Single stage reversed phase liquid chromatography-mass spectrometry for the characterisation of triglyceride positional isomers.

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1.0 INTRODUCTION

Triglyceride (TAG) isomer separation has been achieved previously using multi-dimensional LC, combining reversed phase liquid chromatography (RP-LC) and silver-ion chromatography (Ag^+-LC). This combination allowed resolution of TAG isomers by use of the two different retention mechanism operating under these distinct HPLC modes[1]. The first method to determine positional isomerism in TAG fatty acids (FA) (Brockerhoff, 1967)[2] employed pancreatic lipase to partially hydrolyze TAGs. There is, however, a problem with migration of FAs during digestion[3].

In animal fats, FA contents and positional distribution in TAGs molecules varies widely with species, tissue, diet and environment. FA composition was shown to differ between anatomical locations of adipose tissue in pork fat[4] and the present study aims to further analyse the TAG compositions of subcutaneous and intermuscular pork fat from four different anatomical locations from pigs of the same breed, age and gender but without a controlled diet.

2.0 AIMS

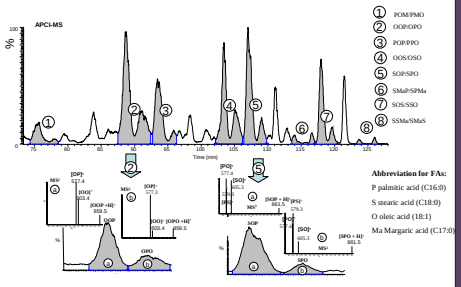
- Separate and identify the TAG positional isomers using a single stage RP-LC system incombination with APCI ITMS
- Develop a UPLC method to improve the resolution and speed of analysis of TAGs.
- Apply the improved method and principal components analysis (PCA) to differentiate fats from different animal species and different locations on the body.

3.0 METHODOLOGY

- EXTRACTION - Fats were extracted with chloroform-methanol(2:1)[5].
- HPLC and UPLC SEPARATION - 2 Spherisorb 3 µm ODS2 columns connected in series for HPLC and 2 RSLC acclaim 120 C18 2.2 µm columns in UPLC. Gradient elution with acetonitrile and dichloromethane.
- MS DETECTION – Thermo-Finnigan LCQ ion trap and Bruker HCT ultra ETD 11. APCI in +ve mode and protonated molecules ($[M+H]^+$) dissociated in MS/MS using an isolation width of 4 *m/z* units and collision energy of 35 eV.

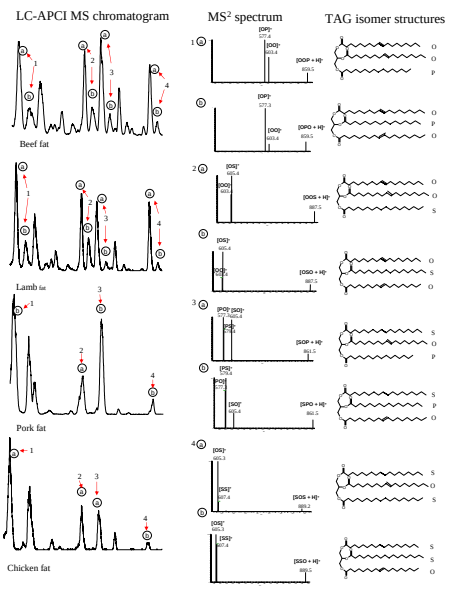
4.0 SEPARATION OF POSITIONAL ISOMERS IN LC-APCI MS

4.1 Separated TAG positional isomers in beef fat



- The complete separation of eight groups of TAG isomers in animal fat by single stage RPLC has been demostrated for the first time.
- Isomers produce identical ammoniated and protonated molecules; The dissociation of ammoniated TAG isomers produce the same diglycerides (DAGs) ion in MS^2 but in differing relative intensities.
- OOP and OPO isomers produce a protonated molecule at *m/z* 859.5 DAGs ions occur in the ratio $[OP]^+$: $[OO]^+$ of about 2:1 in OOP and 5:1 in OPO.
- Product ion abundances are very important for distinguishing between isomers.

