

Untargeted 4D Lipidomics combined with Chemometrics, as a reliable tool for the classification of pork meat cuts

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Introduction

Pork meat represents a significant contributor to the global meat consumption and plays a crucial role in providing essential nutrients to human diets. Despite its widespread consumption, the lipid profile of pork meat is still an area of ongoing research and development, as it can vary greatly depending on several factors such as the breed of the animal, its feeding regime, and the muscle fiber type. Lipids are a critical component of pork meat, as they not only provide energy but also play a role in maintaining the structure and function of cellular membranes. Phospholipids, triglycerides, and diglycerides are the three main lipid classes present in pork meat and are composed of a mixture of different types of fatty acids. Moreover, the lipid composition of pork meat has a significant impact on its flavor, texture, and nutritional value. Understanding the lipid profile of pork meat is important for improving its quality and ensuring its health benefits for consumers.

Methods

In the present work, meat samples of different pork meat cuts, were analyzed by RP-UPLC-TIMS-TOF-MS in positive ionization mode. The obtained mass features were annotated using MetaboScape 2023 (Bruker Corp.) internal lipid annotation tool & LipidBlast spectral library and used to construct a Hierarchical Clustering Analysis (HCA) and an Orthogonal Partial Least Squares - Discriminant Analysis (OPLS-DA) model for the classification of the pork meat cuts based on their lipid profiles. VIPs were extracted from the model, to acquire the lipid molecules that better differentiate the abovementioned meat cuts

