Cutting into tallow: A lipidomic exploration of animal-based and alternatively sourced meat

Using the SCIEX 7500 system

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Alternatively sourced meat has been available at the grocery store in many forms for several years. Black bean “burger” patties or tofu “turkey” have been choices of protein sources for consumers, but with the rising concern about global climate change, the call for more sustainable sources of protein is increasing. Since 2019, the retail sales of plant-based meat alternatives have grown 27%[1], as these alternatives to meat are readily available at popular fast food chains, restaurants and local grocery stores.

In addition to their protein source, many factors define these alternatives to meat products besides just another source of protein. Synthetic biology companies investigate the smell, mouthfeel and visual qualities of their plant-based products. The taste and texture provide the consumer with an overall experience that is similar to the experience of eating meat. Fats, from either a single vegetable oil or from several sources, are often added to develop the flavor profile of the meat alternative. Depending on the source, however, the added fats may have beneficial or harmful effects on consumers’ health.[2]

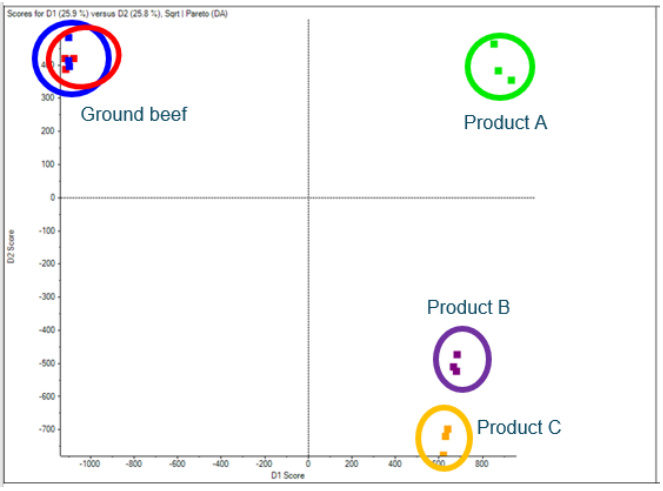

Figure 1. Supervised PCA analysis of various meat and alternative meat products. PC1 vs. PC2 loading plot shows clear differentiation between meat and alternative meat products. Biological replicates cluster tightly, highlighting the reproducibility of the method. Differentiation can also be seen between the various alternative meat products.

Here, the lipid profiles of 5 different types of meat and meat alternatives were analyzed for their lipid profiles. Using the global lipidomic profiling method[3], over 2000 lipids were screened and then narrowed down to quantify over 1000 lipid species in a single injection. A total of 21 lipid classes were monitored for this study, with the changes in carbon length composition, fatty acid composition and degree of desaturation are reported for select classes. The final lipid panel enabled clear differentiation between animal-based meat and meat alternatives (Figure 1).

Key features of lipidomic profiling in meat and alternatively sourced meat

- High-throughput lipidomic profiling method that provides a customizable MRM list that can be adapted based on matrix of interest or lipid class, such as oxidized lipids
- High sensitivity detection using the SCIEX 7500 system
- Enables user to profile and quantify changes in lipid profiles before and after cooking (Figure 1)
- Method can be utilized to report changes in chain length composition, fatty acid profiles and degree of desaturation by lipid class
- Enables users to identify potential markers for animal diet type in beef samples or added oils in meat alternative samples

Methods

Sample preparation: Raw 85% lean, 15% fat ground beef and grass-fed beef were tested alongside 3 commercially available products that are described as Products A, B and C. A Bligh-Dyer extraction was performed on 3 replicates of each sample type. To control for extraction efficiencies, 10 µL each of Lipidyzer and Avanti SPLASH Lipidomix standards were spiked in. Each replicate was normalized by tissue weight.

