

# HPLC method optimization for triglycerides analysis of cocoa butter with different sample pretreatment

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## Introduction

Cocoa butter tryacylglycerol profile (TAG)

"mouth melting feeling" of chocolate



Standard methods for TAG profile



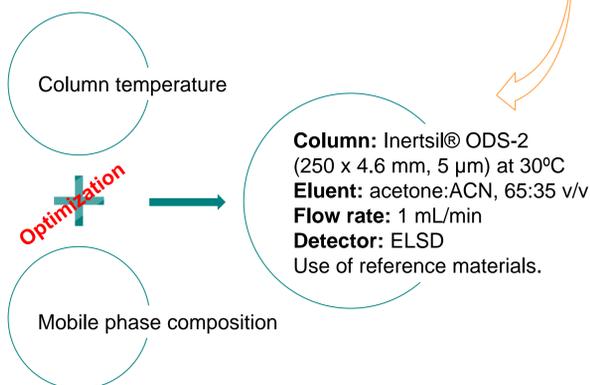
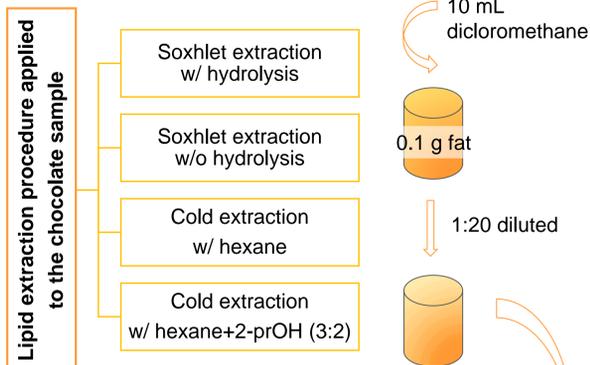
Need of low boiling points and possible fat oxidation

Aim of the work

Method Optimization

Comparison of four lipid extraction methods in chocolate

## Material and methods



One-way ANOVA has been applied to results using Past v.3.06 software.

## Results

### Optimization of working conditions

#### Study of column temperature

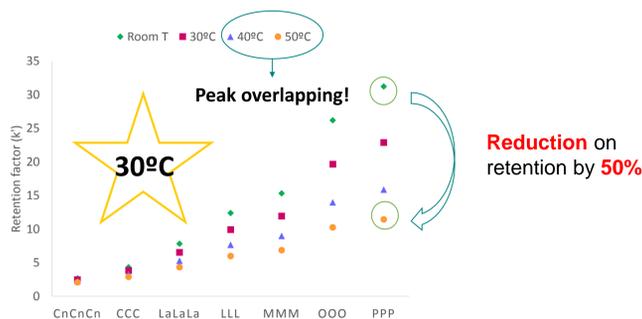


Figure 1. Capacity factor ( $k'$ ) values for seven standards of TAGs at different temperatures of the column.

#### Study of mobile phase composition

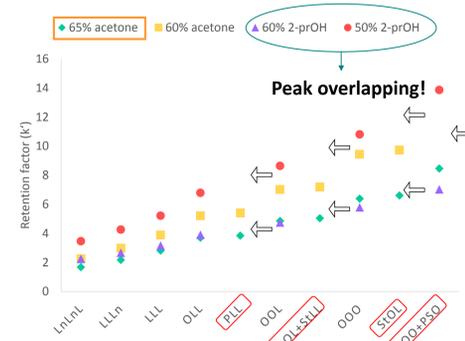


Figure 2. Capacity factor ( $k'$ ) values for TAG peaks of soya oil using a mobile phase of ACN combined with acetone or isopropanol in different proportions

### Chromatographic analysis of cocoa butter

#### Sensitivity

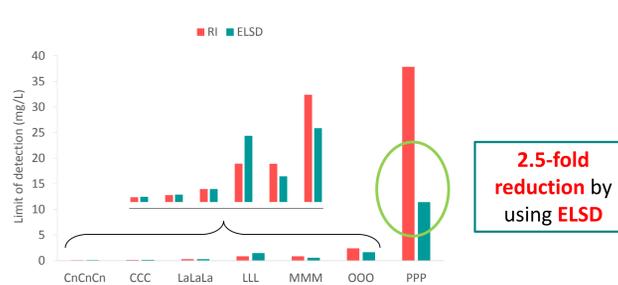


Figure 3. Comparison of limits of detection for two detection systems, Refractive index (RI) and evaporative light scattering detector (ELSD)

#### Calibration - Model fit

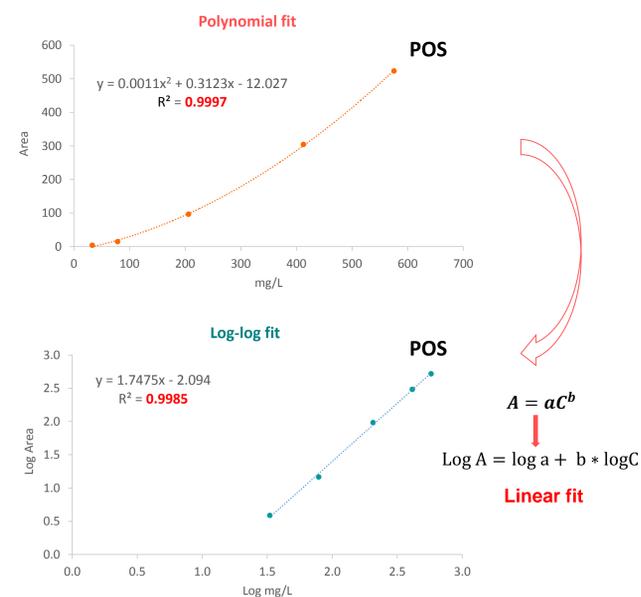


Figure 4. Comparison of polynomial and log-log fittings of data obtained

#### Quantification of TAGs

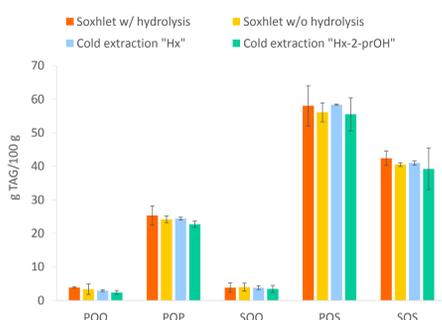


Figure 5. Mean content ( $n=3$ ) of TAG species in cocoa butter obtained from different lipid extraction methods.

- ✓ Test of Anderson-Darling :  $p > 0.05$ , **Normality of the distributions**
- ✓ Test of Levene:  $p > 0.05$  **Homogeneity of variances**
- ✓ One-way ANOVA:

F	POO	POP	SOO	POS	SOS
p-value	0.004	0.008	0.500	0.1790	0.090

- ✓ Tukey's post hoc test, there were not significant differences on chemical composition of SOO, POS and SOS in cocoa butter obtained through four extraction methods. POO and POP concentrations differ between "hot" and "cold" lipid extraction methods.

## Conclusions

Optimization of an existing method for TAG chromatographic separation has been successfully carried out for cocoa butter analysis. Non significant differences on major TAGs were found in a sample which was obtained through "hot" and "cold" extraction. Cold oil extraction could save time and time, and preserve target compounds which may undergone thermal decomposition. In order to confirm results achieved in this work, the four different lipid extraction procedures and chromatographic TAG analysis will have to be applied to a large number of samples

## Acknowledgement

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