Summary
This application note shows results from multiple analytical techniques, comparing four brands of dark chocolate.

Background
Chocolate has several possible variations in preparation, leading to white, milk and dark chocolate. Cacao pods (an example is shown in Figure 1) are allowed to ferment, then dried and roasted. Cacao nibs are then removed from the shell and ground into chocolate liquor, which has two principal components: cocoa solids and cocoa butter. While there are bounds on the acceptable ingredient ratios in milk and white chocolate in the United States, dark chocolate is not specifically named. Semisweet chocolate must contain no less than 35% by weight of chocolate liquor, with the balance being cocoa butter, sugar and other flavorings. Dark chocolate is a subset of semisweet chocolate. The processing of the chocolate will have an effect on the structure and mouthfeel. The chocolate mass is finely ground through conching and refining. This process can last from 4-72 hrs, and can reduce the particles in the chocolate to less than 20 µm. Chocolate can be tempered, which consists of holding it a specific temperature in order to control crystallization upon cooling. The fats in cocoa butter are able to crystallize into six different structures, but some of these cause the chocolate to appear crumbly and to melt too easily while one gives the chocolate a shiny look and a strong snap when broken. Dark chocolate should be tempered at 31-33 °C in order to achieve the best crystal structure. Some manufacturers will also use emulsifiers in their chocolate to give a smooth feeling in the mouth. Chocolate typically contains caffeine and theobromine, which are thought to be responsible for some of immediate energy boosts associated with chocolate consumption (although the sugar probably doesn’t hurt).
Samples
Green & Black’s Organic 70% Cacao (GBO), Lindt 70% Cacao (LIN), Ghirardelli Twilight Delight (72% Cacao) (GHI) and Taza Stoneground (70% Cacao) (TAZ) were all purchased from commercial retail establishments.

Procedure and Results

Scanning electron microscopy (SEM)
SEM was performed with an EVO LS15 SEM in secondary electron mode at an accelerating voltage of 5 kV. The samples were fractured at room temperature and the fracture surfaces were coated with gold using a Cressington 108 sputter coater.

The TAZ sample showed the presence of large particles embedded in the continuous matrix (Figure 2). These particles could be either sugar crystals or cocoa particles, with the continuous phase being cocoa butter. Since cocoa butter crystals tend to be needle-like spikes on the order of 5 \( \mu m \), these particles are not cocoa butter crystals.

The LIN (Figure 3 and Figure 4) and GBO (Figure 5 and Figure 6) samples showed much smaller crystals, typically less than 10 \( \mu m \) across. The GHI (Figure 7 and Figure 8) had no apparent particles at the magnifications used for this study, although the sample proved to be challenging to image due to sample movement under the SEM beam.

Energy dispersive spectroscopy (EDS) was conducted on all the samples. A representative spectrogram is shown in Figure 9. All samples only showed the presence of carbon and oxygen. The unlabeled peak at 2.1 kV is gold from the gold-coating process.

According to the manufacturer, TAZ chocolates are ground using an Oaxacan stone mill known as a molino. This process takes approximately 24 hours. It is possible that this processing method and time are what sets the texture of this chocolate apart from the others in the SEM micrographs. The LIN, GBO and GHI had smaller or no visible particles, which was likely achieved through conching for longer time periods.

Figure 2: TAZ SEM micrographs at 116x. The large embedded particles (yellow arrows) are between 50-200 \( \mu m \) in size.

Figure 3: LIN SEM micrograph at 100x. No large particles are observed.

Figure 4: LIN SEM micrograph at 400x. Small particles less than 10 µm are observed.
Figure 5: GBO SEM micrograph at 133x. No large particles are observed, although some smaller particles are evident, along with some pores that are 75-100 µm in diameter (yellow arrows).

Figure 6: GBO SEM micrograph at 423x. No large particles are observed, although some smaller particles <10 µm are present, along with some porous structure.
Figure 7: GHI SEM micrograph at 133x. No large particles are observed.

Figure 8: GHI SEM micrograph at 341x. No small particles are observed.
Figure 9: Representative EDS spectrogram showing elemental composition of the chocolates.

**Hardness**
Shore A hardness testing was conducted with a Rex Durometer Type A (Model 1600, CPG ID 11680). The gauge was pressed into the molded surface and a reading was taken after 15 seconds. As shown in Figure 10, the hardness values of these chocolates were all very close, indicating that the compositions and crystal structure were similar. The fact that there is more variability in the TAZ sample shows agreement with the general structure observed in SEM.

