GC-MS Analysis of Cocoa Butter Triglycerides

INTRODUCTION

Cocoa butter is the edible fat extracted from cocoa beans that is used in the manufacture of chocolate. Cocoa Butter Equivalents (CBE) are a substance derived from palm oil and shea butter that are used as a substitute for cocoa butter in chocolate. European regulations govern the labeling of chocolate as containing CBE less than 5%. US regulations require that Cocoa Butter Equivalents be specifically labeled with wording such as "chocolate flavored" coating. Analysis of the triglyceride content of chocolate is a quality control measure. Analysis of a standard sample of cocoa butter triglycerides is presented here as an example of the use of the JEOL Q1500 quadrupole GC-MS system for the detection of lipids separated by using a high-temperature GC column (Restek Rtx-65TG).

EXPERIMENTAL

A certified cocoa butter reference standard (IRMM-801) was purchased from LGC and prepared as a solution in isooctane according to the directions supplied with the standard. A Restek Rtx-65TG high-temperature GC column was installed in the Agilent 7890B gas chromatograph with a 1 m deactivated fused silica guard column, and the column was directed into the JEOL MS-Q1500GC quadrupole mass spectrometer for analysis. The GC conditions (Table 1) were adapted from the European standard for the analysis of Cocoa Butter Equivalents (https://ec.europa.eu/jrc/sites/jrcsh/files/EUR%2020831%20EN.pdf). One microliter of sample was introduced into the gas chromatograph as a split injection using a 10:1 split. The low split ratio causes a modest amount of overloading for the high-abundance components, but was used to make it easier to detect the low-level triglycerides in the total ion current chromatogram. Mass spectra were acquired in scan mode using the conditions shown in Table 2.

RESULTS

Figure 1 shows the total ion current chromatogram with the major lipids used for determining cocoa butter equivalents (Table 3). All components show good chromatographic separation except for triolein (OOO) and 1,3-distearoyl-2-linoleoyl glycerol (SLS), which coelute at 29.8 minutes. The mass spectra for the five major triglycerides (Figure 2) are not in the mass spectral database but do exhibit typical triglyceride electron ionization mass spectra with characteristic alkyl

Table 1. GC Conditions

GC	7890B (Agilent)
Column	Rtx-65TG, 30.0 m, 0.25 mm i.d., 0.10 µm (Restek, Cat#17008)
Inlet liner, septum	Topaz 4.0 mm ID Single Taper Inlet Liner w/ Wool, Thermolite Plus Septum (Restek)
Inlet Temp.	350 °C
Carrier Gas Type, Flow	He, 1.000 mL/min constant flow
Mode	Split 10:1
Septum Purge Flow	3.0 mL/min
Saver flow, time	15 mL/min, 5.0 min
Injection Volume	1.0 µL
Oven Program	100 °C → (50 °C/min) → 330 °C → (2 °C/min) → 350 °C (hold 5 min)

Table 2. Mass Spectrometer Conditions

JMS-Q1500GC	
Ion Source Temp.	300 °C
Interface Temp.	300 °C
Ionization Mode	EI+, 70 eV, 100 µA
Measurement Mode	Scan mode
Scan range (*m/z*)	40-925
Step size(*m/z*)	0.1
Scan Time	583 ms
Acquisition Rate	1.714 Hz
Relative EM Voltage	500 V

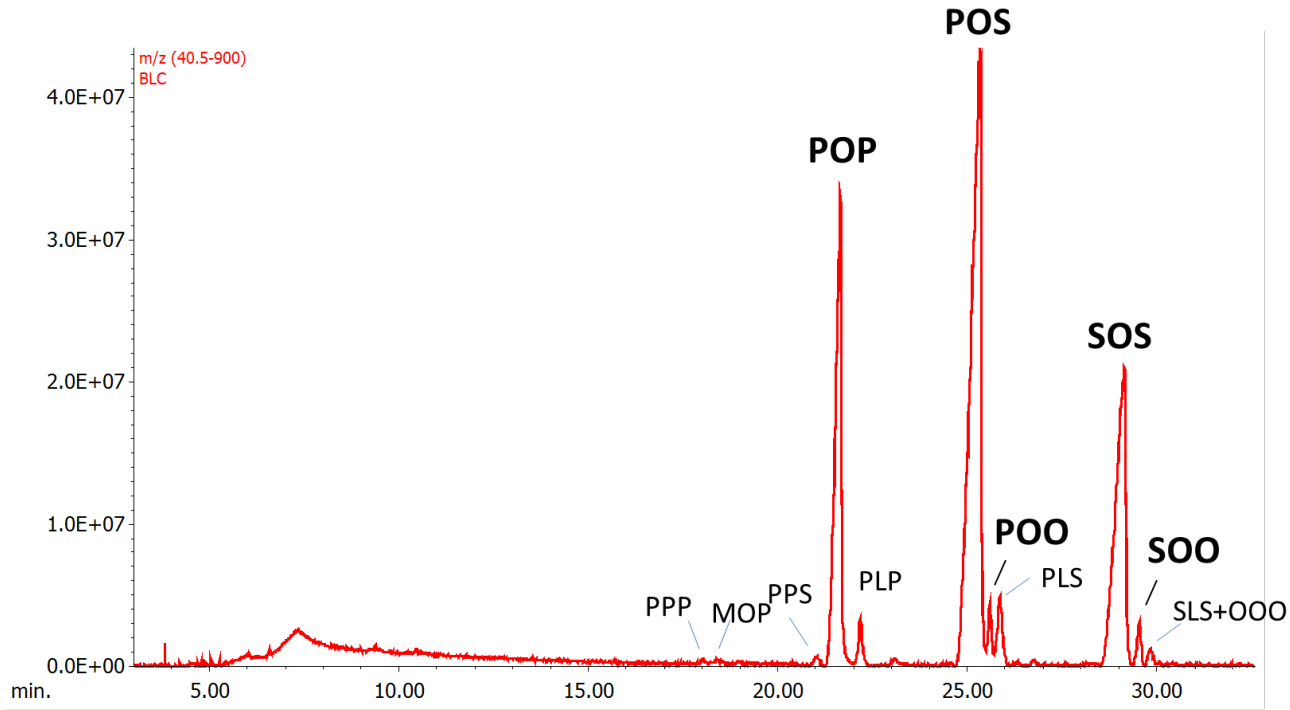

Figure 1. Baseline corrected total ion current chromatogram of CBE standard showing the five triglycerides used for analyzing CBE content in chocolate marked in bold. Minor triglycerides are labeled with a smaller font.