Chromatography: Separations were performed on an ExionLC AC system using a Phenomenex Luna 3.0 µm NH2 column (2 x 100 mm). PN: 00D-4377-B0

Mass spectrometry: A SCIEX 7500 system with an OptiFlow Pro ion source (electrospray ionization probe) was used for data acquisition in positive and negative mode. A targeted assay was built using the Scheduled MRM algorithm (sMRM) for quantification. For details on the LC-MS/MS methods, see references.[4]

Data processing: All data was processed using SCIEX OS software using the MQ4 algorithm. Automated computation of the time scheduled MRM methods was performed using the sMRM Pro Builder v1.4.[5]

Global lipidomic profiling

There are various LC-MS/MS methodologies in which to probe the lipidome, however many can be time consuming from method setup to data analysis. Here, a high-throughput, targeted lipid screening protocol was utilized to broadly and rapidly profile 21 lipid classes and screen more than 2200 lipid molecules. After using the sMRM Pro Builder template to focus the analyses,[5] the final sMRM method screened more than 1100 lipids in a 17-minute chromatographic run time in a single injection. Although several lipid classes were included in the final analyses, this study focuses specifically on triacylglycerols (TAGs). The clustering observed in Figure 1 can be attributed to the changes in this lipid class. A total of 498 TAG species were monitored in the final sMRM method out of the total 520 transitions included in the panel. The final lipid count by class can be seen in Figure 2.

Triacylglycerol and fatty acid analysis

When analyzing lipid profiles in food samples the total carbon length of triacylglycerols (sum of all 3 fatty acid chain lengths on the glycerol backbone) can be indicative of the origin of the fat. Figure 3 shows the TAG profiles for each sample type, organized by carbon chain length. The meat alternatives show more diversity in their total carbon length profiles, spanning from C30 to C58, with largest summed average peak areas in carbon lengths C30 to C44. This difference in distribution can be attributed to the oils (often added for taste and texture) in the alternative meats being sourced from plants. Coconut oils are rich in short chain TAGs, whereas soybean, canola and corn oils have longer chain length lipids.[6] Soybean, canola and corn oils are rich in linoleic acid and can be observed in Figure 3 at sum composition C54 or in Figure 4 at fatty acid C18:1.

Total carbon length does not always indicate how healthy a fat source can be. The fatty acids monitored from all TAG molecules were summed based on average peak area for the replicates in Figure 4. While the meat alternatives had a higher proportion of saturated fats compared to the ground beef, the saturated fats were short-chain fatty acids, which have been shown to be beneficial.[7] The ground beefs profiles are mostly comprised of C16 and C18 fatty acids with an emphasis in saturated C16 and monounsaturated C18. The C20 and C22 series were predominantly present in the ground beef samples, especially as the number of double bonds increases. This is one health benefit of the ground beef samples, as foods with polyunsaturated fats tend to have positive health benefits.[8]

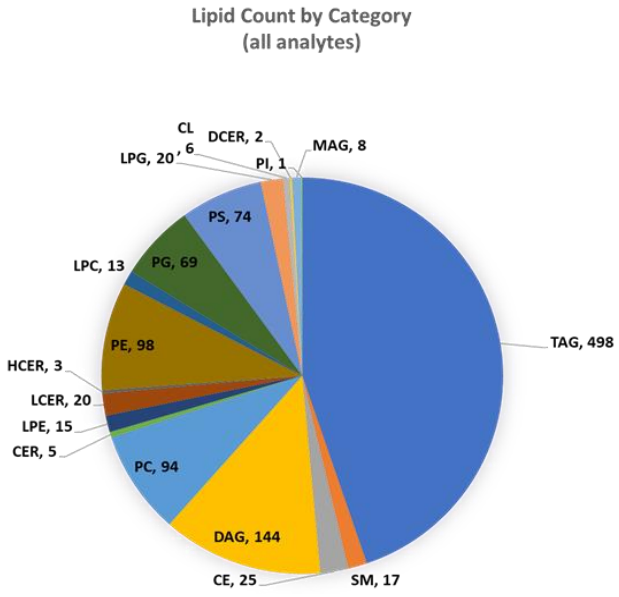

Figure 2. Distribution of lipids in final sMRM assay. Using the sMRM Pro Builder template, lipid species were filtered and the final sMRM list was curated and assayed. The final lipid count, by class, is shown.