![Hardness Test Results](image)

**Rheometry**
Three samples (GBO, LIN, GHI) were analyzed under identical conditions using an AR-G2 shear rheometer (TA Instruments, CPG ID #11460). In each case the sample was melted directly from brick form within the rheometer plate with the rheometer tracking the melting of the sample as it occurred until a gap of approximately 1 mm was achieved. After melting to physiological temperatures (37°C), the samples were analyzed using Small Amplitude Oscillatory Shear (SAOS) and then steady shear. In the SOAS step, a stress sweep was used to determine the linear region of the material, which is a region where determined properties of the sample are independent of the size of the deformation applied when tested at small amplitudes.

![Rheometry Test Results](image)
Procedure
1. Control normal force while melting, and track viscosity until gap reaches less than 1 mm
2. Stress sweep to determine non-linear region
3. Frequency sweep
4. Steady shear at 37 °C
5. Heat to 50 °C
6. Hold for 10 mins
7. Freq sweep
8. Stress sweep
9. Steady shear
10. Cool to 20 °C
11. Freq sweep

The chocolates examined here all showed pronounced non-linearities above a shear stress of approximately 1 Pa (see Figure 11). At this point a sharp transition occurred to a different region with a much lower modulus. One can clearly see the cause of this change when the actual displacement waveform is examined (see insets Figure 11). At low stress, although the deformation is noisy (blue curve) it is visibly sinusoidal, where at higher stress the curve has become rounded, with a flat top, indicating harmonics are present and the deformation is non-linear. This observation suggests a complex, strain dependent material.

![Figure 11: Oscillatory shear stress as a function of modulus for the three samples. Insets show deformation profiles.](image-url)
Figure 12: SAOS frequency sweep at 1% strain. Hollow symbols are $G'$, closed symbols are $G''$.

Under steady shear at both 37 °C and 50 °C (Figure 13) all samples appeared essentially the same with a strong shear-thinning response reaching a high shear rate plateau at approximately 10 - 100 s$^{-1}$. Temperature did not appear to alter the structure of the chocolate, just reduce the viscosity, as would be expected for most materials.

Figure 13: Steady shear at both 37 °C (open symbols) and a 50 °C (closed symbols) for the specimens.
Using small amplitude oscillatory shear, the temperature was first ramped from solid through the melt to 37 °C. Because the chocolate was loaded directly as a solid brick, care had to be taken to compensate for the melting sample. For this reason in the initial heating step the rheometer was operated under normal force control to close the gap as the sample melted. It was then set to stop at a specific gap (500 µm) to complete the run. The apparent modulus at low temperatures (Figure 14) is essentially the same for all samples. The LIN sample is the first to show a melting transition (as evidenced by a drop in modulus) at approximately 30 °C. All samples appear fully melted according to the modulus data at 35 °C, with all showing an apparent increase in modulus at higher temperatures. However, this is likely due to the rapidly changing gap as the rheometer compensates for the molten chocolate.

Figure 14: Temperature ramp to 37 °C from the brick form. Closed symbols gap, open symbols complex modulus.

Taking the molten chocolate and cooling the samples back to room temperature (Figure 15), no samples appear to undergo a pronounced solidification transition, although all appear to show slightly increasing modulus with failing temperature. It is well known that controlled thermal history modifies the crystallization and melting point of chocolates (this is the basis of tempering of chocolate) and this figure suggests that the relatively slow cooling used here is essentially tempering the chocolate.
Figure 15: Temperature ramp from 37 °C to room temperature. Open symbols G’, closed symbols G”.

Measuring the SAOS at room temperature after the cooling described in Figure 15 does show differences. The LIN is markedly different in Figure 16, exhibiting a mostly elastic response, with a relatively flat frequency dependence, suggesting a rubbery system. The other two samples show a pronounced frequency dependence, but this may result from incomplete solidification.

Figure 16: Frequency sweep at 20 °C after cooling from the melt. Open symbols G’, closed symbols G”.
Differential Scanning Calorimetry (DSC)

All samples were prepared according to CPGSOP0043: a clean razor blade was used to section a piece of freshly-opened, untouched chocolate sample approximately 5 to 10 mg in mass. Each sample was then placed into a previously massed (balance CPG ID # 11770) standard aluminum pan and secured using the T-Zero pan press. The samples were weighed an additional time, and the mass of each sample was calculated. Lastly, each sample was placed into the DSC and its position was recorded.

