the animal fats belonged to different animal species. This method is further applied to identify animal species in raw and cooked conditions. The method for analysis of triacylglycerols from animal fats of the present invention is further provided with examples as illustrated below:

Examples

Materials and Methods

A. Sampling

Different anatomical locations of adipose tissue from pigs

A total of 24 samples of adipose tissue from pork fat were taken from four different locations from three different pigs. The four locations were; ham, shoulder, loin and belly. The adipose tissue samples (about 5 g) were taken from subcutaneous and intermuscular sections at each of the locations, following Timon et al., 2001. All samples were extracted immediately.

Raw/uncooked meat samples

Meat samples from beef (labelled fresh British beef sirloin steak), chicken (labelled fresh British chicken) and lamb (fresh British lamb chops) were purchased from a local supermarket. Chicken samples were deboned (skin and muscle were used) whereas beef and lamb were used as purchased. All samples (about 5 g) were homogenised in the laboratory and extracted on the day of purchase.

Cooked meat products

Three different methods of cooking were studied to determine the effect of each on the TAG profiles in the meat products:

Microwave open cooking: Different homogenised samples (≈ 5 g) of beef, lamb and chicken as specified in raw/uncooked meat samples, and pork ham were cooked in a microwave oven (700 W, full power, 4 min) and extracted immediately.

Cooked and roasted cooking: Samples of cooked and roasted pork, beef and chicken were purchased from a local supermarket, labeled as; 'cooked and roasted pork formed from cuts of pork', 'sliced cooked and roasted beef' and 'reformed, cooked and roasted chicken with added pea fibre' on their packaging. The samples (about 5 g) were homogenised and extracted on the day of purchase.

Curry and hotpot: Samples of beef and chicken curry (labeled as 'beef curry: pieces of beef in a spicy curry sauce' and 'chicken curry: chicken pieces in a mild curry sauce') and lamb hotpot (labeled as 'Cumbrian lamb hotpot slow-cooked traditional kitchen recipes') were purchased from a local supermarket. The samples (about 5 g) were homogenised and extracted on the day of purchase.

B. Extraction procedure - *Accelerated Solvent Extraction (ASE)*

The samples of raw and cooked meat products were extracted according to Dionex Application Note 334 i.e. Rapid determination of fat in meat using accelerated solvent extraction (ASE).

C. RP HPLC APCI MS separation and detection of TAG profiles

The procedure for separation of TAGs profiles and detection by reversed phase high performance liquid chromatography (RP HPLC) coupling with atmospheric pressure chemical ionization tandem mass spectrometry (APCI) and ion trap mass spectrometer. A developed mobile phase gradients (combination of acetonitrile, dichloromethane and 10 mM ammonium acetate in methanol) are shown in **Table 1**. Two Waters Spherisorb ODS2 (150 mm x 4.6 mm, 3 µm) columns connected in series were used for the elution of the TAGs and the injection volume was 25 µL and the flow rate 1 mL/min in the present invention. An Ultimate 3000 (Dionex) HPLC was used in combination with a Bruker HCT ultra ETD II ion trap. The APCI source was operated in the positive ion mode. The vaporiser temperature was set to 450°C and drying temperature to 150°C. Nitrogen was used as a dry gas at a flow rate of 3 L/min and nebulizer gas at 50 psi. Scans were obtained from *m/z* 330 – 1000. The smart tune and auto MS^n to MS^3 were selected. The TAG profiles were then analysed by application of PCA.

Results

Figure 1(A) to 1(D) illustrates a RP HPLC-APCI MS Chromatogram of TAGs from raw and cooked meat products of beef, lamb, chicken and pork in accordance of the present invention. Reference is made to **Table 2** for peak identifications. The individual TAG peak areas of all eighteen identified compounds for beef, all seventeen for lamb, all twenty four for chicken and all twenty one for pork are expressed relative to the total peak area and the data represent means of five replications.

Figure 2 (A) illustrates PCA scores plot of TAGs for raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention. The first two principal components in the PCA scores plot show that the distribution of different animal species according to their TAGs and with 79% of the variance being explained; 58% from the first component (PC1) and 21% from the second one (PC2). All animal species are separated well and no overlap is observed between them despite the substantial variation in the pork fat samples.

