Lipidomics investigation of traditional Greek yoghurt skin (petsa) utilising RP-UHPLC-TOFMS following exhaustive liquid-liquid extraction

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Introduction

Nowadays, yoghurt is one of the most consumed dairy products and lately, it has gained great popularity due to its high nutritive value.

Traditional Greek yoghurt is considered quite different from all other yoghurt products, since during its production, no standardisation or homogenisation is applied to milk. The lack of the aforementioned procedures results in the formation of a layer at the surface of yoghurt, called yoghurt skin. Skin is rich in fat and provides traditional yoghurt with special organoleptic features.

In this study, we present the first attempt to analyse the overall lipidome content of traditional yoghurt skin after its separation from the rest of the yoghurt mass. This will aid our understanding of the skin's nutritional content and identify components of high nutritional value.

Methods

Yoghurt skin was separated from the rest of the traditional Greek yoghurt and was homogenised. Then, an extraction protocol, using ethanol, water and methyl tert-butyl ether was applied. After the end of the extraction, the upper layer was separated and was dried at room temperature and, it was resuspended in a mixture of isopropanol: acetonitrile: water (3:1:1, v/v/v) at specified volumes.

The resuspended samples were centrifuged in order to remove any undissolved material and further dissolved for TOF analysis (dilutions of 1:10, 1:100 and 1:10000).

Raw chromatogram files were processed with MSConvert (Version: 3.0.21147-f422a055e) to mzML file type. Data processing was performed using MS-DIAL ver. 4.70 with annotations using the embedded LipidBlast library(v.68).

Experimental Conditions

Column: ACQUITY UPLC CSH C18 2.1x100mm , 1.7µm

Solvent system:

Mobile phase A: MeCN/H2O (60:40, v:v) with 10mM ammonium formate and 0.1% formic acid

Mobile phase B: IPA/MeCN (90:10, v/v) and 0.1% formic acid

Gradient Conditions:
0-2min 60-57% A
2-2.1min 57-50% A
2.1-12min 50-46% A
12-12.1min 46-30% A
12.1-18min 30-1% A
18-18.1min 1-60% A
18.1-20min 60% A

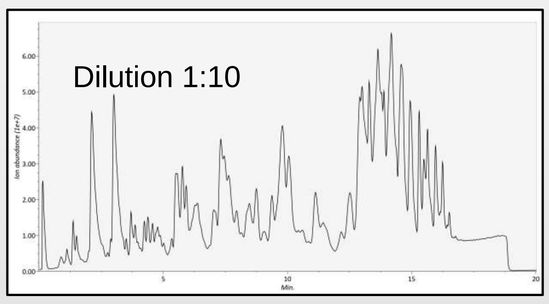
Flow rate: 400 uL/min

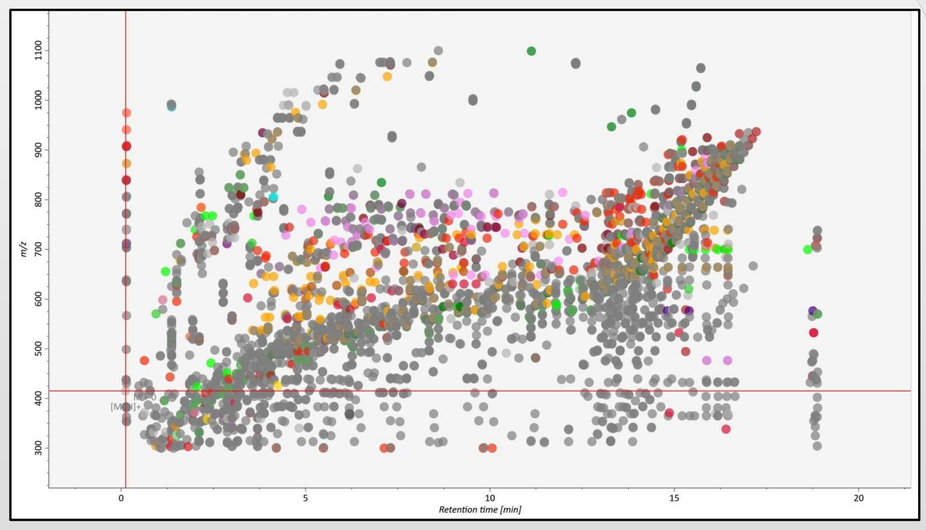
Injection volume: 5 uL

Column Temperature: (40 ºC)

Analysis was performed on a quadrupole timsTOF mass spectrometer coupled to an Elute LC system with autosampler (Bruker, Germany)