Test Method:
1: Equilibrate at 0.00 °C
2: Isothermal for 5.00 min
3: Ramp 10.00 °C/min to 100.00 °C
4: Mark end of cycle 1
5: Isothermal for 5.00 min
6: Ramp 10.00 °C/min to 0.00 °C
7: Mark end of cycle 2
8: Isothermal for 5.00 min
9: Ramp 10.00 °C/min to 100.00 °C
10: Mark end of cycle 3

In initial tests, the chocolate was heated in the DSC from 20 °C (room temperature) above the melt and then cooled and reheated. These data are shown in Figure 17. There is a clear melt transition reaching up to approximately 27 °C for the LIN and TAZ samples, and 29 °C for the other two specimens. This is generally consistent with the order reported in the rheometer data (Figure 14). The concern with these data is however that the “startup hook” present whenever a DSC transitions from isothermal (0 °C/min) to heating makes it difficult to tell what the entire transition looks like. For this reason, further experiments were performed, cooling the sample to 0 °C before heating. These data are shown in Figure 18. As with the ambient heat curves in Figure 17, the LIN and TAZ samples finish melting first. Interestingly though, all samples exhibit onset temperatures at approximately 5 °C, suggesting that some crystals are technically melted within the chocolate, even at room temperature. Second heats did modify these transitions somewhat, indicating a change in crystal distributions, but only for the LIN sample (Figure 19), where the transition generally moved slightly lower in temperature (suggesting smaller crystals) and a slightly sharper peak (suggesting a more defined population of crystals). The melt transitions of the low-temperature DSC runs are provided in Table 1. In general, total enthalpy appears to fall slightly for all samples on the second heat. Only weak variations that may not be significant are seen in the melting temperatures. The peak temperature appears to drop slight for the LIN and GHI, but is invariant for the other two samples. The peak temperature is different for the LIN and GHI, than the other two samples, and both of these samples had slightly higher enthalpies, implying more crystals. In fact, when the thermograms themselves are considered, these two samples appear similar to the other two samples below approximately 25 °C, but have an additional crystal population above this temperature melting in the GBO and TAZ samples. It is notable that the LIN and GHI samples also appear the hardest of the four specimens (Figure 10).

Table 1: Thermal parameters from two heat cycles of four chocolate samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T_{mo} [°C]</th>
<th>T_{mp} [°C]</th>
<th>ΔH_{m} [J/g]</th>
<th>T_{co} [°C]</th>
<th>T_{cp} [°C]</th>
<th>ΔH_{c} [J/g]</th>
<th>T_{mo} [°C]</th>
<th>T_{mp} [°C]</th>
<th>ΔH_{m} [J/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBO</td>
<td>13.4</td>
<td>17.9</td>
<td>36.1</td>
<td>15.6</td>
<td>14.1</td>
<td>26.2</td>
<td>13.0</td>
<td>18.0</td>
<td>35.1</td>
</tr>
<tr>
<td>LIN</td>
<td>14.6</td>
<td>19.3</td>
<td>38.7</td>
<td>16.4</td>
<td>15.2</td>
<td>26.7</td>
<td>14.3</td>
<td>18.3</td>
<td>37.6</td>
</tr>
<tr>
<td>GHI</td>
<td>13.9</td>
<td>19.3</td>
<td>41.2</td>
<td>16.4</td>
<td>14.7</td>
<td>29.3</td>
<td>14.7</td>
<td>18.9</td>
<td>40.6</td>
</tr>
<tr>
<td>TAZ</td>
<td>13.9</td>
<td>18.7</td>
<td>32.6</td>
<td>15.8</td>
<td>14.1</td>
<td>23.5</td>
<td>13.6</td>
<td>18.5</td>
<td>31.9</td>
</tr>
</tbody>
</table>

T_{mo}: melting onset temperature; T_{mp}: melting peak temperature; ΔH_{m}: heat of melting; T_{co}: crystallization onset temperature; T_{cp}: crystallization peak temperature; ΔH_{c}: heat of crystallization.
Figure 17: Comparison of all curves from room temperature to above the melt (first heat).