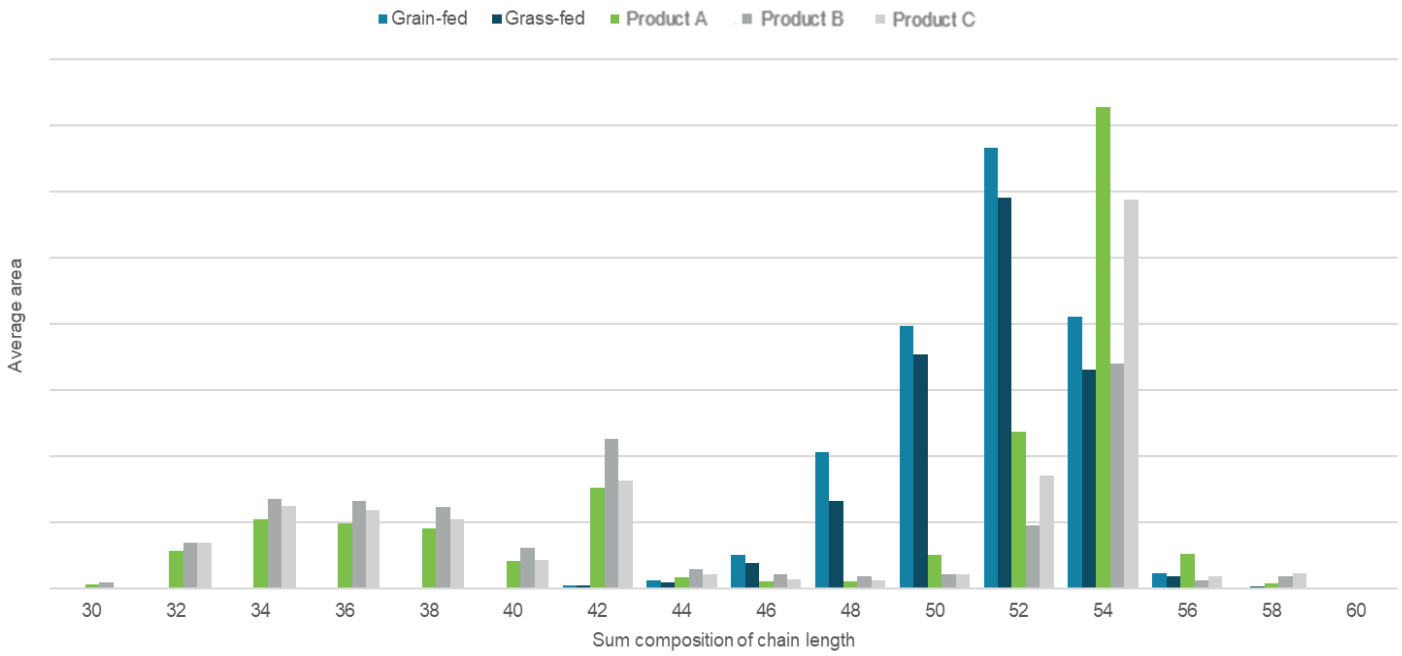

Figure 3. Sum composition of carbon length of triacylglycerols (TAG). The MRM peak areas of all TAGs within each chain length were summed for each sample then averaged within each group analyzed. The short chain lengths C30-44 are more abundant in the meat alternatives than in the ground beef, which is most abundant in chain lengths C46-52. All samples contain C54, with Product B containing the most.