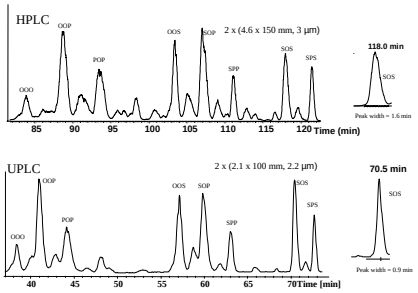
4.2 HPLC TAG isomer profiles in fats of different animal species



- APCI MS/MS was used for determining positional distributions of FA isomer in TAGs with added ammonium acetate in the mobile phase. For the SOP and SPO isomer pair, SOP eluted first, the unsaturated oleic acid being located in the *sn*-2 position, and the MS^2 spectrum shows the least abundant DAG ion $[SP]^+$ at *m/z* 579.4. The isomer with the unsaturated FA in either *sn*-1 or *sn*-3 is retained more strongly than the isomer with the unsaturated FA in *sn*-2 position.
- The prevalence of positional isomers is evident in beef and lamb fat; OPO is the only isomer in pork fat and OOP in chicken fat.

5.0 HPLC vs. UPLC

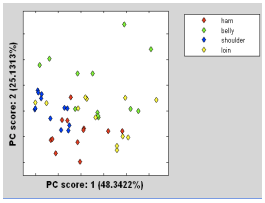
- Although there is good separation of TAGs and their isomers in HPLC, analysis times are long.
- The separations achieved in UPLC were comparable and indicate that it is possible to reduce the run time to almost a half.



- Converting the method from HPLC to UPLC has advantages of improved speed and resolution. The analysis time for OOO to SPS by UPLC is under 74 min, compared with 123 min for HPLC. The peak widths are reduced to half (e.g. 1.6 min to 0.9 min for SOS).
- In general, slightly better resolution is achieved in UPLC with column length 2/3 that in HPLC; the resolution for separation of SPS and SOS is 2.59 for HPLC and 2.88 in UPLC.

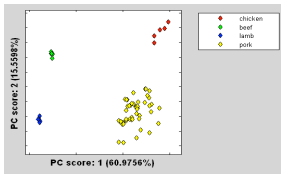
6.0 PCA analysis for species differentiation

The improved method for TAG analysis was used to assess the extent of variation of major TAGs in subcutaneous and intermuscular pork fat from four different locations of adipose tissues: ham, shoulder, loin and belly. The samples were taken from pigs of the same breed, age, gender without a controlled diet.



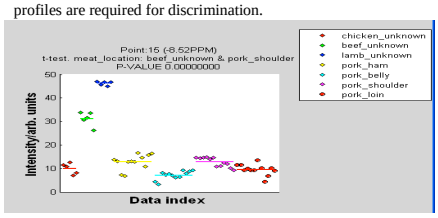
The PCA plot for TAG profiles of different pork fat locations

There is no obvious distinction between fats from different locations and only belly fat shows discrimination between intermuscular (separated from the rest of the data) and subcutaneous fat. The TAGs responsible for the discrimination are OLO, OOO, LPS and OOS.



PCA plot for TAG profiles for different animal species

No overlap between different animal species occurs in PCA despite the substantial variation observed in PCA for pork fat. Thus, PCA of fat TAG profiles can be used to discriminate different animal species. Analysis of individual TAG relative abundances indicates overlap between species for certain TAGs. For example, OOS for chicken shows overlap with pork loin and partial overlap with the other fat locations. Thus, complete TAG profiles are required for discrimination.



Plot of the relative abundance of OOS in animal fats. Only chicken shows overlap with pork fat and not for beef and lamb.

7.0 CONCLUSIONS

- TAG isomers in animal fats have been separated using single stage RP-LC system.
- APCI with ammonia in the mobile phase provides an alternative to ESI for MS/MS analysis of FA positional isomers in TAGs.
- A UPLC method has been developed offering reduced run time and improved resolution of TAGs.
- The application of PCA to the TAGs data was successful in discriminating samples between species; pork, chicken, beef and lamb fat, but not for different locations in pork fat.

8.0 REFERENCES

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3) Malone M., *et al.*, 2004, *Lipids.*, 39(3), 273-284.
4) Monziols M., *et. al.*, 2007, *Meat Sci.*, 76, 54-60.
5) Pedro EDe., *et.al.*, 1997, *Meat Sc.*, **45(1),** 45-51.

9.0 ACKNOWLEDGEMENT

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