Figure 18: Comparison of melt transitions for all four samples (first heat) from 0 °C.
Experimental: Lipid Analysis

The four chocolate samples were ground using a mortar and pestle to increase material surface area prior to extraction. Liquid extraction in a hexane/IPA mixture was used to remove fat from the ground chocolate samples for subsequent analysis by GC/MS. Approximately 5.0 g of ground chocolate were taken from each sample, and placed in a 100 ml round flask. After addition of 1 ml of water, the sample was mixed on a vortex mixer for 30 seconds. 30 ml of hexane were added and the sample was vortexed for 4 additional minutes and sonicated for 10 minutes. Subsequently a 10 ml aliquot of isopropanol was added and the mixture was vortexed for 2 minutes and sonicated for 10 minutes. The extract was filtered in a vacuum filter. The residue in the extraction vessel was washed with a 20 ml aliquot of hexane. The extract and wash solvent were combined in a cleaned, preweighed 100 ml beaker, and 1.0 ml of liquid was filtered into an autosampler vial using a 0.45 µm filter. The rest of extract was left in the hood for evaporation for 30 hours.

The derivatization of triglycerides, needed for GC/MS analysis of fatty acids in order to increase compound volatility, was performed on the approximately 5.0 mg dried extract by adding a boron trifluoride solution (14% in methanol). 1 ml of boron trifluoride solution was added to the solid sample and the resulting mixture was heated to 100 °C for 20 minutes and manually agitated every 3 minutes. The mixture was cooled to room temperature and 1 ml of hexane and 1 ml of DI water were added. The system was mixed using a vortex mixer and the hexane layer was filtered through a 0.43 µm PTFE syringe filter and analyzed by GC/MS.

The GC/MS method parameters used for analysis of Hex/IPA extraction are described below:

- **Column:** ZB5-MS (5% diphenyl, 95% dimethylpolysiloxane), 30 m x 0.25 mm ID, 0.25 µm film thickness
- **Injection Mode:** Split (5:1 split ratio)
- **Inlet Temperature:** 250 °C
- **Column flow rate (helium):** 1.0 ml/min

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Figure 19: First (blue) and second (red) heat curves for the LIN sample.
**Experimental: Caffeine and Theobromine Analysis**

Since theobromine and caffeine were observed in the Hex/IPA extraction (which would adversely affect quantitation of these compounds), petroleum ether was used to extract the chocolates for theobromine and caffeine quantification (both caffeine and theobromine are very poorly soluble in petroleum ether).

Chocolates were ground using a mortar and pestle to increase material surface area prior to extraction. Between 1-1.2 g of ground chocolate were placed in preweighed 15 ml centrifuge tubes. Next, 10 ml of petroleum ether was added to the tube. The tubes were vortexed for 3 minutes, centrifuged, and the solvent was decanted. The extraction, with 10 ml petroleum ether each time, was repeated 2 more times. In third extraction the tubes with the solvent and chocolates were sonicated for 10 minutes. After the third petroleum decantation, residual solvent in chocolate was removed by heating the uncapped centrifuge tubes in a beaker of boiling water. After returning to room temperature, the mass of defatted chocolates was determined and is shown in Table 2. The difference of chocolate, extracted in petroleum ether, was assumed to be fatty acids, sterols and flavonoids as shown in the Hex/IPA extraction chromatogram.

Approximately 250 mg of defatted chocolate residue were placed in 25 ml volumetric flask. 20 ml DI water were added to the flask and the mix was heated in water bath at 80°C for 20 minutes. The mix was cooled down to room temperature and water was added to bring the volume to 25 ml. A portion of the slurry chocolate was centrifuged at 3000 rpm, and 1 ml of the supernatant liquid was filtered using a 0.45µm PTFE syringe filter into an autosampler vial.

The four chocolate samples were each analyzed in three configurations: the first run was from the liquid hexane/IPA extraction; the second from derivatized triglycerides (dried extraction); and the third run from water extraction of fat-free chocolate (theobromine and caffeine determination).

