Application Note

Food Testing

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LC/Q-TOF Analysis and Nontargeted Chemometric Profiling of Meats and Plant-Based Alternatives

Food sensory testing using the Agilent 1290 Infinity II LC and Agilent 6546 Q-TOF

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Abstract

Meat-alternative sources of protein, including plant-based and cell-based foods, are gaining popularity globally due to a combination of consumer interest, regulatory changes, and global food systems. For example, as Singapore aims to achieve 30% of its food production levels through self-production by 2030, many established food companies and startups are developing meat-substitute products. The main drivers of Singapore's food production target are around health and environmental concerns. Historically, plant-based meat substitute foods have struggled to achieve the same texture and taste as animal meats. However, recent analogs of plant-based meats are significantly more similar in taste, texture, and composition as traditional meats due to technological advances in production methods. This application note describes a nontargeted profiling method to characterize chemical components of unknown foods, using a high-resolution accurate mass LC-Q/TOF. Also, various statistical tools are presented that translate accurate mass LC/Q-TOF data into more easily understandable information. Principal component analysis (PCA) of the data can be used to identify compounds, abundance distribution of the compounds in different samples, and how the compounds correspond to target taste profiles. Heat maps and hierarchical clustering of raw ingredients show similar distribution of proteins with target taste profiles.

Introduction

Food sensory evaluation is a key method to assess the flavor quality of foods because it measures what consumers perceive. It is, however, subjective. As technology advances, more objective and measurable methods such as liquid chromatography with mass spectrometry (LC/MS) will be used. The five basic tastes (i.e., sweet, salty, sour, bitter, and umami) can now be characterized by LC/MS and the data can be used for the optimization of the overall taste of foods.

Alternative meats are meant to substitute animal-based meat. However, key barriers to consumer adoption have been identified as taste, texture, and nutrition. Testing is critical to ensuring that equivalent health benefits and experience for customers of eating alternative meat foods is achieved. Therefore, there are many studies that compare the difference in nutrition and taste levels between animal-derived meat and meat-alternative products.[1]

Targeted analysis is focused on known groups of nutrients or flavor compounds. The results from targeted analytical methods and sensory evaluation tests may differ as compounds that are not in the targeted list may contribute to the overall taste. In contrast, nontargeted high-resolution accurate mass analysis is not restricted to a specific group of compounds. In an unbiased manner, compounds in the proteins can be profiled, identified, and comparisons made between alternative meat and real meat. As in food sensory analysis testing, nontargeted LC/MS methods do not analyze a particular flavor profile but are unbiased, and focus on total compound profiles, much like taste buds.

Apart from finding the different compounds that contribute to various taste profiles, their abundance in each protein is equally important. Although standards are not often available for quantitative analysis, the relative intensity differences of compounds in the various proteins can be used to tell them apart. A person may only distinguish flavors when there is a drastic abundance difference in some compounds. In this study, quadrupole time of flight (Q-TOF) LC/MS and statistical software were used to identify and differentiate flavor profiles. The method will help the development of equivalent flavor profiles in plant-based protein foods.

Experimental

Solvents

Agilent ultrapure LC/MS grade methanol (part number 5191-4497), acetonitrile (part number 5191-4496), and water (part number 5191-4498) were used. Formic acid for LC/MS (Fluka from Honeywell) and ammonium formate for LC/MS (LiChropur, MerckMillipore) were also used.

Materials

Agilent InfinityLab solvent bottles with cap (part number 9301-6528) were used for the mobile phase. The open-top caps were fitted with an Agilent InfinityLab Stay Safe cap, GL45, one port, one InfinityLab vent valve, 3.2 mm od fitting PTFE insert (part number 5043-1217). The O-ring from the heavy-duty vacuum bottle cap was used to seal the PTFE insert in the bottle. The standard PTFE solvent line was threaded through the PTFE insert. An Agilent stainless steel 12 to 14 µm solvent bottle inlet filter (part number 01018-60025) was then fitted to the solvent line.

Samples

The plant-based meats described in Table 1 were commercially available products. The real meats included minced raw products that were bought from a market.

Table 1. Plant-based alternative meat samples and sample codes.