Figure 2 (B) illustrates PCA scores plot of TAGs for raw and cooked meat products of pork, chicken, beef and lamb in accordance of an embodiment of the present invention. The PCA scores plot is constructed including only the TAGs OOPo* to SSS for beef curry, the TAGs labelled with 'vegetable oil' are excluded prior to PCA whereas all the TAGs identified from the raw and cooked meat samples for chicken, lamb and pork are analysed. The first two principal components in the PCA scores plot show the distribution of raw and cooked meat according to their TAGs and with 74% of the variance being explained; 55% from the first component (PC1) and 19% from the second one (PC2). All of the raw and cooked meat products group well by species and are separated well between species with no overlap between them. Notably, all raw and cooked lamb samples overlapped, the microwave pork and beef samples overlapped with the corresponding raw meat and chicken curry overlapped with raw chicken. It is particularly noteworthy that the cooked meats plot close or overlap to the raw meat for each species, indicating similarity in their TAG profiles. This shows that the discrimination of different species is possible for both raw and cooked meat products, revealing that the difference between cooking methods are less than the variations between species.

Table 2: Mean Percentages of Tags from raw and cooked meat products of pork, chicken, beef and lamb

TAGs	Peak No.	Pork			Chicken				Beef				Lamb		
		Raw	Microwave	Roasted	Raw	Microwave	Roasted	Curry	Raw	Microwave	Roasted	Curry	Raw	Microwave	Hotpot
LLLn*	1	0.68	0.35	0.11	1.73	0.28	0.54	1.48	-	-	-	-	-	-	-
LLL	2	1.95	1.33	0.32	2.19	0.47	1.61	1.81	-	-	-	-	-	-	-
OLnL*	3	1.72	1.51	0.51	5.90	1.63	1.70	7.89	-	-	-	-	-	-	-
PLLn*	4	1.38	1.05	0.41	3.25	1.24	1.34	1.20	-	-	-	-	-	-	-
LLO*	5	6.75	7.10	1.80	8.63	3.79	7.68	10.10	-	-	-	-	-	-	-
OLnO*	6	-	-	-	3.53	2.12	1.38	10.71	-	-	-	-	-	-	-
LLP*	7	7.05	5.77	2.06	4.14	2.71	4.83	2.11	-	-	-	-	-	-	-
LnOP*	8	1.98	1.86	1.43	3.49	2.90	2.04	1.83	-	-	-	-	-	-	-
OLO*	9	9.03	11.06	4.63	13.61	10.59	13.39	23.55	-	-	-	-	-	-	-
OOPo*	10	0.10	0.12	0.05	2.97	3.42	2.95	0.36	3.66	4.01	2.24	1.45	-	-	-
LPO*	11	18.84	18.85	11.61	11.43	10.94	13.52	5.30	-	-	-	-	-	-	-
OPPo*	12	0.21	0.21	0.13	2.72	3.88	3.58	0.27	10.06	10.14	7.35	3.26	-	-	-
PPL*	13	1.48	1.04	1.07	1.61	2.35	3.42	0.47	-	-	-	-	-	-	-
POM*	14	-	-	-	0.67	0.98	1.88	0.13	5.00	4.22	3.83	1.68	3.50	1.28	2.79
PMO*	15	-	-	-	-	-	-	-	0.26	0.32	0.14	0.15	0.66	0.34	0.30
OOO	16	5.62	6.74	6.82	7.19	10.12	8.73	23.36	7.02	8.31	6.11	37.06	3.32	1.80	5.31
OOP*	17	-	-	-	11.54	17.03	13.48	5.24	30.49	31.60	28.37	20.06	20.11	8.31	19.24
OPO	18	17.73	18.18	30.89	-	-	-	-	3.02	3.32	1.65	2.40	7.07	4.38	3.93
LPS*	19	5.82	4.38	3.60	0.70	1.21	1.23	0.28	-	-	-	-	-	-	-
POP	20	-	-	-	5.53	8.93	6.97	0.79	10.43	9.68	11.88	4.92	7.77	5.67	6.97
PPO*	21	3.03	2.51	5.47	-	-	-	-	0.43	0.40	0.30	0.35	1.27	0.92	0.61
SMP*	22	-	-	-	0.60	0.99	1.04	0.12	2.26	1.49	2.16	0.93	3.22	2.04	2.54
OOS*	23	3.82	5.26	5.53	3.04	5.74	3.66	2.14	9.19	10.58	11.46	10.58	10.12	13.13	12.24
OSO	24	-	-	-	-	-	-	-	1.49	1.58	1.27	1.77	5.70	9.06	4.21
SOP*	25	-	-	-	3.67	6.01	3.59	0.51	9.73	9.21	13.62	7.67	16.29	22.47	16.53
SPO*	26	11.08	11.34	19.20	-	-	-	-	0.51	0.53	0.42	0.58	2.43	2.63	1.36
PPS*	27	0.53	0.46	1.55	0.82	1.25	0.75	0.11	2.00	1.21	2.45	1.40	3.98	3.42	4.67
SOS	28	-	-	-	-	-	-	-	3.14	2.51	4.56	3.56	8.16	18.92	10.80
SSO*	29	0.23	0.28	0.28	0.76	1.03	0.50	0.18	0.17	0.22	0.19	0.35	1.44	1.64	1.15
SPS*	30	0.80	0.76	2.57	0.28	0.45	0.26	0.07	1.08	0.68	1.69	1.37	3.66	2.97	5.32
SSS	31	-	-	-	-	-	-	-	-	-	0.32	0.48	1.52	1.03	2.36