The GC/MS method parameters used for analysis of water extraction are described below:

Column: ZB5-MS (5% diphenyl, 95% dimethylpolysiloxane), 30 m x 0.25 mm ID, 0.25 µm film thickness

Injection Mode: Split (5:1 split ratio)
Inlet Temperature: 250 °C
Column flow rate (helium): 1.0 ml/min
Oven Temperature Program: Ramp 10°C/min, 50 °C – 315 °C, hold 20 min at 315 °C
Solvent Delay: 2.50 min
Detection Mode: Selective Ion Monitoring (SIM)
Ions Monitored: m/z 180, 181, 193, 194
Dwell Time: 33 ms
Quantitation Ions: 180, 194

Blanks tested included Hexane/IPA in first run; hexane in second run and distilled water in third run. The resulting spectra were screened against the 2011 NIST/EPA/NIH mass spectral library using the NIST Mass Spectral Search Program v2.0g. The best library match is reported for each peak along with the Match Factor for the unknown and library spectrum. A perfect match results in a value of 999; spectra with no peaks in common result in a value of 0. As a general guide, 900 or greater is an excellent match; 800–900, a good match; 700–800, a fair match. Less than 600 is a very poor match. However, unknown spectra with many peaks will tend to yield lower Match Factors than similar spectra with fewer peaks. Also, even an excellent match should not be considered as a definitive identification of the compound—true confirmation should be done by testing the sample alongside an analytical standard of the compound in question.
900, a good match; 700–800, a fair match. Less than 600 is a very poor match. However, unknown spectra with many peaks will tend to yield lower Match Factors than similar spectra with fewer peaks. Also, even an excellent match should not be considered as a definitive identification of the compound—true confirmation should be done by testing the sample alongside an analytical standard of the compound in question.

Calibration curves of caffeine and theobromine in water were prepared gravimetrically to quantify the concentrations of caffeine and theobromine in Lindt, Green & Black, Ghirardelli and Taza chocolate samples, and are shown in Figure 23 and Figure 24.

**Results: Lipid Analysis**

Parts of total ion chromatograms of the Lindt, Green & Black, Ghirardelli, and Taza chocolate samples are shown in Figure 20 through Figure 22. Peaks are tabulated in Table 3 through Table 5, by retention time. The best library match for the measured mass spectrum of each peak is shown with its CAS number, match factor, and chemical structure.

The compounds identified in the Hexane/IPA extraction runs consist of fragrant food additives; caffeine and theobromine; some fatty acids and their ethyl esters; and sterols. All the compounds in Table 3 were found in all four of the chocolate samples except the isovanillin compound that was found only in the Taza chocolate.

The majority of the compounds identified in the derivatized triglycerides (esterification of lipids) runs consist of fatty acid methyl esters (FAMEs), except for Nonanal dimethyl acetal which is a food additive acetal and the oleic acid amide.

**Results: Caffeine and Theobromine Analysis**

Comparisons of relative size of the caffeine peaks in the chocolate extracts to the caffeine peak in a 20 ppm caffeine stock solution are shown in Figure 25. Comparisons of the theobromine peaks in the chocolate extracts to the theobromine peak in 94 ppm and 188 ppm theobromine stock solutions are shown in Figure 26.

The concentrations of caffeine and theobromine found in ppm from the calibration curves and as calculated in mg per serving for each chocolate sample are shown in Table 6.
Figure 20: Overlay showing part of total ion chromatogram of 4 samples and a blank. This ion chromatogram shows peaks of fatty acid and sterol compounds in Hexane/IPA extraction before evaporation.

Figure 21: Overlay showing part of total ion chromatogram of 4 samples and a blank. This ion chromatogram shows peaks of fatty acids from derivatized triglycerides in dried extractions.
Figure 22: Total ion chromatogram of 4 samples showing peaks of caffeine and theobromine in water extraction.

Table 2: The amount of de-fatted chocolate after petroleum ether extraction.

<table>
<thead>
<tr>
<th>Chocolate mass [mg]</th>
<th>Defatted chocolate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before extraction</td>
<td>After extraction</td>
</tr>
<tr>
<td>LIN</td>
<td>1.1971</td>
</tr>
<tr>
<td>GBO</td>
<td>1.1994</td>
</tr>
<tr>
<td>GHI</td>
<td>1.0277</td>
</tr>
<tr>
<td>TAZ</td>
<td>1.1923</td>
</tr>
</tbody>
</table>

Table 3: GC/MS results for TAZ chocolate sample from the first run of liquid hexane/IPA extraction. The table is sorted by retention time. The best library match for each peak is listed with the compound’s CAS number, spectral match quality, and chemical structure.