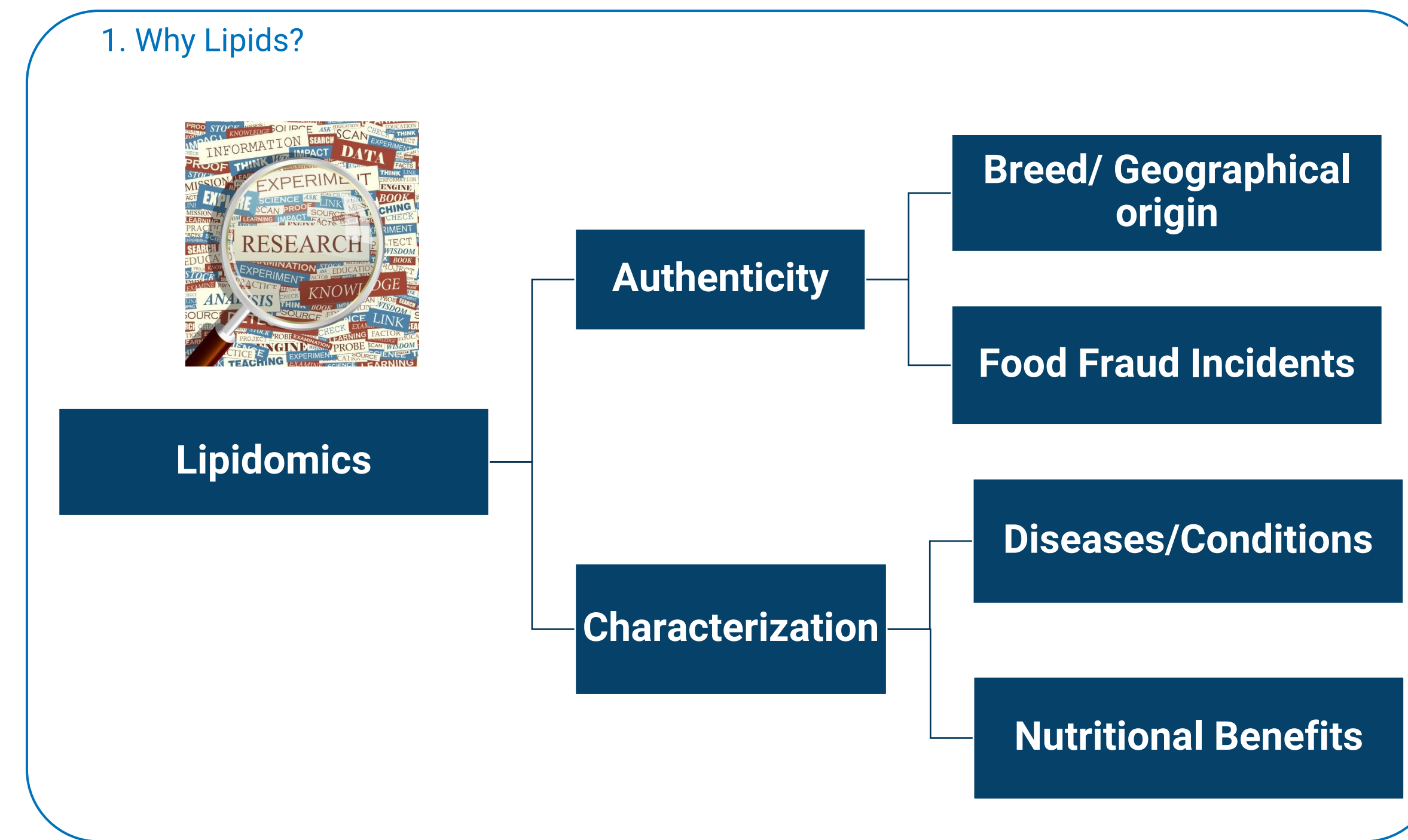


Fig. 1: Lipidomics approach advantages and aspects

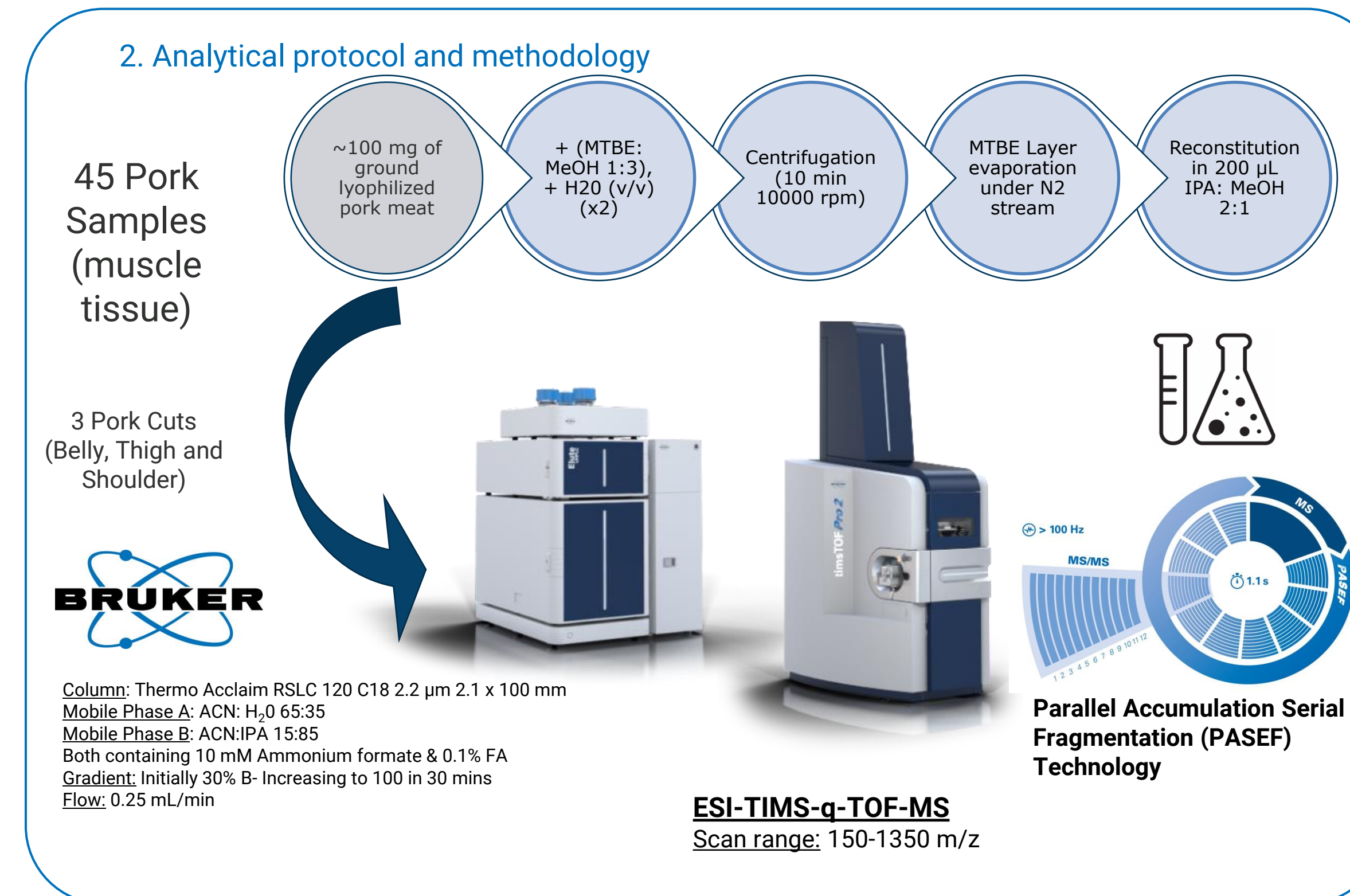


Fig. 2: Extraction protocol using MTBE and Methanol & Instrumental Analysis using RP-UPLC-TIMS-TOF-MS in DDA mode (PASEF technology)