The profiles of TAGs in animal species highlight a number of components that are important for species discrimination. In particular, the regioisomers PPO*/POP and SPO*/SOP* discriminate pork from beef and lamb. Good separation of TAGs containing highly unsaturated FAs (e.g. OLnO, OLnL and PLLn) results in effective discrimination of chicken from pork. The inspection of the profiles of TAGs in animal species highlighted a number of components that are important for species discrimination. The important TAGs for discriminating different animal species are: POP, OOP* and OPPo* for beef, PMO*, SOS, OOS*, PPS*, OSO, SPS* and SSS for lamb, OLnO* and OLnL* for chicken and OPO, PPO*, SPO* and LPS* for pork fat.

Figure 3 (A) illustrates a loadings plot for i) PC1 and ii) PC2 of TAGs from raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention. The loadings plot reveals TAGs with high positive and high negative loadings values. Those with positive loadings values (e.g. LLLn*, LLL, LLO*, OLO*, LPO*, LPS* and SPO*) are significantly higher in chicken/pork than in beef/lamb. By contrast, the TAGs with negative loadings values (e.g. OOP*, OOS*, SOP* and SOS*) are significantly higher in beef/lamb than in chicken/pork. The loadings plot for PC1 reveals that LPO*, SMP* and SOP* have slightly higher magnitude and contribute most to the discrimination on PC1.

Figure 3 (B) illustrates plots of PC1 vs. PC2 loadings values from PCA scores plot of TAGs from raw meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention. The plots reveal the TAGs that are important for species discrimination by the similarities in their coordinate values to the regions of the scores plot in which the species are located. POP, OOP* and OPPo* are identifiable as the important TAGs for recognition of beef. This coincides with the significantly higher levels of those TAGs in beef than in other species (Region A). Seven TAGs are important in discriminating lamb from other species (PMO*, SOS, OOS*, PPS*, OSO, SPS* and SSS; Region B). Only two TAGs are important for discrimination of chicken: OLnO* and OLnL* (Region C). Four TAGs, OPO, PPO*, SPO* and LPS* are important descriptors for discriminating pork (Region D).

A loadings plot for i) PC1 and ii) PC2 of TAGs from raw and cooked meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention is illustrated in **Figure 4 (A)**. The loadings values of PC1 and PC2 for the scores plot of TAGs for raw and cooked meat products are similar to the raw meat in different animal species and have the same important descriptors for discrimination.

Figure 4 (B) illustrates plots of PC1 vs. PC2 loadings values from PCA scores plot of TAGs from raw and cooked meat of pork, chicken, beef and lamb in accordance of an embodiment of the present invention. The important descriptors are the same as those identified for the raw samples alone.

From the above results, it is concluded that the meat products of different species (beef, pork, chicken and lamb) cooked by microwave, roasting and currying are separated well in the PCA scores plot. The present invention confirms that the discrimination of meat from different animal species is possible for both raw and cooked meat products, and reveals that the differences produced by the various cooking methods are less than the variations observed between species. The loadings values for the scores plot of TAGs for raw and cooked meat products are similar to the raw meat in different animal species and have the same important descriptors for discrimination.

The techniques developed in the present invention have made important advances in the analysis of TAGs demonstrating clear improvements in their chromatographic resolution and, by utilizing APCI in combination with ammonium acetate to identify regioisomers, enabling their distributions to be characterised more accurately.

The foregoing detailed description and examples are merely illustrative of the preferred embodiments. They are by no means meant to be the exclusive description of the inventive concept hereby disclosed. It will be recognized by one of ordinary skill in the art that certain aspects of the practice of the invention are readily susceptible to modification or practice by alternative, known means.

