SHIMADZU
Excellence in Science

Application Note

No. 86

Food

Exploring Wagyu Brand Indicators with UV-Vis, GC-MS, a Microwave-Drying Moisture Meter, and a Meat Lipid Measuring Device

Masami Nishimura[1], Yumi Unno[2], Yuki Nakagawa[2], Keiko Matsumoto[2], Kyohei Hikichi[3], Masaki Baba[4]

Food

■ Abstract

A gas chromatograph mass spectrometer (GCMS-TQ™8040 NX), an ultraviolet and visible spectrophotometer (UV-1280), a meat lipid measuring device, a microwave-drying moisture meter, and a visual evaluation were conducted on 61 beef cattle of Tottori Wagyu. Based on the 603 items, eMSTAT Solution™ performed statistical analyses such as U-test, ANOVA, PLS-DA, SVM, and random forest. In this application note, Wagyu brand indicators (e.g., by the pedigree or the farm) were explored using statistical analyses.

1. Introduction

Wagyu beef is limited to four types of domestic beef produced in Japan: the black, the brown, the polled, and the shorthorn. There is a difference in meat quality among the four types. The black is small and marbled with much fat, while the brown has a good balance between fat crossings and lean meat. The polled is chewy, and the shorthorn is lean with many proteins.

Wagyu beef is not only consumed in Japan but is also popular overseas as a luxury food. In the past 10 years, the export volume has increased approximately 9 times and is expected to continue increasing (Fig. 1).

However, the definition of Wagyu is different in each country. For example, in the United States, which is the largest export destination, the definition of Wagyu is not a purebred individual.

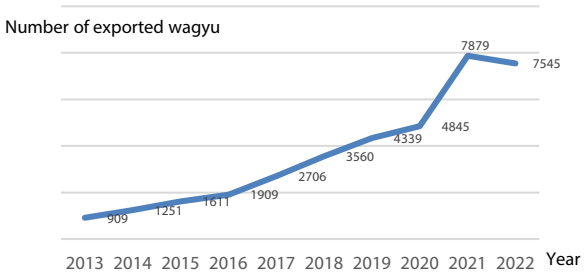


Fig. 1 Export volume of wagyu beef
(Source: ministry of finance trade statistics [1])

It is possible to sell a cow with more than 50 % of the genes derived from Wagyu as Wagyu. According to the American Wagyu Association, there are currently about 40000 Wagyu cattle raised in Texas, New Mexico, Oregon, Idaho, and other states. Still, less than 5000 of them are purebred due to the progress of crossbreeding with Angus cattle[2]. For purebreds to compete with low-unit crossbreds in overseas markets, one of the measures to be taken is establishing brands based on prefecture, production area, and pedigree.

Tottori Wagyu, a Japanese Black breed, won first place in Japan in the beef herd in Ward 7 at the 11 National Wagyu Achievement Association, a national competition held every 5 years to promote local brands. Tottori Wagyu is known for its high content of oleic acid, an unsaturated fatty acid, low melting point of fat, and good meltdown in the mouth. It also won the first prize in the beef section at the joint event held in fiscal 1966.

1 Tottori Prefectural Animal Husbandry Experiment Station
2 Shimadzu Corporation
3 CEM Japan Corporation
4 Soma Optics, Ltd.