Data Treatment and Chemometrics

A. Peak Picking and Lipid Molecules Annotation

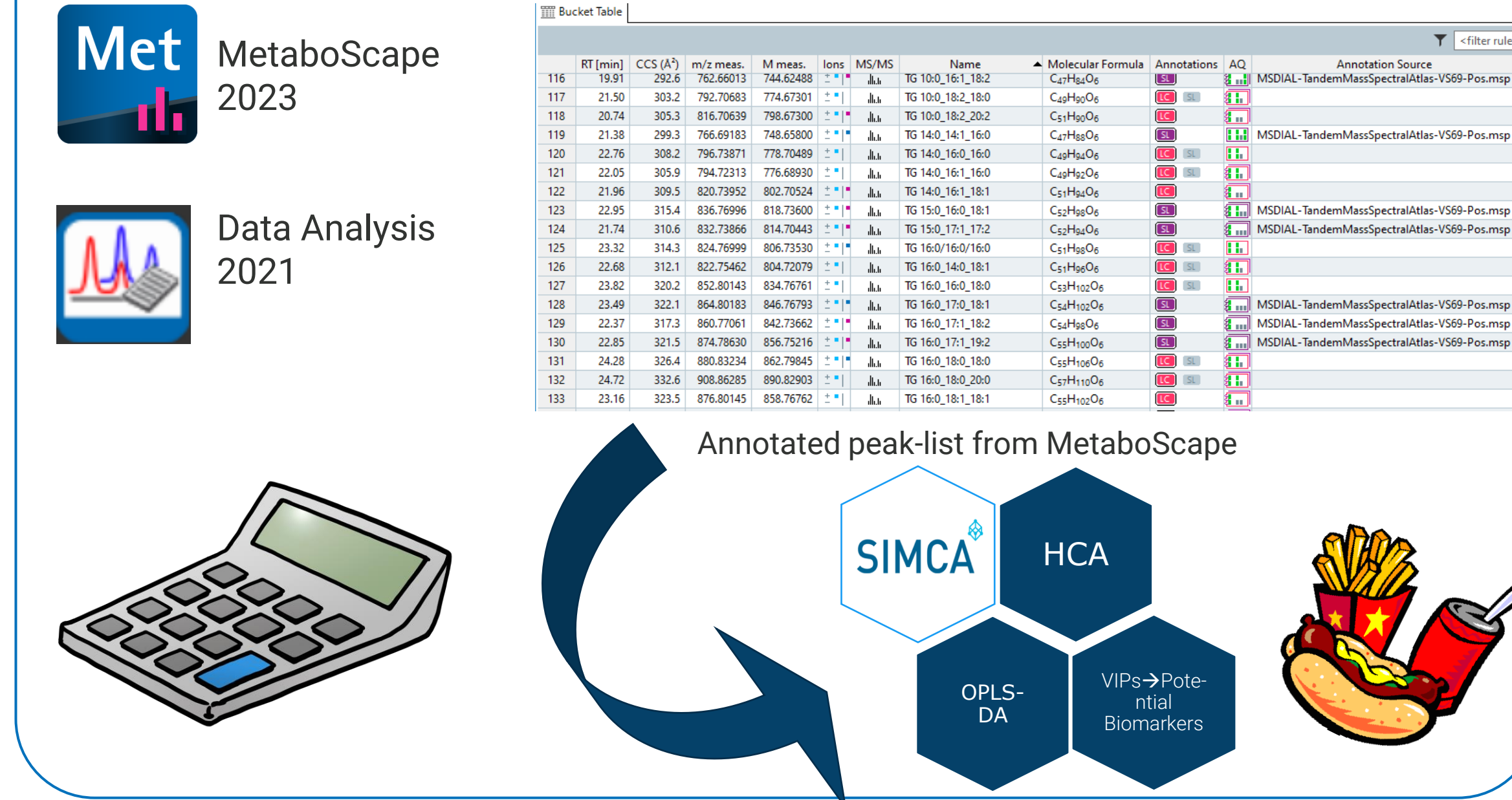


Fig. 3: Annotated peak list from MetaboScape 2021 and chemometrics

B. Chemometric Analysis and VIPs

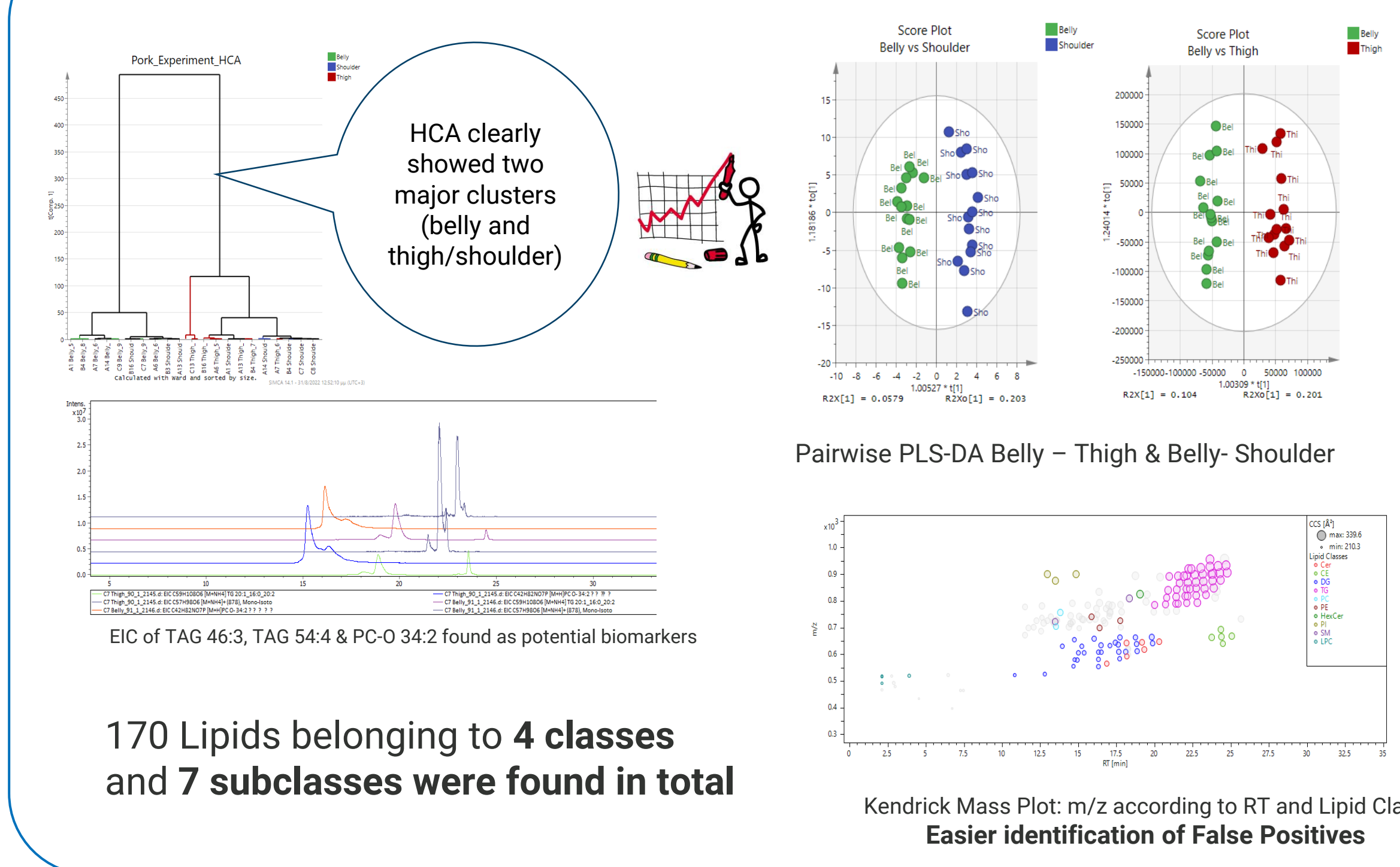


Fig. 4 a) HCA and PLS-DA score plots b) EIC of TAG 46:3, TAG 54:4 & PC-O 34:2 c) Kendrick Mass Plot of lipid species in the samples

Results

In this study, more than 2000 mass features were detected using the PASEF technology and 170 lipid molecules, belonging to four classes and seven lipid subclasses, were identified. The use of PASEF technology, resulted into more clean signals and higher sensitivity. Kendrick Mass Plot, internally available in MetaboScape 2023, facilitated the identification of false positives. The mass spectrometric data derived from the above-mentioned procedure were used for advanced chemometrics. A smart selection of scaling methods was adopted. Chemometrics using HCA showed the formation of two major clusters (belly and thigh/shoulder), while the same result was visible to the OPLS-DA, in which, the samples were not clearly discriminated. The respective OPLS-DA between Thigh-Belly and Shoulder-Belly led to a robust and accurate classification & prediction model, successfully classifying pork meat samples according to meat cut based solely on the lipid content. Seventy lipid compounds, were suggested as characteristic authenticity markers responsible for the discrimination the pork meat samples in both instances. The robustness of the abovementioned models, was calculated by using the permutation test and by calculating the misclassification table. The results obtained showed a correct classification of approximately 97%.



Conclusions

- MS/MS information acquired from more than 2000 m/z
- UPLC-Q-TOF-MS based Lipidomics coupled with supervised and unsupervised chemometrics, can correctly classify different pork meat cuts
- 170 Lipid Molecules annotated using MetaboScape's Lipid Annotation tool and Lipid Blast Spectral Library
- ~ 70 Compounds with VIP Score > 1.0 were identified

Technology