References

1. ***Fauconnot et al., 2004;***

Fauconnot L., Hau J., Aeschlimann JM., Fay L.B., Dionisi F., Quantitative analysis of triacylglycerol regioisomers in fats and oils using reversed-phase high-performance liquid chromatography and atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 2004; **18**: 218-224.

2. ***Dugo et al., 2006;***

Dugo F., Kumm T., Fazio A., Dugo G., Mondello L., Determination of beef tallow in lard through a multidimensional off-line non-aqueous reversed phase-argentation LC method coupled to mass spectrometry. *J. Sep. Sci.* 2006; **29**: 567-575.

3. ***Kallio et al., 2001;***

Kallio H., Yli-Jokipii K., Kurvinen J.P., Sjovall O., Regioisomerism of triacylglycerols in lard, tallow, yolk, chicken skin, palm oil, palm olein, palm stearin, and a transesterified blend of palm stearin and coconut oil analyzed by tandem mass spectrometry. *J. Agric.* & *Food Chem.*2001; **49**: 3363-3369.

4. ***Mottram et al., 2001***

Mottram H.R., Crossman Z.M., Evershed R.P., Regiospecific characterisation of the triacylglycerols in animal fats using high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. *Analyst.* 2001; **126**: 1018-1024.

5. ***Leskinen et al., 2007***

Leskinen H., Suomela J.P., Kallio H., Quantification of triacylglycerol regioisomers in oils and fat using different mass spectrometric and liquid chromatographic methods. *Rapid Commun. Mass Spectrom.* 2007; **21**: 2361-2373.

6. ***Byrdwell, 2005***

Byrdwell W.C., Atmospheric pressure ionization techniques in modern lipid analysis. *Modern Methods for Lipid Analysis by Liquid Chromatography/Mass Spectrometry and Related Techniques*. 2005; Chapter 1: 1-18, Chapter 7: 299-412.

7. ***Timon et al., 2001***

Timon M.L., Ventanas J., Carrapiso A.I., Garcia J.C. , Subcutaneous and intermuscular fat characterization of dry-cured Iberian hams. *Meat Science*. 2001; **58**:85-91.

8. ***Dionex Application Note 334***

Rapid determination of fat in meat using accelerated solvent extraction (ASE).

(there is no detail about year or anything, it came together with the instrument. I forward to you the pdf and might be we can put in the web address?) **The web address is sufficient. Please provide the web address in this case.)**

CLAIMS

1. A method for analysis of triacylglycerols from animal fats comprising
providing a preparation of a sample comprising triacylglycerols;
applying the preparation of sample as determined by chromatography having a separation material;
separating and detecting the triacylglycerols content of said preparation of the sample;
collating the detection data into a data analysable form; and
analysing the data using chemometrics for identifying and determining animal fats belonged to different animal species;
wherein the sample is prepared using an accelerated solvent extraction.

2. The method as claimed in claim 1 wherein a mobile phase gradient for separation of triacylglycerols used in a reverse phase high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (RPHPLC/MS-APCI) is a combination of acetonitrile, dichloromethane and ammonium acetate in methanol.

3. The method as claimed in claim 1 wherein the animal species are cow, pig, chicken and lamb in raw condition and cooked condition.

4. A method for identifying animal species in raw and cooked conditions comprising
providing a preparation of a sample comprising triacylglycerols;
applying the preparation of sample as determined by chromatography having a separation material;
separating and detecting the triacylglycerols content of said preparation of the sample;
collating the detection data into a data analysable form; and
analysing the data using chemometrics for identifying and determining animal fats belonged to different animal species.

5. The method as claimed in claim 4 wherein the sample is prepared using an accelerated solvent extraction.

6. The method as claimed in claim 4 wherein the chromatography is a reverse phase high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (RPHPLC/MS-APCI).

7. The method as claimed in claim 6 wherein a mobile phase gradient for separation of triacylglycerols used in the reverse phase high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (RPHPLC/MS-APCI) is a combination of acetonitrile, dichloromethane and ammonium acetate in methanol.

8. The method as claimed in claim 6 wherein the atmospheric pressure chemical ionization is operated in a positive ion mode.

9. The method as claimed in claim 4 wherein the chemometrics is a principal component analysis (PCA).

10. The method as claimed in claim 4 wherein the animal species are cow, pig, chicken and lamb.

11. The method as claimed in Claim 4 wherein the raw and cooked conditions are raw meat and cooked meat.