# Classification of Poultry Meat Cuts Based on Approach of Untargeted Lipidomic Analysis and Advanced Chemometrics

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## Introduction

Chicken meat is a significant source of lipids in human diets and its fat composition is of growing interest to consumers, due to recommendations to reduce total fat intake and increase polyunsaturated fatty acids. Chicken meat contains a higher proportion of PUFAs than other meat species, which have been linked to reduced risks of cardiovascular diseases, inflammation, and immunological disorders. As the human body cannot synthesize significant amounts of PUFAs, they must be obtained through the diet. Poultry meat is the second most widely consumed meat globally and its lipid content is primarily influenced by the composition of the animal's diet.

## Methods

In this study, RP-UPLC-TIMS-TOF-MS in positive ionization mode was used to analyze meat samples from various poultry muscle cuts. MetaboScape 2023 (Bruker Corp.) internal lipid annotation tool and LipidBlast spectral library were used to annotate the obtained mass features, which were then used to construct a hierarchical clustering analysis (HCA) and an orthogonal partial least squares discriminant analysis (OPLS-DA) model for the classification of pork meat cuts based on their lipid profiles. VIPs were extracted from the model to obtain the lipid molecules that distinguish the aforementioned meat cuts more precisely.

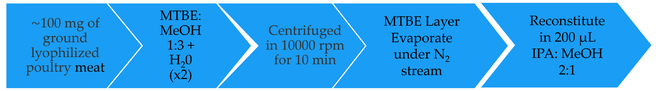
### 1. Aim of study

Meat is an important source of lipids in the human diet and its consumers are increasingly interested in fat composition. Thus, nutritional guidelines recommend reducing total fat intake, especially saturated fat, and increasing polyunsaturated fat .

In every diet plan, poultry meat plays an important role. Especially chicken breast is the flagship of any "clean" diet. As a result, the price of chicken breast is two (2) times higher than this of chicken thigh. Thus, it is crucial to compare the lipid profiles of breast vs thigh in order to 1. **verify differences to their content** and 2. study the chemical differentiation in terms of the total content of MUFAs PUFAs and SFA content as indicators of the **nutritional value of each cut**



### 2. Analytical protocol and methodology





**Parallel Accumulation Serial Fragmentation (PASEF) technology**

24 Samples, 12 of each poultry meat cut , Breast and Thigh

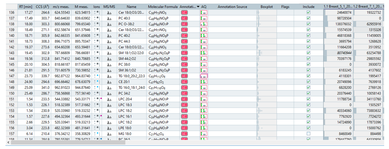
**Column**: Thermo Acclaim RSLC 120 C18 2.2 µm 2.1 x 100 mm
**Pre column:** Van guard Acquity UPLC BEH C18 (1.7 µm, 2.1x 5 mm)
**Mobile Phase A**: ACN: $H_20$ 65:35
**Mobile Phase B**: ACN:IPA 15:85
**Both containing 10 mM Ammonium Formate & 0.1% FA**
**Gradient:** Initially 30% B- Increasing to 100 in 30 mins
**Flow:** 0.25 mL/min
**Source temp:** 200°C
**Scan range:** 150-1350 m/z

Fig. 1: Extraction protocol using MTBE and Methanol & Instrumental Analysis using RP-UPLC-TIMS-TOF-MS in DDA mode (PASEF technology)

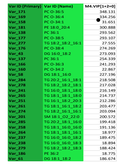
### 3.Data Treatment and Chemometrics

MetaboScape 2023

**170 Lipid Molecules Annotated using Metaboscape's Lipid Annotation tool and Lipid Blast Spectral Library**



SIMCA

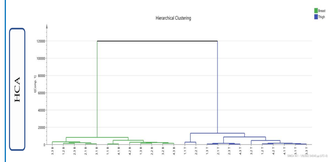


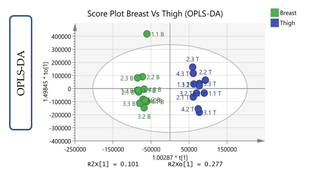
**74 Compounds with VIP Score > 1.0**

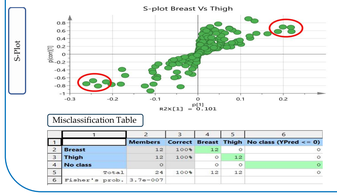
HCA | OPLS-DA | S-PLOT | Misclassification Table

Fig. 2: Annotated peak list from Metaboscape 2021 and chemometrics
Fig. 3 : Compounds list with VIP score >1.0 for the classification

### 4. Poultry meat authenticity assessment using untargeted metabolomic approach




Misclassification Table

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | | Members | Correct | Breast | Thigh | No class (YPred <= 0) |
| 2 | Breast | 12 | 100% | 12 | 0 | 0 |
| 3 | Thigh | 12 | 100% | 0 | 12 | 0 |
| 4 | No class | 0 | | 0 | 0 | 0 |
| 5 | Total | 24 | 100% | 12 | 12 | 0 |
| 6 | Fisher's prob. | 3.7e-007 | | | | |

Pairwise PLS-DA Belly : Breast -Thigh

DataAnalysis

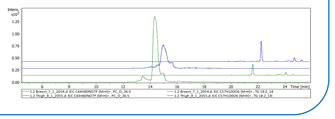


Fig. 4 a) HCA and PLS-DA score plot b) S-plot c) Misclassification Table d) EIC of PC-O-36:5 and TG 18:2-18

## Nutrition Value

- T-Test performed with 0.05 significant level. More than 70 lipids have < 0.05.
- Fold Change shows that Breast contains 25% lower saturated fat than Thigh
- PUFA/FSA & MUFA/FSA are higher in Breast than in Thigh

## Results

In this study, more than 1700 mass features were detected using the PASEF algorithm and more than 200 lipid molecules, were identified. By using PASEF, the signals acquired, were cleaner, presenting more sensitive results than the traditional MS methods. MetaboScape 2021b was used for the data treatment, utilizing the internal lipid annotation toll and the lipid blast spectral library. Moreover, the use of Kendrick Mass Plot by the generated data, resulted in an easier identification of false positives. The mass spectrometric data obtained were utilized for advanced chemometric analysis. Both, unsupervised and supervised techniques were used for this matter. An HCA model was constructed, based on the annotated lipid molecules. The results show a clear differentiation between the two clusters, as expected. An OPLS-DA model was also constructed, where the discrimination of the above-mentioned samples was clear. The VIPs were extracted in order to further study and understand the molecules that differentiate the poultry samples. Seventy lipid compounds, were suggested as potential biomarkers for the discrimination of poultry meat cut. Permutation and misclassification test were conducted, resulting in 100% correct classification.

## Conclusions

- Successfully separate the two (2) major poultry meat cuts with their lipid profile using supervised & unsupervised chemometric techniques
- More than 1700 Mass features with MS/MS spectra detected
- Using MetaboScape Lipid Annotation Tool and Lipid Blast Spectra Library >200 Lipid molecules are annotated
- Permutation test validate the robustness of the model
- Breast has higher nutrition value than Thigh

Technology