Untargeted UHPLC-TOF/MS-based lipidomics approach for identifying differences in egg yolks from hens fed diets supplied with xylanase

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Introduction

Eggs are one of the most widely consumed foods and a major source of dietary lipids, which have high nutritional value. The lipid profile of egg yolks varies depending on the feeding regime of the hens. Since poultry cannot develop enzymes capable of digesting dietary non-starch polysaccharides (NSPs), adding NSPases to wheat and rye diets improves nutrient availability and bird performance. Xylanase is a NSP degrading enzyme that breaks down long-chain arabinoxylans into small-chain xylo-oligomers, releasing nutrients for animal digestion and lowering digesta viscosity associated with high arabinoxylan intake. In this study, a UHPLC-TOF/MS lipidomic profiling approach was developed for the analysis of egg yolks from hens fed with modified diets with or without the enzyme xylanase for the first time.

Methods

Samples: 120 egg samples were collected from hens fed an ordinary diet and diets supplied with different amounts of xylanase.

Instrument: UHPLC Elute system (Bruker Daltonics) equipped with an UPLC CSH C18 column. MS data were acquired using a TIMS TOF mass spectrometer (Bruker Daltonics) in positive and negative ionization modes.

Sample preparation

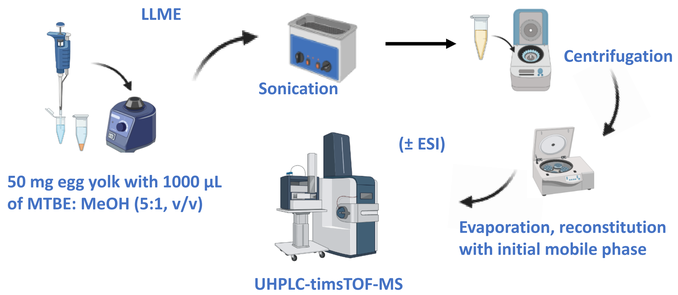

Figure 1: Sample preparation procedure for the lipidomic analysis of egg yolks.

Metabolite structural alignment

METLIN, HMDB, Lipid Maps

Metabolite identification

MS-DIAL

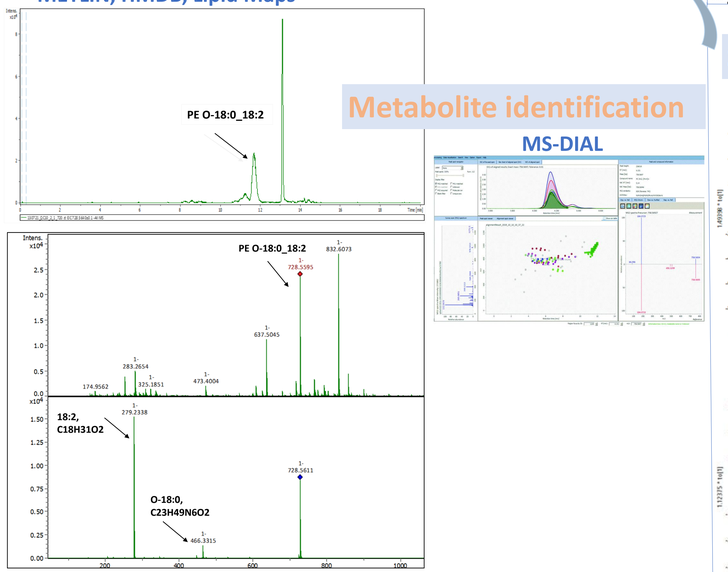

Figure 4: Chromatographic peak of phosphatidylethanolamine (PE O-18:0_18:2) together with MS and MS/MS spectrum. Precursor and MS fragments facilitated peak annotation.

Data acquisition

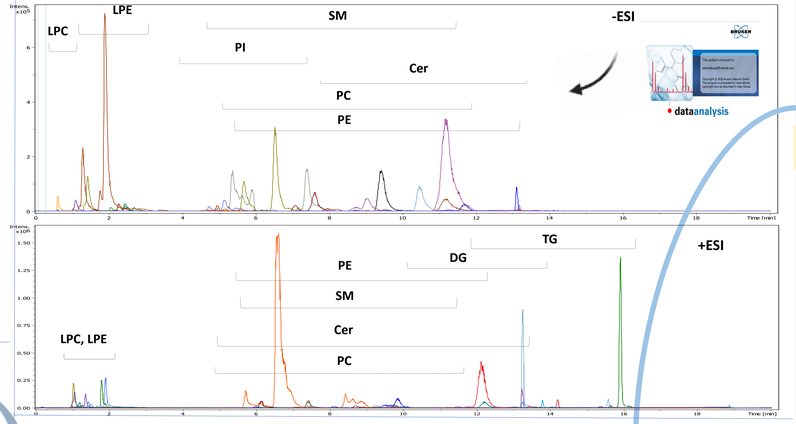

Figure 2: Extracted ion chromatograms in ±ESI modes.