<table>
<thead>
<tr>
<th>Retention Time [min]</th>
<th>Match Quality</th>
<th>Best Library Match</th>
<th>Comments</th>
<th>Chemical Structure</th>
<th>Cas #</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.38</td>
<td>831</td>
<td>Pyrazine, tetramethyl-</td>
<td>Food flavoring.</td>
<td><img src="#" alt="Chemical Structure" /></td>
<td>1124-11-4</td>
</tr>
<tr>
<td>6.84</td>
<td>650</td>
<td>Phenylethyl Alcohol</td>
<td>Aromatic alcohol that occurs widely in nature.</td>
<td><img src="#" alt="Chemical Structure" /></td>
<td>60-12-8</td>
</tr>
<tr>
<td>7.26</td>
<td>764</td>
<td>Pyranone</td>
<td>Found in nature as part of the fragrant compound coumarin.</td>
<td>28564-83-2</td>
<td></td>
</tr>
<tr>
<td>10.85</td>
<td>659</td>
<td>Isovanillin</td>
<td>Isomer of vanillin, it is a fragrant phenolic aldehyde compound.</td>
<td>621-59-0</td>
<td></td>
</tr>
<tr>
<td>11.19</td>
<td>700</td>
<td>Sucrose</td>
<td>Disaccharide combination of glucose and fructose.</td>
<td>57-50-1</td>
<td></td>
</tr>
<tr>
<td>15.75</td>
<td>700</td>
<td>Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-</td>
<td>Diketopiperazine with the flavor of roasted malt. It is thought to give chocolate the bitter test from the interaction with theobromine during roasting.</td>
<td>5654-86-4</td>
<td></td>
</tr>
<tr>
<td>16.09</td>
<td>935</td>
<td>Caffeine</td>
<td>A bitter xanthine alkaloid of the cacao and several other plants.</td>
<td>58-08-2</td>
<td></td>
</tr>
<tr>
<td>16.36</td>
<td>949</td>
<td>Theobromine</td>
<td>A bitter xanthine alkaloid of the cacao and several other plants.</td>
<td>83-67-0</td>
<td></td>
</tr>
<tr>
<td>17.19</td>
<td>897</td>
<td>Palmitic acid</td>
<td>A saturated fatty acids of chocolate triglycerides.</td>
<td>57-10-3</td>
<td></td>
</tr>
<tr>
<td>17.52</td>
<td>781</td>
<td>Ethyl palmitate</td>
<td>Ethyl ester of palmitic acid</td>
<td>628-97-7</td>
<td></td>
</tr>
<tr>
<td>18.9</td>
<td>908</td>
<td>cis-Vaccenic acid</td>
<td>An unsaturated Omega – 7 fatty acid.</td>
<td>506-17-2</td>
<td></td>
</tr>
<tr>
<td>19.11</td>
<td>810</td>
<td>Stearic acid</td>
<td>A saturated fatty acids of chocolate triglycerides.</td>
<td>57-11-4</td>
<td></td>
</tr>
<tr>
<td>19.39</td>
<td>776</td>
<td>Ethyl stearate</td>
<td>Ethyl ester of stearic acid.</td>
<td>111-61-5</td>
<td></td>
</tr>
<tr>
<td>20.86</td>
<td>837</td>
<td>Oleic acid amide</td>
<td>An amide of unsaturated oleic acid.</td>
<td>301-02-0</td>
<td></td>
</tr>
<tr>
<td>26.11</td>
<td>788</td>
<td>β-Tocopherol</td>
<td>Methylated phenols that have vitamin E activity.</td>
<td>148-03-8</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: GC/MS results for TAZ chocolate sample from the second run of derivatized triglycerides (dried extraction). The table is sorted by retention time. The best library match for each peak is listed with the compound’s CAS number, spectral match quality, and chemical structure.