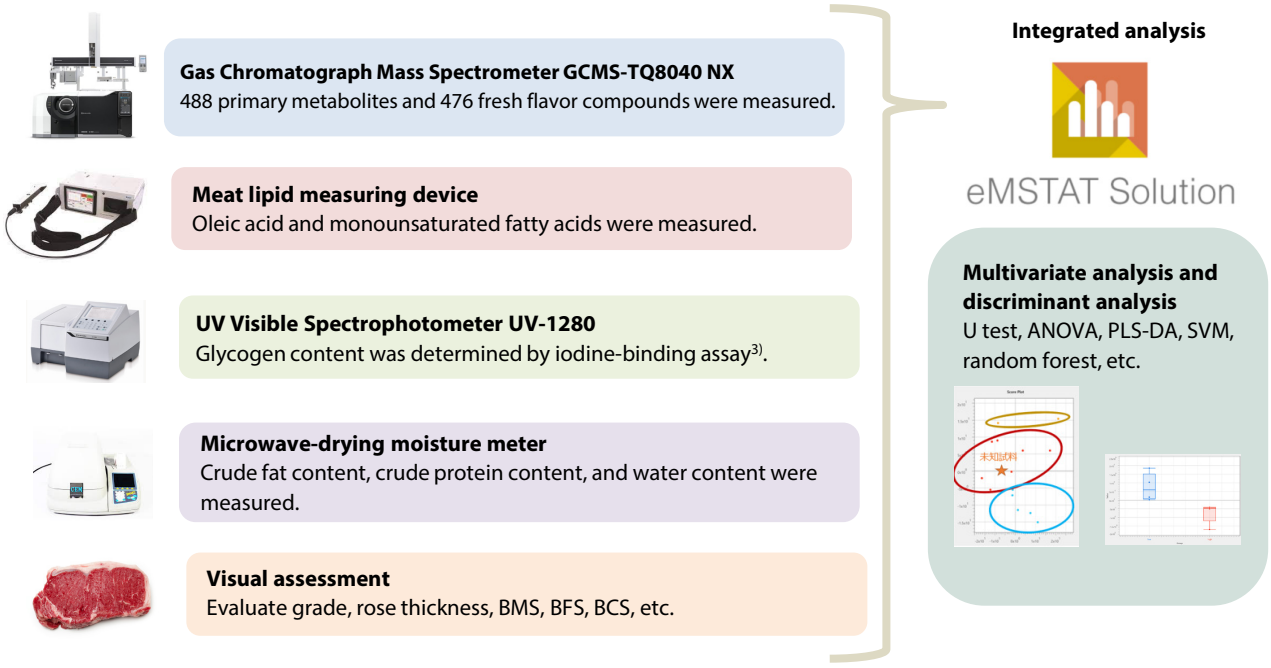


Fig. 2 Instrument and method used for the multi-faceted evaluation

Therefore, in this application, 61 rare and valuable Tottori Wagyu beef cattle, which are shipped only around 1900 head per year (0.7 % of Japan), were evaluated using a gas chromatograph mass spectrometer (GCMS-TQ8040 NX), an ultraviolet/visible spectrophotometer (UV-1280), a meat lipid measuring device (Soma Optics, Ltd.), and a microwave-drying moisture meter (CEM Japan Co., Ltd.). In addition to the beef carcass rating (i.e., visual evaluation) conducted according to the Japan Meat Rating Association, eMSTAT Solution was used to search for ingredients that can serve as brand indicators by farmer and pedigree (Fig. 2).

2. Experiments

603 explanatory variables were obtained using the following 5 instruments and methods to evaluate beef samples.

1) 488 primary metabolites, including organic acids, sugars, nucleic acids, fatty acids, and amino acids, were measured using GCMS-TQ8040 NX and Smart Metabolites Database™ Ver. 2. The Smart Aroma Database™ and SPME Arrow were used to measure 476 fresh aroma components at 40 ˚C. 399 primary metabolites and 170 fresh aroma compounds were detected.

2) Oleic acid and monounsaturated fatty acids were measured by a meat lipid measuring device. The muscle fat was measured on the cut surface between the sixth and seventh ribs of the carcass according to a standard set by the Japan Meat Rating Association. Oleic acid is currently used as a brand indicator for Tottori Wagyu beef. Tottori Wagyu beef with an oleic acid content of 55 % or more and meets the brand requirements is certified as Tottori Wagyu Olein 55.

3) Glycogen content was measured by the iodine-binding assay[3] using UV-1280. Glycogen is one of the animal starches and is tasteless and odorless. However, it is known that glycogen decomposes into sugars during the ripening process and is involved in the strength of Japanese beef's umami. Sensory evaluation tests conducted pervious to this study have confirmed a high correlation with the strength of the umami[4].

4) Crude fat content, crude protein content, and water content were measured using a microwave-drying moisture meter. The crude fat content is a predicted value based on the measured water content, but it is confirmed to be highly accurate compared to the conventional method. Water content is used to correct glycogen levels after water correction.

5) Graders of the Japan Meat Rating Association visually assessed beef color (Beef Color Standard (BCS)), degree of fat hybridization (Beef Marbling Standard (BMS)), and beef fat color (Beef Fat Standard (BFS)), and assessed loin core area, rose thickness, subcutaneous fat thickness, yield grade, and meat quality grade.