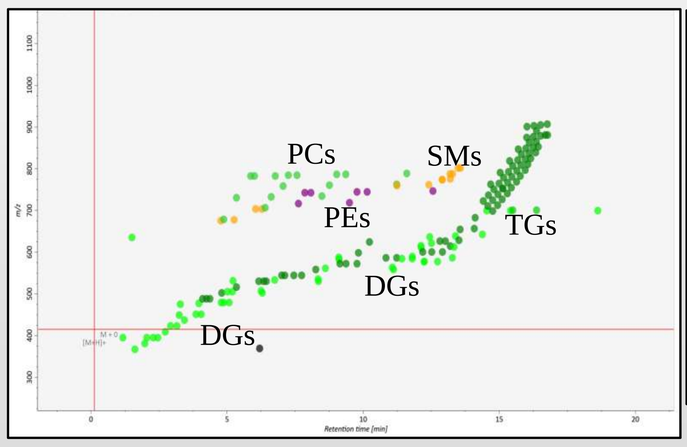
Results



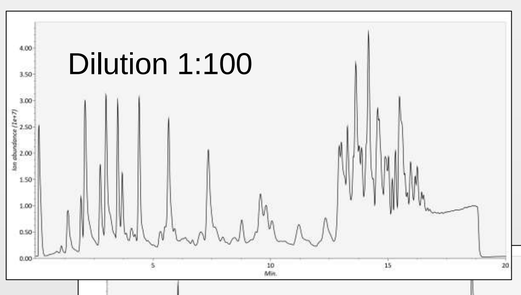


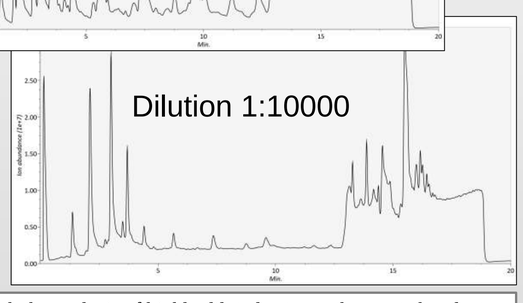
Top: Total Ion Current (TIC) chromatogram of the sample with 1:10 dilution. The sample displays a rich lipid content. **Right:** Peak viewer from MS-Dial displaying all the peaks that were assigned by the software. X-axis represents retention time and y-axis represents m/z ratio. Coloured dots are peaks with an identification (referenced or not) and grey peaks indicate unidentified components. Approximately 4000 peaks are currently assigned in total.

Yoghurt skin separated from the rest of the yoghurt mass. In this study yoghurt skin accounted for about 6-7% of the total yoghurt mass.

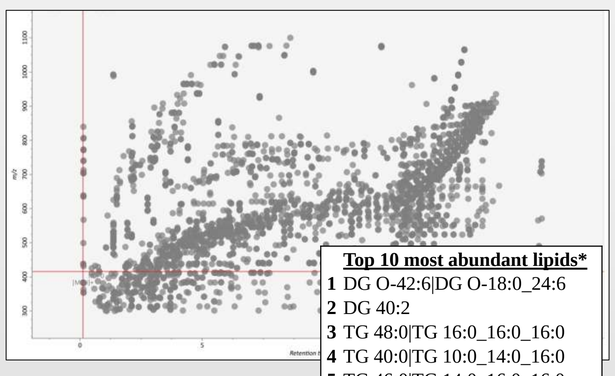


Peak viewer display from MS-Dial (dilution 1:10) indicating only the peaks that were annotated with reference spectra. A rough indication of the following classes is included: **Triacylglycerols (TGs)**, **Diacylglycerols (DGs)**, **Phosphatidylcholines (PCs)**, **Sphingomyelins (SMs)**, **Phosphatidylethanolamines (PEs).**





Through the analysis of highly diluted extracts the most abundant components of the sample can be identified. In the case of yoghurt skin, the most abundant components are, as expected, TGs and DGs. These analyses indicate high concentration differences between the various sample components.



Top 10 most abundant lipids*	
1	DG O-42:6|DG O-18:0_24:6
2	DG 40:2
3	TG 48:0|TG 16:0_16:0_16:0
4	TG 40:0|TG 10:0_14:0_16:0
5	TG 46:0|TG 14:0_16:0_16:0
6	TG 42:0|TG 12:0_14:0_16:0
7	TG 44:0|TG 12:0_14:0_18:0
8	TG 50:0|TG 16:0_16:0_18:0
9	DG 42:5
10	TG 48:1|TG 14:0_16:0_18:1

Type	Percentage of total peaks
Annotated with reference	4.2
Annotated without ref.	21.5
Unknown	74.3

Peak viewer plot of the unknown peaks in the yoghurt skin extract. The majority of the components in the sample remain unidentified using the embedded LipidBlast database. Only 4.2% of the lipidome can be annotated with reference spectra leaving 74.3% completely unknown.

**as annotated by MS-DIAL and the LipidBlast library.*

Conclusions

Extraction of lipids from traditional yoghurt skin aimed for lipidomics analysis is a difficult task and special extraction methods are required along with careful sample handling to ensure that a representative sample is processed. Here, the authors report a method that, to their knowledge, is the first attempt to handle this type of sample for lipidomics analysis. Both sample preparation and analysis were successful in revealing the overall picture of the lipidome content of traditional Greek yoghurt skin. Several lipid classes were identified by utilising reference spectra; our results demonstrate that these classes appear to be in significantly different concentrations, thus, additional work is required to remedy these differences analytically.

Future work may also include identifying the distribution of lipids between the skin and the rest of the yoghurt mass and comparing yoghurt skins from samples of different animal origin.

References

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