Sample Code	Description of Food Product
PBC 1	Plant-based chicken
PBC 2	Plant-based chicken
PBB 3	Plant-based beef
PBB 4	Plant-based beef
PBP 5	Plant-based pork
PBP 6	Plant-based pork
PBP 7	Plant-based pork

Sample preparation

All sample collection and preparation steps were done in polyethylene or polypropylene containers. Fifteen and 50 mL high-performance polypropylene centrifuge tubes with plug caps (VWR International Ltd., UK) were used throughout. Agilent 2 mL screw top amber glass autosampler vials (part number 5182-0716) with screw caps (part number 5185-5862) were used. The samples were weighed in a centrifuge tube, 70/30 methanol/water was

then added to the samples at a ratio of 1:2. The samples were vortexed for 10 minutes and centrifuged at 4,000 rpm for 15 minutes. The samples were re-extracted under the same conditions. The extracts were then filtered into the autosampler vials using an Agilent 0.45 µm polyethersulfone (PES) filter (part number 5190-5276).

Instrumentation

An Agilent 1290 Infinity II LC consisting of an Agilent 1290 Infinity II high speed pump (part number G7120A) was used as the HPLC. The system also featured an Agilent 1290 Infinity II multisampler (part number G7167B) fitted with an Agilent InfinityLab sample thermostat and Infinity multiwash option. The LC included an Agilent 1290 Infinity II multicolumn thermostatted column compartment (part number G7116B). An Agilent 6546 Q-TOF MS system (part number G6546A) was used for accurate mass measurements. The mass spectrometer was run in "Data Independent All Ions Fragmentation" scan acquisition mode where all ions passed through the Q-TOF collision cell operating under positive ion polarity.

Data analysis was done using Agilent MassHunter Qualitative Analysis 10.0, Profinder 10.0, and Mass Profiler Professional 15.1 software.

Table 2. Agilent 6546 LC/Q-TOF LC/MS system (G6546A) operating conditions.

HPLC Conditions	
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)
Injection Volume	5 µL
Mobile Phase	A) 10 mM NH_4F + 0.1% FA in DIW B) Acetonitrile
Initial	A) 98 % 10 mM NH_4F + 0.1% FA in DIW B) 2 % acetonitrile
Gradient	Time (min) %A %B 0.30 98.0 2.0 7.27 20.0 80.0 10.27 1.0 99.0 12.00 1.0 99.0 12.10 98.0 2.0 15.00 98.0 2.0
Flow	0.4 mL/min
MS Conditions	
ESI	Positive
Source Parameters	
Gas Temperature	300 °C
Gas Flow	11 L/min
Nebulizer	35 psi
Sheath Gas Heater	350 °C
Sheath Gas Flow	11 L/min
Capillary	3,500 V
V Charging	1,000

Results and discussion

The LC/Q-TOF data were acquired using an All Ions full scan from *m/z* 100 to 1,700 Da and fragmentation spectrum at three different collision energies (10, 20, and 40 V). For compound identification, the accurate mass data were searched against a custom MS fragmentation library consisting of compounds that may impact taste. These compounds included amino acids, short peptides, nucleotides, fatty acids, and various vitamins.

As shown in Figure 1, an overall view of the raw data shows some slight differences between the spectra of the actual meats and substitute meats. Also, it would be time-consuming to screen through the spectral library to identify an individual compound via a library match. Therefore, statistical analysis tools become useful in converting the raw data from the nontargeted analysis into more useable information.

For principal component analysis (PCA), three injections of each extract were performed to check the repeatability of data by observing the clustering of samples. Generally, it was observed that the replicates for each food sample were tightly clustered, indicating a high degree of repeatability in the method (Figure 2). Under the score plot view in Figure 2, each dot represents an injection of a sample. Protein samples were assigned distinct colored data points by target flavor profiles and individual products were assigned different shapes to differentiate them. This score plot view in Figure 2 shows which trends in the sample set contribute to the differences between flavor profiles and particular products. Also, the plot can show if different samples are similar by sharing the same general region in the PCA scores plot. The alternative protein foods were found to cluster well in their targeted flavor profile and there were significant differences between flavor profiles of each food-type, as expected. In contrast, in the PCA loading plot view (Figure 3B), each dot represents a compound. This plot provides information on which compounds impact the scores plot in the PCA. Compounds with the highest loadings (indicated by their symbols) on a principal component correlate with higher abundances of those compounds in the samples.