3. ANOVA analysis of differences between five fattening farms

Even in the same production area, fattening farmers differ in fattening techniques such as raw cattle selection ability (bloodline and qualities), rearing density, feed mass, and feeding method, and it is known that the characteristics of beef produced differ[5]. ANOVA (analysis of variance) included in eMSTAT Solution was conducted to detect differences among five fattening farms. Since the beef samples compared here were from the same region, 492 of the 603 test items (e.g., glycogen water correction values) showed no statistical difference, and the remaining 111 items (approximately 18 %) showed a statistically significant difference. (Fig. 3, Fig. 4).

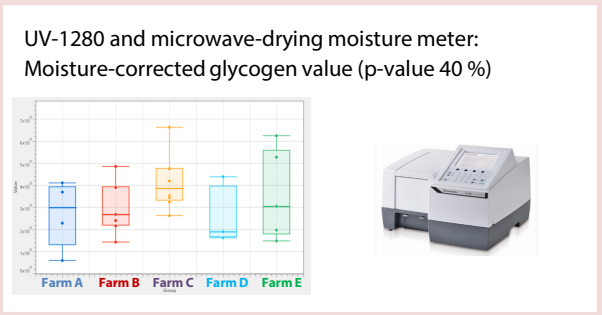


Fig. 3 Test items with no statistically significant differences (box-and-whisker plot)

Fig. 5 shows an example of a test item with a statistically significant difference. Glutamate, an amino acid, is known as a delicious ingredient in foods, but it also plays a vital role as an energy source. Oleic acid is also known as a component involved in the quality of mucus. The farmer C produces beef with higher glutamic acid and oleic acid content than the other fattening farmers. Linalool, which plays an important role as a fresh flavor component, was highly detected in fattening farmers B, D, and E. The farmer A had the highest rose thickness. In this way, characteristic items in each farmer were identified.

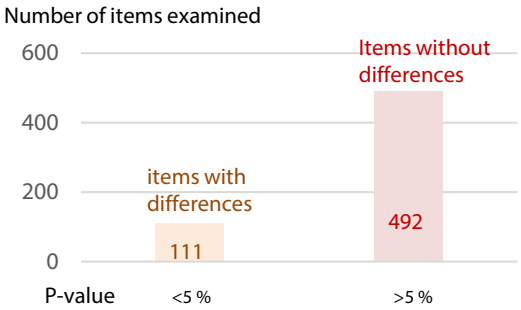
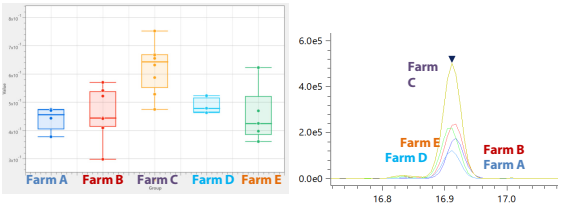
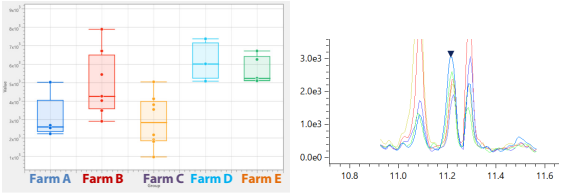


Fig. 4 Summary of p-value results from ANOVA analysis

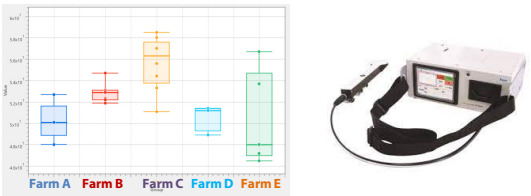
GC/MS liquid injection:
Glutamate (p-value 0.4 %)



GC/MS SPME:
Floral fragrance Linalool (p-value 0.3 %)



Meat lipid measuring device:
Oleic acid (p-value 0.3 %)



Visual Assessment:
Rose thickness (p-value 0.3 %)

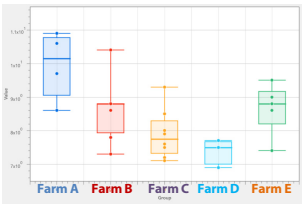


Fig. 5 Test items with statistically significant differences (p-value ≤ 5 %) (box-and-whisker plot)