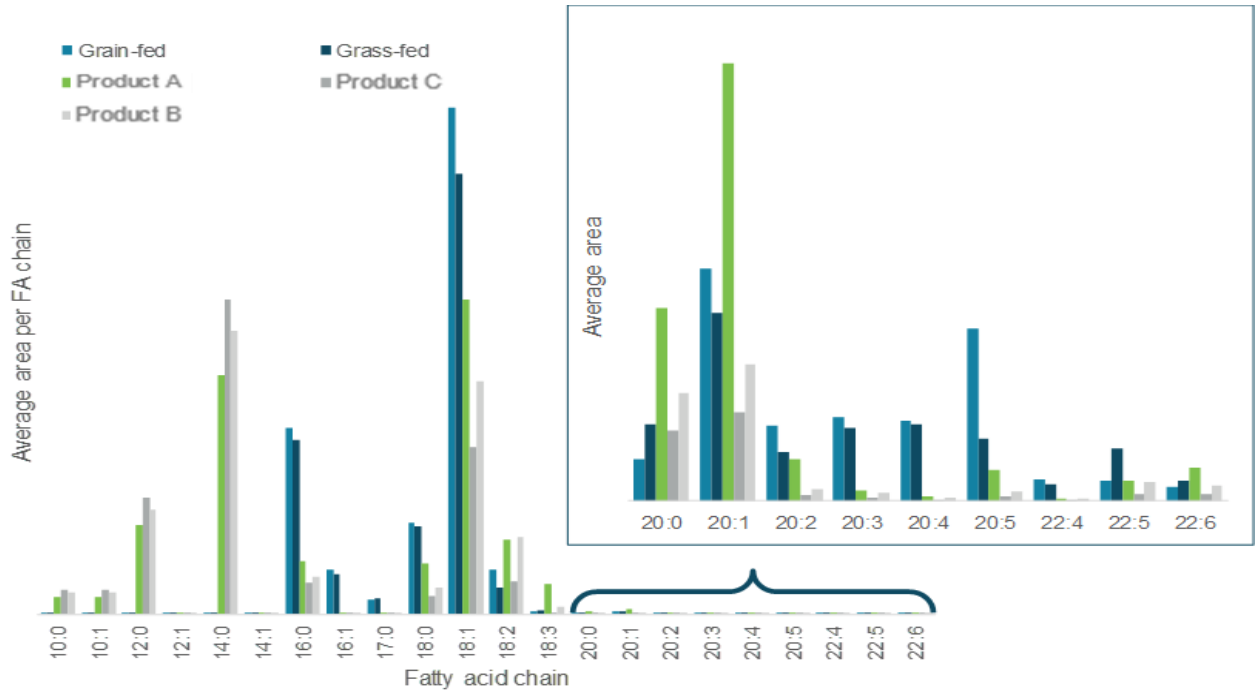

Figure 4. Fatty acid profiles of TAG species monitored. Each acyl chain length that was monitored in the TAG species was summed for the replicates and then averaged for each group and reported by that fatty acid chain. The grain-fed and grass-fed beef are shown in the light and dark blue bars and show more medium-chain saturated and monounsaturated and long-chain polyunsaturated fats. The meat alternatives have lipid profiles with more short-chain and long-chain saturated fats.

Unique markers for differentiation of lipid source

While looking at the overall composition of the lipidome profile can be a good indicator of the origin of the fat, a unique marker to identify the exact source can be utilized in targeted methods for origin identification or origin authentication. Figure 5 shows a unique marker for Product A meat alternative and Figure 6 shows two unique markers for grass-fed beef. Markers in the alternative meats could also be used to ensure the quality of the fats being added, as well as being able to monitor any potential changes in the lipid profiles from handling or treatment of the food that could affect the taste or texture of the product.

The markers for grass-fed beef could potentially be used to authenticate the diet the animal has been fed. Mammals do not produce the enzymes that synthesize fatty acid 18:3, which is present in the green leafy tissues of plants. Therefore, this fatty acid is unique to animals on a grass-fed diet. This fatty acid could likewise be a unique marker for oils or fats added to meat alternatives.