Statistical analysis (PCA, OPLS-DA)

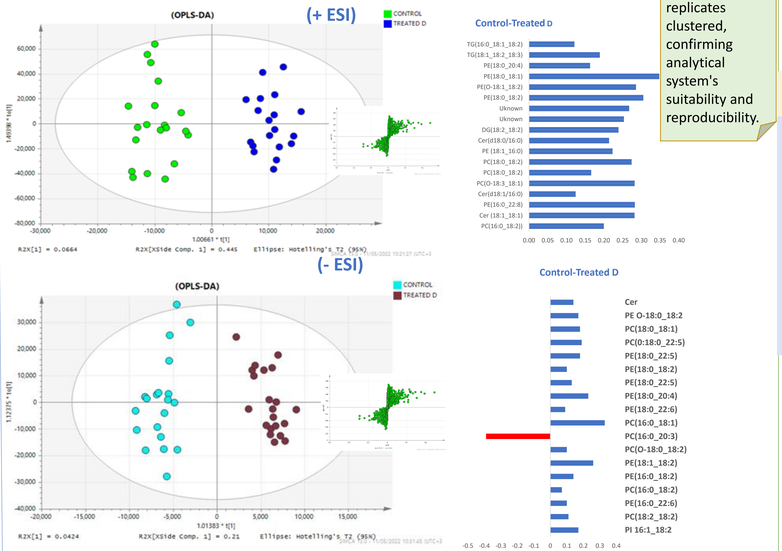

Figure 3: OPLS-DA plots for the discrimination of control and treated groups in ±ESI modes.

Principal component analysis (PCA) showed QC replicates clustered, confirming analytical system's suitability and reproducibility.

Peak peaking

A total of 6806 and 2209 ions, in negative and positive mode, respectively, met the quality control (QC) requirements

Results

Due to the high peak capacity of UPLC and high scanning speed of high-resolution MS, the analysis permitted unbiased analysis of a multitude of lipids including almost all classes in egg yolk samples.

- Orthogonal projection to latent structures discriminant analysis (OPLS-DA) showed a clear discrimination for treated samples (groups B, C, D) and the control ones (group A).
- Cross-validation ANOVA testing (CV-ANOVA) was performed as a significance test of the OPLS models ($p < 0,05$) in ± ESI.
- Features of five lipid species-classes (phosphatidylcholines (PC and PC O), phosphatidylethanolamines (PE and PE O), ceramides (Cer), phosphatidylinositols (PI), fatty acids (FA) were found significant for the discrimination of the study groups in -ESI.
- In +ESI, phosphatidylcholines (PC and PC O), phosphatidylethanolamines (PE and PE O), triglycerides (TG), diacylglycerols (DG), and ceramides (Cer) were found to differentiate between the two groups (control-treated).

Conclusion

- **Clear discrimination between egg yolks from hens fed diets supplied with different levels of xylanase (treated groups B, C, D) and the control ones (group A) by (OPLS-DA) .**
- **(PC and PC O), (PE and PE O), (Cer), (PI), fatty acids (FA) were found significant for the discrimination of the study groups in -ESI.**
- **In +ESI, (PC and PC O), (PE and PE O), triglycerides (TG), (DG), and (Cer) were found increased in egg yolks from hens fed diets supplied with xylanase.**

References

- Pirgozliev, V.; Bedford, M.R.; Acamovic, T. Effect of Dietary Xylanase on Energy, Amino Acid and Mineral Metabolism, and Egg Production and Quality in Laying Hens. British Poultry Science 2010, 51, 639–647.
- Taylor, A.E.; Bedford, M.R.; Pace, S.C.; Miller, H.M. The Effects of Phytase and Xylanase Supplementation on Performance and Egg Quality in Laying Hens. British Poultry Science 2018, 59, 554–561.

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