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

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Introduction
Cocoa butter triglycerides profile (TAG)

“mouth melting feeling” of chocolate

Primary importance for chocolate industry

Standard methods for TAG profile

HPLC

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
Soxhlet extraction w/o hydrolysis

10 mL dichloromethane

0.1 g fat

1:20 diluted

Cold extraction w/o hexane

Cold extraction w/ hexane+2-proOH (3:2)

Lipid extraction procedure applied to the chocolate sample

Column: Inertsil® ODS-2 (250 x 4.6 mm, 5 µm) at 30ºC
Eluent: acetone:ACN, 65:35 v/v
Flow rate: 1 mL/min
Detector: ELS

Use of reference materials.

Optimization of working conditions

Study of column temperature

Study of mobile phase composition

Peak overlapping!

Reduction on retention by 90%

Peak overlapping!

30ºC

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

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.

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

Sensitivity

2.5-fold reduction by using ELSD

Quantification of TAGs

Figure 4: Comparison of polynomial an log-log fittings of data obtained

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 temperature, and preserve target compounds which may undergo 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.

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

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