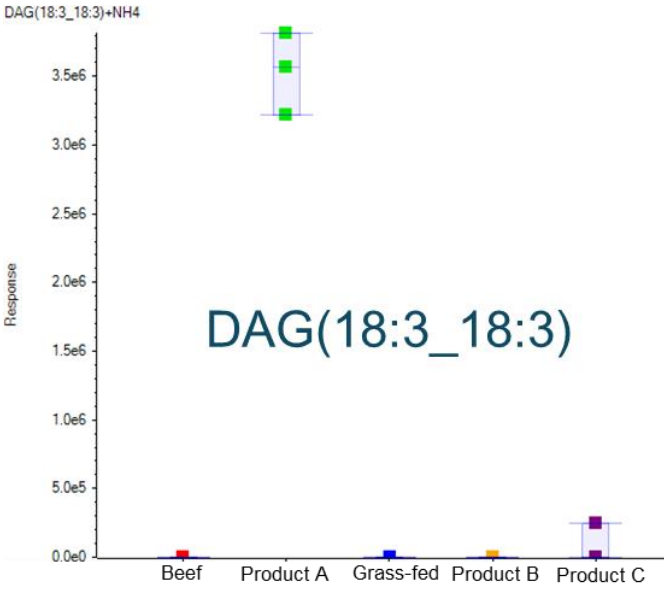

Figure 5. Unique marker in Product A. Using MarkerView software, a diacylglycerol (DAG) species, DAG(18:3_18:3) was found to be significantly increased in the Product A samples compared to the other samples.

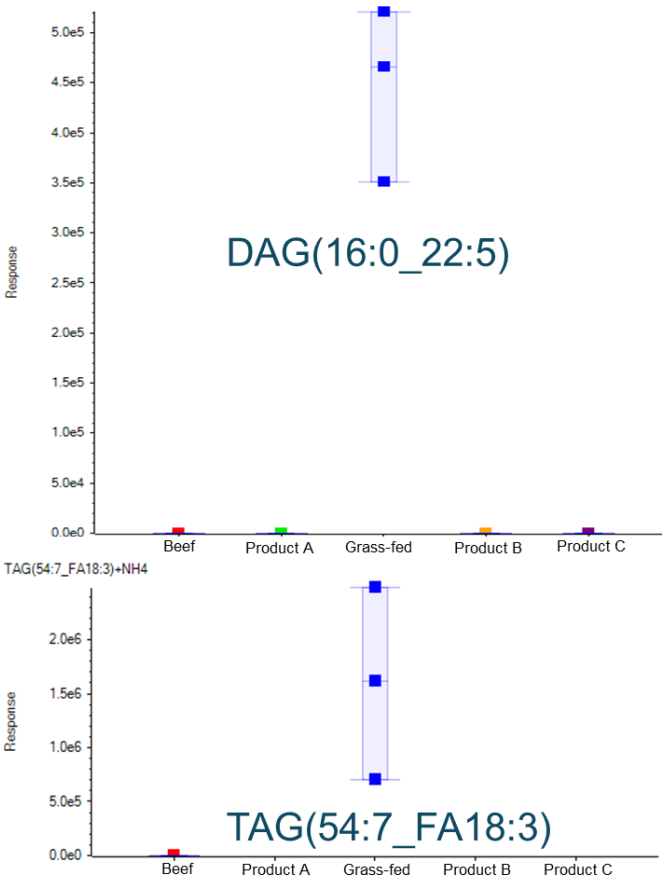

Figure 6. Increased neutral lipids in grass-fed beef. Two markers, DAG(16:0_22:5) and TAG(54:7_18:3), were identified only in grass-fed beef samples.

Conclusions

- The SCIEX 7500 system enables users to profile and identify the lipid composition of meat and meat alternative samples
- Utilizing the global lipidomic profiling method, users are able to report total carbon length profile changes, fatty acid profile changes, and lipid class changes in diverse set of fat sources
- MarkerView software provides useful visuals to determine lipid classes of interest for further study
- Fat sources from meat alternatives can be profiled to establish dietary benefits and to monitor for changes that could affect taste and texture
- The global profiling method can be used to authenticate the animal diet by targeting markers specific for grass-fed or grain-fed meat

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