Figure 2 shows an overview of the distinct types of meat and their plant-based alternatives. From the two-dimensional (2D) PCA plot of nonvolatile compounds, each meat (e.g., chicken) and its plant-based equivalent (e.g., PBC 1 and PBC 2) are more similar to each other compared to the other meats (e.g., beef or pork).

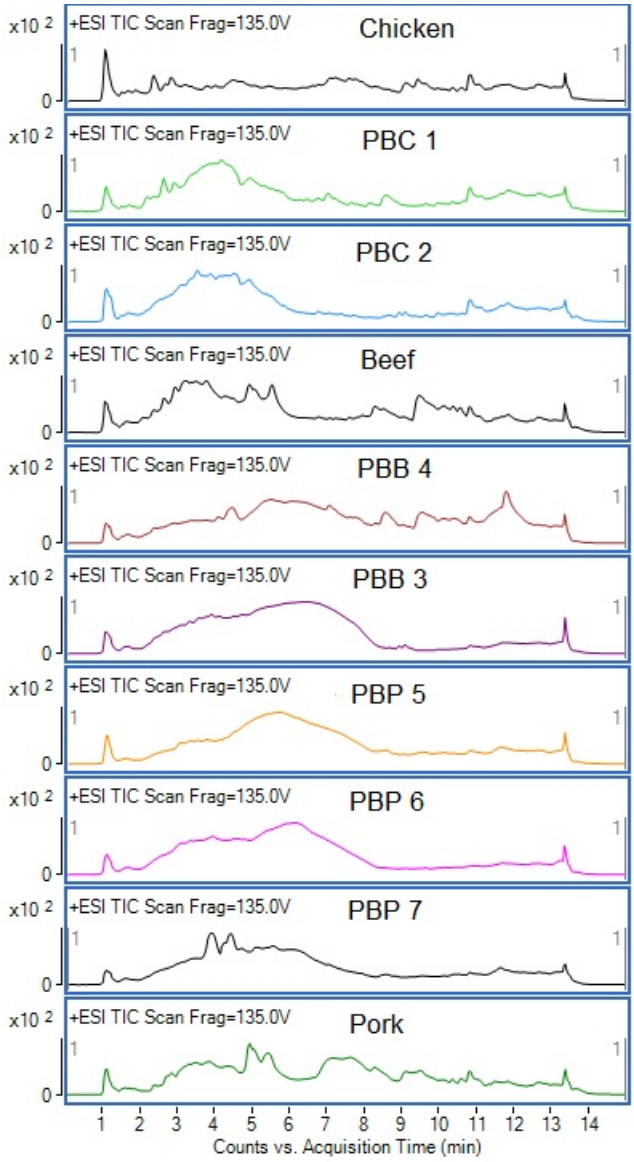

Figure 1. TIC overview of actual meats and plant-based meat equivalents.

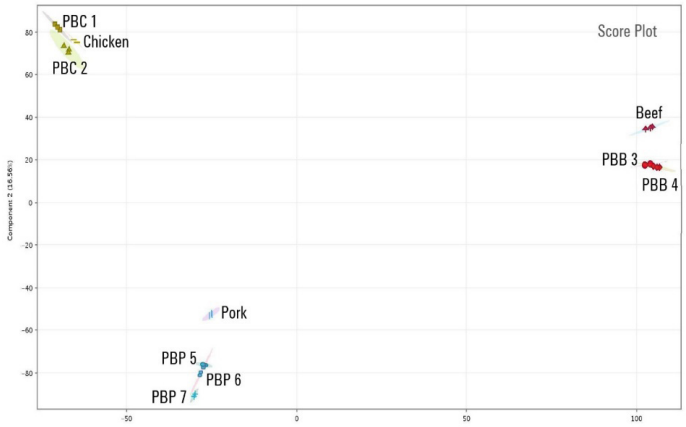

Figure 2. 2D PCA score plot of pork, beef, and chicken and their plant-based equivalents.

Viewing the PCA score plot and loading plot side by side for one meat-type makes it easy to correlate the compounds associated with the group of nutrients or flavor compounds. Figure 3A shows a 3D score plot for chicken, with different groups separated along each axis. The loading plot (Figure 3B) provides information on the compounds that cause the differences in the score plot.

