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An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products

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Introduction

There is increasing demand for genuine cocoa butter (CB) in chocolate products in developed nations, however, this demand has created a shortage of CB and raised its costs. To overcome this, chocolate manufactures sometimes add vegetable-derived fats to some chocolate products to reduce costs while still maintaining desirable physical characteristics. It is of current interest to have a reliable method to detect, identify, and quantify the triacylglycerol (TAG) components of cocoa butter substitutes, replacers, and equivalents (CBEs) in chocolate products. Traditionally GC was used for this task, but due to the low volatility of triacylglycerides and their susceptibility to thermal decomposition, retention time is the only identifying factor for the TAGs and typical GC analyses of this type can take 40 minutes. LCMS is able to not only provide faster throughput, but also has the additional advantage of allowing characterization of the TAG, including qualitative regiospecific analysis. We have developed a single, UHPLC column-based LCMS method to analyze the TAG components in commercial chocolate and chocolate-like products. This analysis has a runtime of 17minutes, making it suitable for relatively high throughput. Additionally, the method was very repeatable, with an interday variability of <7% for the absolute area counts of the three major TAGs in CB (POP,POS,SOS).

Materials and Method

A Shimadzu Nexera UHPLC coupled to a Shimadzu LCMS-8040 triple quadrupole mass spectrometer was utilized for this analysis. A pure CB standard was used as a reference. Chocolate and chocolatey products were purchased in retail stores over a range of cocoa content.

Sample Preparation

For analysis, we slightly modified a sample preparation method originally used for algal oils. For analysis, 5mg of sample was weighed and then dissolved in a 3:1 Toluene-Isopropyl Alcohol solution. We then sonicated the mixture for 5 minutes. The solution was filtered through a Thomson filter vial (P/N 35538-100) to remove sugars and other insoluble materials and diluted 5-fold using 3:1 Toluene-IPA and injected into the UHPLC-MS system.

Chromatography

Instrument	: Shimadzu Nexera UHPLC system
Column	: Shimadzu Shim-Pack XR-ODSIII (200x2.1mm,)
Mobile Phase A	: LC/MS Acetonitrile
Mobile Phase B	: 1:1 Dichloromethane-Isopropyl Alcohol
Gradient Program	: 48% B (initially) – gradient to 51% B (0-8.0 min) – gradient to 54% B (8.0 – 11.0 min) – gradient to 74% B (11.0-14.0 min) – hold at 74% B (14.0-15.0 min) – reequilibrate at 48% B (15.1-17 min)
Flow Rate	: 0.33 mL/min
Column Temperature	: 30°C
Injection Volume	: 1 µL

Mass Spectrometry

Instrument	: Shimadzu LCMS-8040 Triple Quadrupole Mass Spectrometer
Ionization	: APCI
Polarity	: Positive
Scan Mode	: Q3 Scan

Results

Retail Chocolates from Hershey's, Lindt and Tcho, as well as a chocolatey candy - Charleston Chew - were compared against pure cocoa butter. The chocolates used were selected to cover a range of Cocoa content and purity. We specifically chose to use Hershey's Mr. Goodbar and Charleston Chews because they listed the use of vegetable oils in their ingredients list. As you can see in the chromatograms, the products that market themselves as pure chocolate have similar chromatograms in comparison to the pure CB.

We used an MS library that was provided to us by Dr. John Carney and Mona Koutchekinia to identify the types of TAGs contained in the chocolates using the spectral information captured in the Q3 scans. A minimum similarity of 70 was required for a result to be considered a match. In order to identify usage of CBEs, we applied the equation: %POP<44.025-0.733*%SOS, which was determined by the European Commission Joint Research Centre, which can detect around 2% CBE usage in CB content, or approximately 0.4% CBE content in chocolate. The chocolate products we tested all agreed with the expected results: All of the dark chocolate products we tested passed this specification, as well as Hershey's Milk Chocolate. The two products which had a higher %POP than is allowable, Mr. Goodbar and Charleston Chew, were selected specifically for the inclusion of vegetable oils. It may be informative to further test the accuracy of this testing method by adulterating cocoa butter with known quantities of CBEs. The data has been summarized in Table 1.