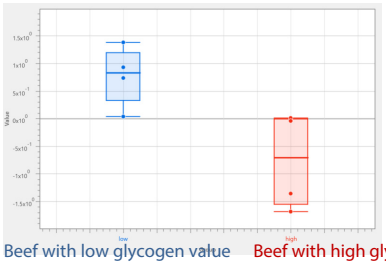
4. Evaluation of glycogen-derived components by U-test

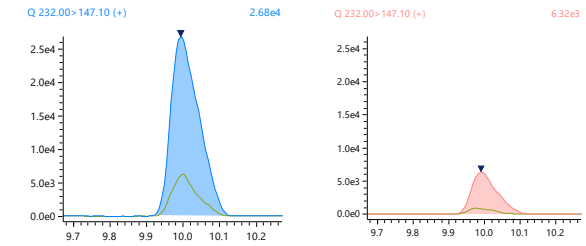
Glycogen is a polymer of many α-D-glucose molecules polymerized by glycosidic bonds, also called animal starch. The glycogen in beef is broken down by anaerobic metabolism after slaughter to form lactic acid, which lowers the pH of beef. Beef with degraded glycogen and low pH has low water retention and a hard texture.

To investigate the relationship between glycogen content after water correction and primary metabolites and fresh flavor compounds, beef with high (>6.5) and beef with low (<1.5) glycogen contents after water correction were compared in n=4 each using the U-test.

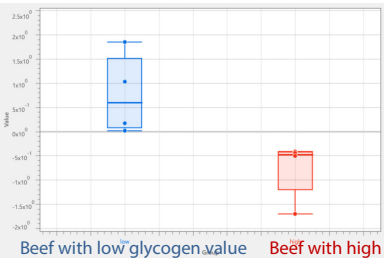
The U-test value for lactate was calculated to be 0.69, and no statistical difference could be confirmed. However, a statistically significant difference was confirmed for pyruvate, which is also a breakdown product of glycogen (Fig. 6).

Primary metabolite: pyruvate (U-test value 0.029)





Fresh flavor compound: 2-Acetylpyridine (U-test value 0.029)



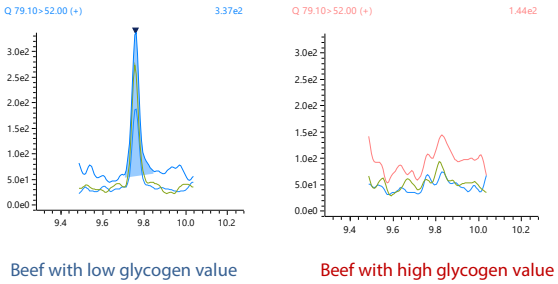


Fig. 6 U-test results of glycogen-derived compounds (Box-and-whisker plots and MRM chromatograms)

In addition, a statistically significant difference was observed in the flavor compound 2-acetylpyridine, produced by glucose which is a breakdown product of proline and glycogen (Fig. 6).

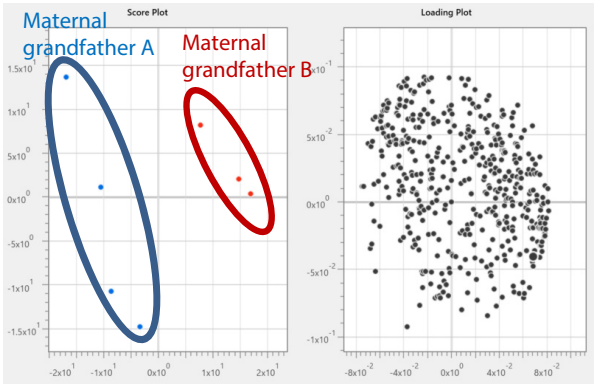
Both pyruvate and 2-acetylpyridine were highly detected in beef with low glycogen content, suggesting that glycogen degradation was advanced or that the original glycogen content was low.

5. Characterization of two pedigrees by PLS-DA

Two groups of samples (n=4 and n=3) with the same father and different maternal grandfathers were analyzed by PLS-DA (Fig. 7). PLS-DA is a statistical method used to compare two groups. While PCA maximizes only the variance of the data, PLS-DA maximizes the degree of association between the objective variable and the data, so even a sample group that PCA cannot separate may be separated with PLS-DA.