A heat map is a data visualization technique that shows the abundance of a compound on a color scale, with red representing high abundance and blue low abundance. Heat maps allow users to quickly see compound abundance differences of a particular set of flavor profiles, as shown in Figure 4.

Free amino acids that form on the surface of meat at typical cooking temperatures provide the “grilled-meat” flavors that consumers like.[1] It is important, therefore, that manufacturers of plant-based beef foods control the abundance of various amino acids in their products. Figure 4 shows that plant-based beef products, PBB 3 and 4, contain some of the bitter amino acids in higher abundance than real beef. These amino acids may affect the final taste of these products. Profile data of amino acids in foods can be used to select base ingredients that provide a similar abundance of a class of flavor compounds to the desired one.

The plant-based chicken, PBC 2, has more short peptides, while sample PBC 1 has more nucleotide flavor enhancer, which may come from soy or bean-based ingredients. Real chicken meat is known to contain fatty acids, amino acids, and acetyl carnitine, as shown in Figure 3. Chicken is at the top right of the plot, between Y and Z-axis. PBC 1 is near the origin of the three axes, and PBC 2 is at the bottom end of the X-axis. Compounds such as glutamine-glutamine (glu-glu), adenosine diphosphate (ADP), inosine-5-diphosphate (IDP), and valine-glutamine (val-glu) would provide plant-based meats with more of the umami flavor of real chicken.[2]

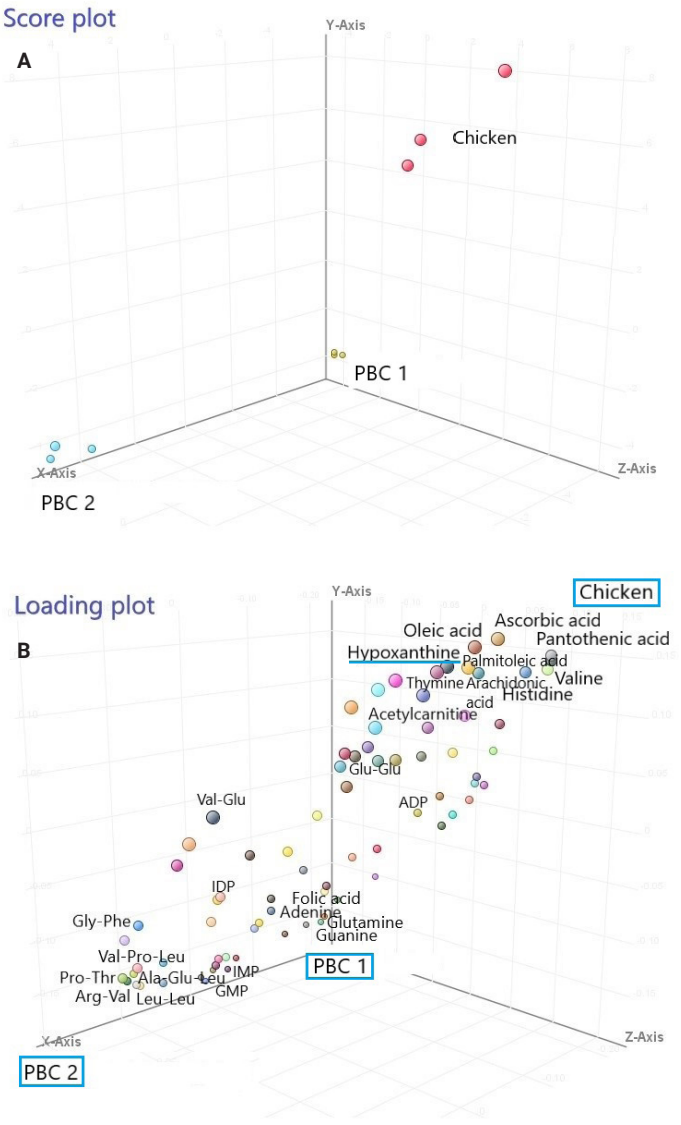

Figure 3. 3D PCA score plot (A) and loading plot (B) of chicken and its plant-based alternatives, PBC 1 and PCB 2.

Higher levels of these flavors in PBC 1 and PBC 2 would be shown by a shift in the compound data points up the Y-axis, closer to the region of chicken.