Table 1: Percentage of the major TAGs in CB in various chocolate products

Product	%POP	%POS	%SOS	%POP needs to be less than
Cocoa Butter	23.7%	46.9%	29.5%	43.8
Lindt 85% Cocoa	16.9%	46.4%	36.6%	43.8
TCHO 70% from Ghana	17.8%	46.1%	36.1%	43.8
TCHO 65% from Ecuador	20.9%	46.2%	32.9%	43.8
Hershey's Special Dark	20.0%	47.1%	32.9%	43.8
Hershey's Milk Chocolate	18.6%	46.6%	34.8%	43.8
Hershey's Mr Goodbar	44.8%	21.1%	34.1%	43.8
Charleston Chew	100.0%	0.0%	0.0%	44.0

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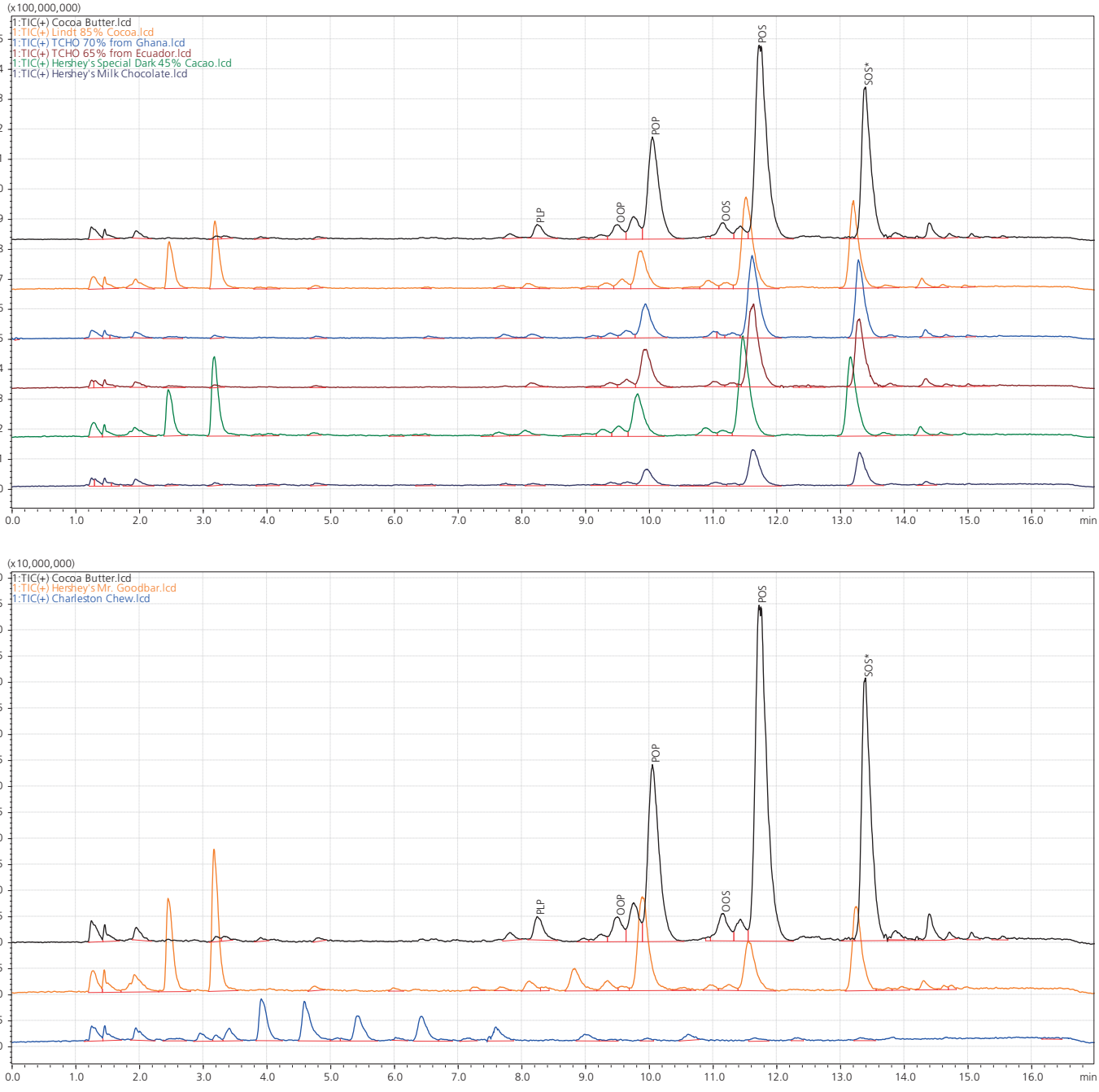

Figure 1. Chromatograms of the various chocolate products analyzed versus pure cocoa butter

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Conclusions

We have developed a 17 minute method for the rapid determination of CBE usage in chocolate products by using a UHPLC column and Q3 ion scans to analyze samples and then matching spectral information with an MS library of ion ratios for identifying TAGs.
Further studies could add a calibration curve to enable quantification of TAGs. This method should also provide a base method which can be modified to support TAG analysis in other product types.

References

Co ED, Koutchekinia M, Carney J et al. *Matching the Functionality of Single-Cell Algal Oils with Different Molecular Compositions*. 2014.
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