<table>
<thead>
<tr>
<th>Retention Time [min]</th>
<th>Match Quality</th>
<th>Best Library Match</th>
<th>Comments</th>
<th>Chemical Structure</th>
<th>Cas #</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.11</td>
<td>836</td>
<td>Nonanal dimethyl acetal</td>
<td>Food additive acetal.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>18824-63-0</td>
</tr>
<tr>
<td>14.72</td>
<td>834</td>
<td>Myristic acid, methyl ester</td>
<td>Ester of myristic acid. Triglyceride of myristic ac. Constitutes 75% of nutmeg butter.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>124-10-7</td>
</tr>
<tr>
<td>16.65</td>
<td>870</td>
<td>Palmitoleic acid, methyl ester</td>
<td>Ester of unsaturated palmitoleic acid.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1120-25-8</td>
</tr>
<tr>
<td>16.85</td>
<td>953</td>
<td>Palmitic acid, methyl ester</td>
<td>Ester of saturated palmitic acid.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>112-39-0</td>
</tr>
<tr>
<td>17.84</td>
<td>875</td>
<td>Margaric acid methyl ester</td>
<td>Ester of saturated margaric acid. Found in the mammalian’s milk.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1731-92-6</td>
</tr>
<tr>
<td>18.48</td>
<td>948</td>
<td>Linoleic acid, methyl ester</td>
<td>Ester of polyunsaturated omega-6 linoleic acid.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>112-63-0</td>
</tr>
<tr>
<td>18.55</td>
<td>950</td>
<td>Oleic acid, methyl ester</td>
<td>Ester of monounsaturated omega-9 oleic acid.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>112-62-9</td>
</tr>
<tr>
<td>18.79</td>
<td>956</td>
<td>Stearic acid, methyl ester</td>
<td>Ester of saturated stearic acid.</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>112-61-8</td>
</tr>
<tr>
<td>20.56</td>
<td>893</td>
<td>Eicosanoic acid, methyl ester</td>
<td>Ester of saturated eicosanoic acid which is a minor</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1120-28-1</td>
</tr>
</tbody>
</table>
constituent of peanut and corn oils, or obtained by the hydrogenation of polyunsaturated arachidonic acid.

<table>
<thead>
<tr>
<th>Retention Time [min]</th>
<th>Match Quality</th>
<th>Best Library Match</th>
<th>Comments</th>
<th>Chemical Structure</th>
<th>Cas #</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.86</td>
<td>700</td>
<td>Oleic acid amide</td>
<td>Formed from unsaturated oleic acid and an amine. It is an endogenous substance.</td>
<td><img src="stem" alt="Chemical Structure" /></td>
<td>301-02-0</td>
</tr>
<tr>
<td>22.21</td>
<td>700</td>
<td>Behenic acid, methyl ester</td>
<td>Ester of saturated behenic acid. It is component of behen, canola and peanut oils.</td>
<td><img src="stem" alt="Chemical Structure" /></td>
<td>929-77-1</td>
</tr>
</tbody>
</table>

Table 5: GC/MS results for TAZ chocolate sample from the third run of water extraction of fat free chocolate (theobromine and caffeine determination). The table is sorted according to their retention time. The best library match for each peak is listed with the compound’s CAS number, spectral match quality, and chemical structure.

- **5-Hydroxymethyl furfural** (67-47-0): Derived from dehydration of sugars during cooking and caramelization.
- **Sucrose** (57-50-1): Disaccharide combination of glucose and fructose.
- **Lactose** (63-42-3): Disaccharide combination of galactose and glucose.
- **Caffeine** (58-08-2): A bitter xanthine alkaloid of the cacao and several other plants.
- **Theobromine** (83-67-0): A bitter xanthine alkaloid of the cacao and several other plants.
Figure 23: Calibration curves used for quantitative analysis of caffeine extraction in water.

Figure 24: Calibration curves used for quantitative analysis of theobromine extraction in water.
Figure 25: Extracted ion chromatogram (m/z = 194) comparing the relative size of the caffeine peak in the 4 chocolate samples to the caffeine peak in a 20 ppm caffeine stock solution.
Figure 26: Extracted ion chromatogram (m/z = 180) comparing the relative size of the theobromine peak in the 4 chocolate samples to the theobromine peak 94.2 ppm (black) and 189 ppm (pink) theobromine stock solutions.

Table 6: Concentrations of caffeine and theobromine found in GBO, GHI, LIN and TAZ chocolate samples in expressed in ppm unit and calculated per serving for each chocolate.

