Profiling of milk triglycerides by UHPLC-MS

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INTRODUCTION

Fat is generally characterized by its fatty acid profile. The triglyceride profile is used less often, even though it can provide useful information in technology or enable technological processes to be demonstrated.

For milkfat, the triglyceride profile is important, particularly for the technological properties of butter, but also to ensure a certain degree of control over the stability of dairy products over time.

The approach developed in this study is based on liquid chromatography coupled with a tandem mass spectrometer. The method developed was applied to the dairy herd at the CRAW experimental farm.

SAMPLES AND MATERIEL & METHOD

Individual sample for each cow in the dairy herd at the CRAW experimental farm

Fat extraction

100 µL milk sample + 1 mL extraction solvent (Butanol/Methanol/Chloroform 3:5:4) in an Eppendorf of 2 mL

Mixing by vortex at the highest speed for 30 seconds

Centrifugation 10 minutes at 13000 g

Filtration of supernatant on 0.22 µm filter

Chromatographic conditions

UPLC® Acquity (Waters)

Column UPLC® CSH C18 - 2,1 * 100 mm – 1,7 µm

Column temperature : 50 °C

Injected volume : 5 µL

Flow : 400 µL·mL^{-1}

Mobile phase :

A : Acetonitrile / Water (60/40), 10 mM Ammonium Formiate, 0.1% Formic Acid

B : Isopropanol / Acetonitrile (90/10), 10 mM Ammonium Formiate, 0.1% Formic Acid

Step	Time (min)	A %	B %
1	-	60,0	40,0
2	3,00	46,0	54,0
3	3,10	70,0	70,0
4	18,00	99,0	1,0
5	18,10	60,0	40,0
6	20,00	60,0	40,0

Mass conditions

Quattro Premier XE (Micromass)
ESI +
Cone scan voltage
Scan mass : 90 – 1500 uma

Identification of individual triglycerides — 1

Analysis of TIC chromatogram (Total Ionic Current) – Principal component analysis — 2
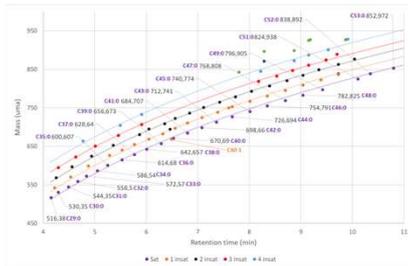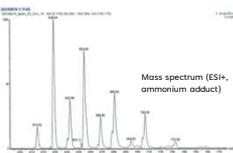
Analysis of the spectral volume reduced to the components with the greatest variability — 3
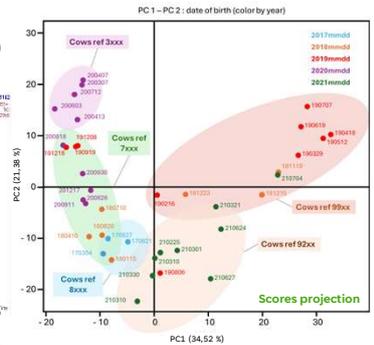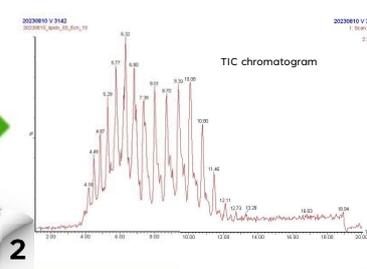
RESULTS

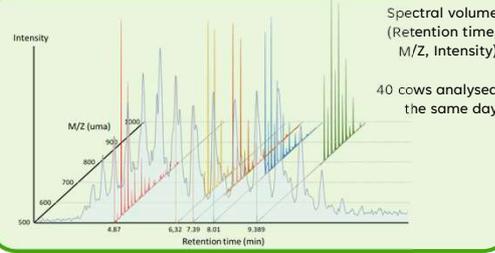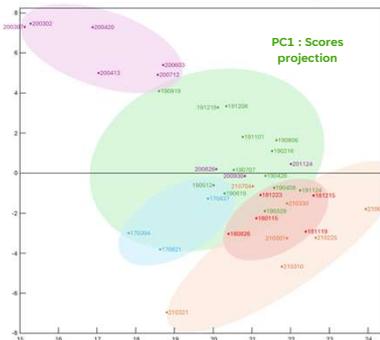**Identification of individual triglycerides (based on mass, number of carbons and number of saturations) – N ≈ 150**

Mass spectrum (ESI+, ammonium adduct)

Analysis of TIC chromatogram (Total Ionic Current) – Principal component analysis

TIC chromatogram

Reduction of the spectral volume to the components (retention time, mass) with the greatest variability

Principal component analysis of this new variable

PC1 : Scores projection

Spectral volume (Retention time, M/Z, Intensity)

40 cows analysed the same day

Volume reconstruction of loadings to identify the triglycerides responsible for discrimination

Loadings plot
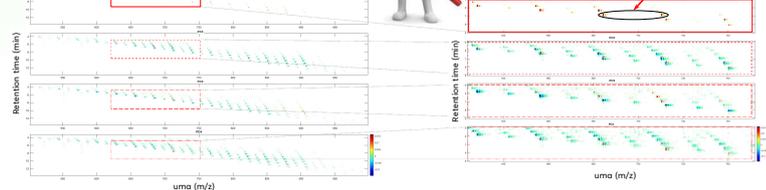
Correspondence between the results of both approaches: identification of the two triglycerides mainly responsible for discrimination

PC 1 – PC 2 : date of birth (color by year)
Scores projection

CONCLUSIONS

The method developed and applied to milk samples from the CRAW experimental farm provided a triglyceride profile based on mass, number of carbons and number of unsaturations.

Principal component analysis of the TIC (total ionic current) chromatograms obtained showed the influence of the age of the cow and its genetics in the discrimination observed.

Analysis of the data reduced to those showing the most variability in relation to the background noise of the spetral volume revealed the triglycerides showing the most variability within the samples. The triglycerides responsible for the discrimination were thus identified.

This approach could be used in particular in projects involving the authentication or detection of fat adulteration (PDO Beurre d'Ardennes, for example). In this context, the chromatographic method could be extended to the other groups that make up fat (free fatty acids, sterols, phospholipids, sphingolipids, ...) and the lipophilic components it contains.

PERSPECTIVES

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