Table 3. Triglycerides Corresponding to Peaks in Figure 1.

Symbol	Name
POP	**1,3-dipalmitoyl-2-oleoylglycerol**
POS	**1-palmitoyl-2-oleoyl-3-stearoylglycerol**
POO	**1-palmitoyl-2,3-dioleoyl-glycerol**
SOS	**1,3-distearoyl-2-oleoylglycerol**
SOO	**1-stearoyl-2,3-dioleoyl-glycerol**
PPP	tripalmitin
MOP	1-margaroyl-2-oleoyl-3-palmitoylglycerol
PPS	1,2-dipalmitoyl-3-stearoylglycerol
PLP	1,3-dipalmitoyl-2-linoleoylglycerol
PLS	1-palmitoyl-2-linoleoyl-3-stearoylglycerol
SLS	1,3-distearoyl-2- linoleoyl glycerol
OOO	triolein

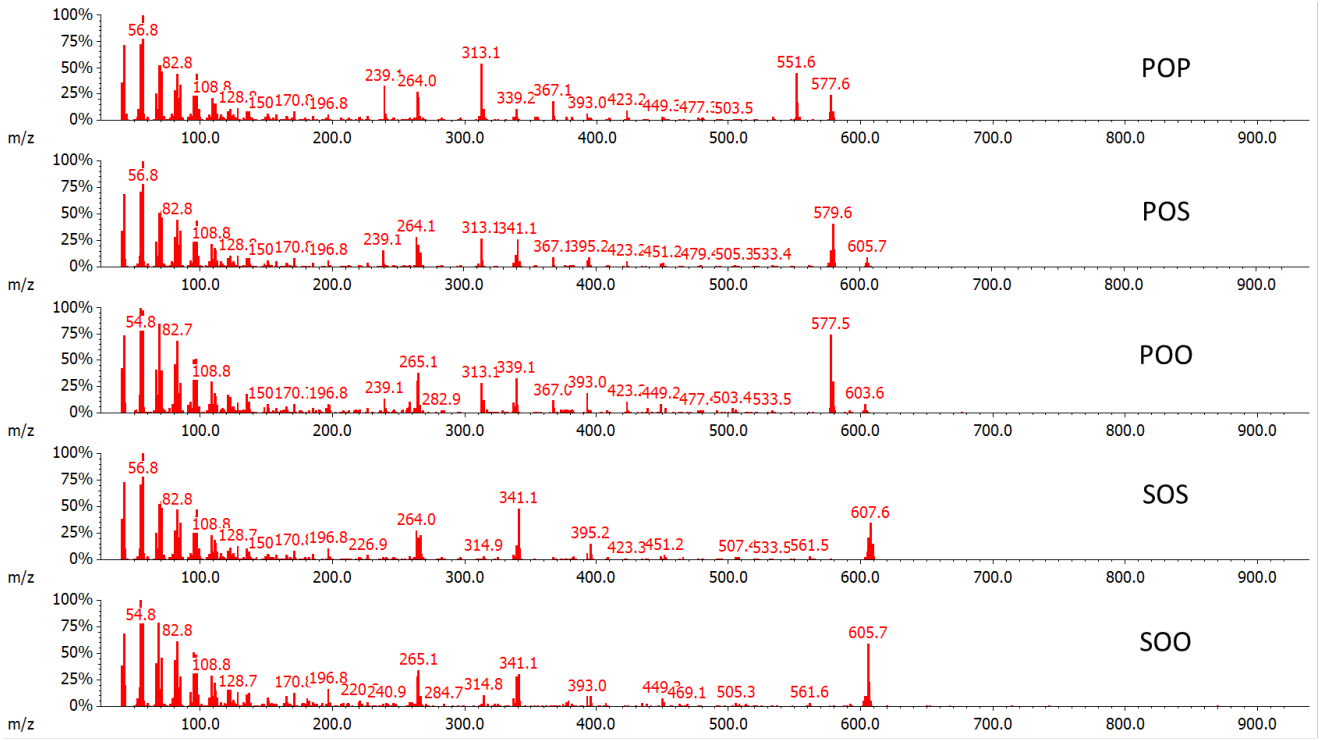

Figure 2. Mass spectra for the five major triglycerides labeled in Figure 1.

fragments and fragments characterized by loss of a fatty acid substituent. Characteristic fragment peaks indicating the presence of specific fatty acid substituents are observed, such as the peaks at *m/z* 239 ($C_{16}H_{31}O^+$) and 313 ($C_{19}H_{37}O_3^+$) for palmitic acid, *m/z* 264 ($C_{18}H_{32}O^+$) for oleic acid, and *m/z* 341 ($C_{21}H_{41}O_3^+$) for stearic acid.

CONCLUSIONS

The JEOL MS-Q1500GC system was used with a high-temperature GC column for the separation and identification of cocoa butter triglycerides. The Q1500 is a robust, easy-to-maintain and easy-to-operate GC-MS system that is suitable for a wide range of applications.