<table>
<thead>
<tr>
<th>Caffeine</th>
<th>Theobromine</th>
<th>Chocolate serving amount</th>
<th>Caffeine</th>
<th>Theobromine</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ppm]</td>
<td>[g]</td>
<td>[mg/per serving]</td>
<td>[ppm]</td>
<td>[g]</td>
</tr>
<tr>
<td>LIN</td>
<td>696</td>
<td>5506</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>GBO</td>
<td>958</td>
<td>7313</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>GHI</td>
<td>746</td>
<td>6061</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>TAZ</td>
<td>971</td>
<td>7129</td>
<td>42</td>
<td>41</td>
</tr>
</tbody>
</table>

TGA-FTIR
Experiments were performed on a TA Instruments Q500 TGA (CPG ID#12850) coupled to a Biorad Excalibur 3000 FTIR bench (CPG ID#10450) with a Pike Scientific Evolved Gas Accessory (EGA) (CPG ID#12880). The EGA was set to 230 °C throughout these experiments. The following method was used:
1. Equilibrate at 30.00 °C (nitrogen purge)
2. Ramp 20.00°C/min to 600.00°C
3. Isothermal for 5.00 min
4. Select Gas: 2 (air)
5. Ramp 20.00°C/min to 800.00°C
6. End of method

Samples were equilibrated at 30 °C, then ramped to 600 C at 20 °C/min. At this point the nitrogen purge was switched to air and the chocolate burned off to determine the final solids content. All chocolates had very similar responses, with replicates basically identical, therefore only representative curves are shown. Percent mass is shown in Figure 27. Transitions are seen at ~220 °C and 390 °C. More informative is the mass/temperature derivative (Figure 28). This plot highlights the two main transitions noted before, but also suggests further transitions at 158 °C, 314 °C, 353 °C and 592 °C. This plot also more clearly isolates the location of the main peaks described above, but highlights that the LIN chocolate appears different at the high temperature, and that the TAZ chocolate appears different at the lower main transition. Considering in more detail the higher peak (424 °C), the LIN has an apparently lower transition, relative to the GBO, GHI and TAZ samples. These plots imply that generally the same materials and weight fractions are being evolved. The percentage mass losses at each of the main transitions are shown in Table 7. Within a few percent, the values are virtually identical at each transition, although the GBO and TAZ brands may lose more mass at the lower temperatures than the other two brands.
Using the Evolved Gas Accessory, the evolved species can be tracked with the temperature in the TGA. The data discussed so far is considered in

![Graph showing TGA data versus FTIR Gram-Schmidt data.](image)

**Figure 29: TGA data versus FTIR Gram-Schmidt data.**

As previously noted, there are no substantial differences between the chocolates, with the major transitions highlighted in Figure 28 in the TGA data, also evident in the FTIR spectra. The FTIR data do reinforce the case that the partial transition observed at ~315 °C is real, with a bump in the Gram-Schmidt visible in the FTIR data. Although it is technically possible to generate library searches on the spectra collected, this is not done here because of the complex nature of these materials. Nonetheless, examining “snap-shot” FTIR spectra from the key transitions highlighted in Figure 28 allows comparison of the similarities of the ingredients being released. These data are shown in Figure 30 to Figure 32. No substantial differences were observed at any of the temperatures, but at 225 and 315 °C the GHI may lack one compound present in the other materials, and at 425 °C the TAZ sample may lack a different compound.
Figure 27: Percent mass for all four chocolates versus temperature.

Figure 28: Mass derivative (against temperature) for all four chocolates versus temperature.

Table 7: Percent mass loss during main transitions.

<table>
<thead>
<tr>
<th>Transition</th>
<th>0-240 °C</th>
<th>240-445 °C</th>
<th>445-800 °C</th>
<th>residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIN</td>
<td>-15.7%</td>
<td>-64.4%</td>
<td>-18.4%</td>
<td>1.6%</td>
</tr>
<tr>
<td>TAZ</td>
<td>-16.6%</td>
<td>-60.9%</td>
<td>-20.8%</td>
<td>2.0%</td>
</tr>
<tr>
<td>GHI</td>
<td>-14.1%</td>
<td>-65.8%</td>
<td>-18.3%</td>
<td>1.7%</td>
</tr>
<tr>
<td>GBO</td>
<td>-17.5%</td>
<td>-61.0%</td>
<td>-19.8%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>
Figure 29: TGA data versus FTIR Gram-Schmidt data.

Figure 30: FTIR spectra from the 225 °C transition. Only GHI appeared different, missing a broad peak at ~2100 cm⁻¹ (arrow).
Figure 31: FTIR spectra from the 315 °C transition. Only GHI appeared different, missing a broad peak at ~2100 cm⁻¹ (arrow).

Figure 32: FTIR spectra from the 425 °C transition. Only TAZ appeared different, missing a peak at ~2400 cm⁻¹ (arrow).