The nucleotide hypoxanthine, which is a naturally occurring purine derivative, plays a critical role in the umami flavor of chicken. However, with its low purine (guanine) content, PBC 2 may be a healthier choice for reducing the formation of uric acid, which can lead to gout.[3]

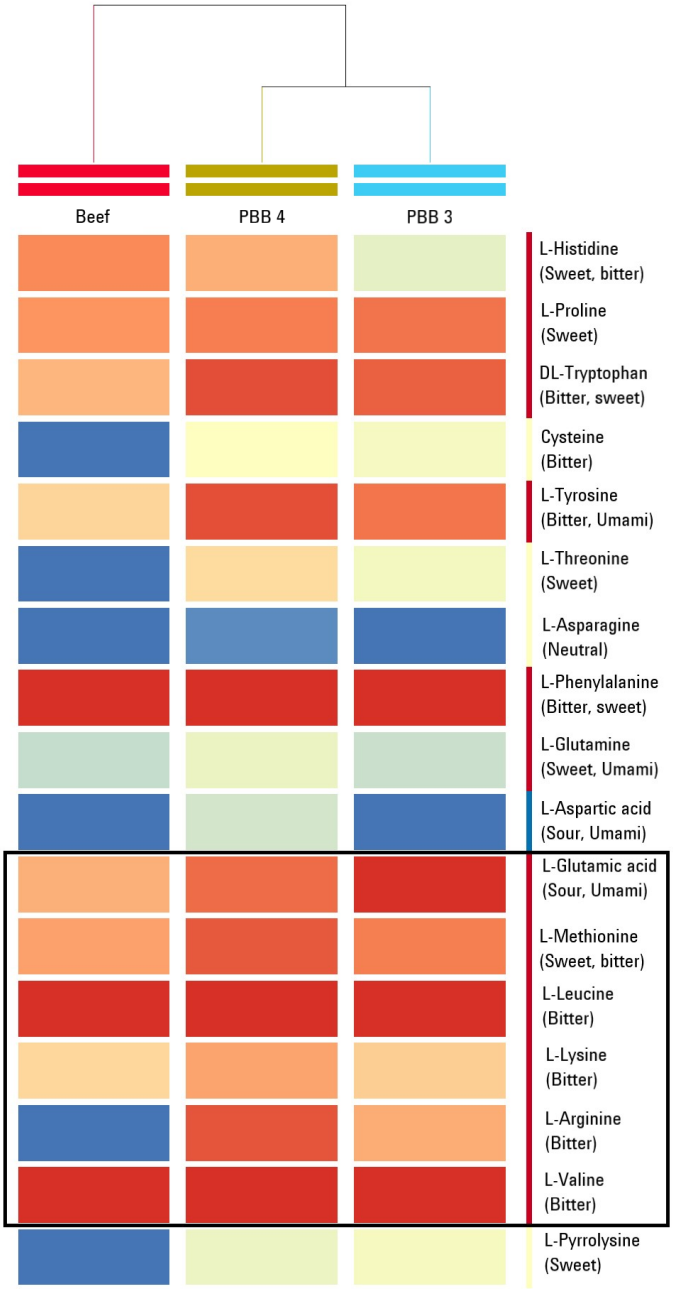

Figure 4. Heat map of amino acids in beef and its plant-based equivalents, PBB 3 and PBB 4.

Conclusion

The flavor, texture, and nutritional value of meat-alternative protein sources are critical to consumer perception, acceptability, and assessment of value.

A nontargeted, data-independent, All Ions workflow using a high-resolution Agilent 6546A Q-TOF LC/MS system successfully profiled and identified many flavor compounds in chicken, beef, pork, and their plant-based alternatives. Agilent Mass Profiler Professional (MPP) software was used to determine relationships among the real meat and alternative plant-based meats using advanced statistical analysis and visualization tools. PCA score and loading plots are useful for comparing compounds in food products. Heat maps are also useful tools for visualizing the profiling of compounds, such as amino acids, in meat and commercially available plant-based meat substitute foods.

The comprehensive LC/MS data acquisition and statistical workflow provides manufacturers of alternate protein foods with critical molecular insights of their products. The profile data would help manufactures to fine-tune a product's ingredients to better replicate the taste of animal-derived meats.

References

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