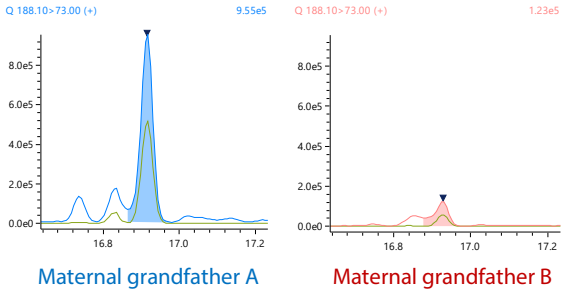
Six items (e.g., hypotaurine) were characteristic of the maternal grandfather A, while 32 items (e.g., 2-ethylfuran) that were characteristic of the maternal grandfather B.

The father is the same.



Example of tested items with high content in maternal grandfather A group

Umami compound: Hypotaurine [6]



Example of tested items with high content in maternal grandfather B group

sweet-smelling 2-ethylfuran

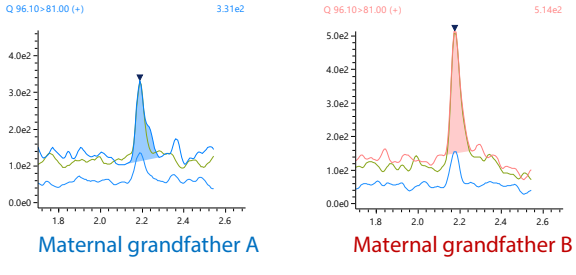


Fig. 7 Pedigree characterization using PLS-DA

6. Characterization of two pedigrees by PLS-DA

Beef is graded according to yield grade and meat quality grade. Yield grades are divided into three grades: A, B, and C. The higher the yield standard value (Value calculated from loin core area, rose thickness, etc.), the higher the grade (A>B>C). Meat quality is also divided into five grades based on visual evaluation of the degree of marbling, color, tightness, and texture of the meat.

The 56 samples graded as yield grade A were divided into 45 samples with meat quality grade 5 and 11 with meat quality grade 4, and a regression model was created using a support vector machine (SVM). When the two unknown samples not used in creating the discriminant model were fed into the model, both were determined to be the meat correct grades with a score of 100 (Fig. 8).

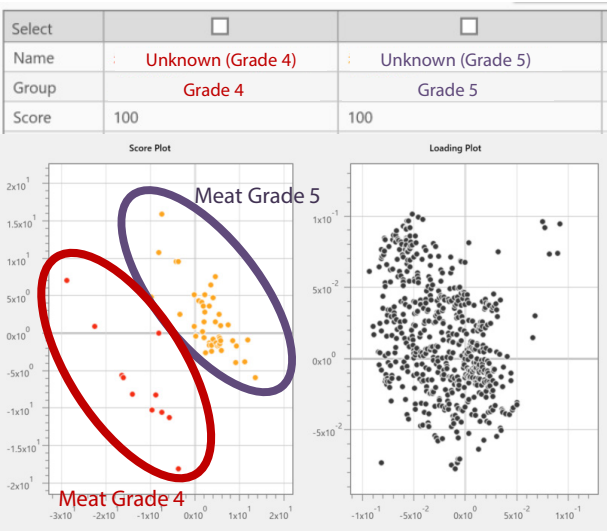


Fig. 8 Meat quality classification of unknown samples by SVM regression model

7. Farmer discrimination model by random forest

A random forest (RF) discriminant model was created using 13 samples from 3 farms producing A5 grade beef.

While SVM is used to discriminate between two groups, Random Forest is a statistical method that can discriminate between two or more groups. Random forest is a method that avoids overlearning and enables robust learning and evaluation even when there are many explanatory variables (number of test items) in this experiment. Using the created discriminant model, the unknown sample (the correct answer is Farmer 2) was discriminated, and a high score (70) was obtained for Farmer 2 (Fig. 10). The discriminant analysis utilized compounds with low p-values, such as linoleic acid (Fig. 9, 11):

Though the area values of the primary metabolites used in the discriminant analysis were corrected with an internal standard, the area values obtained in the chromatogram are shown in the box-and-whisker plot for illustration (Fig. 11).

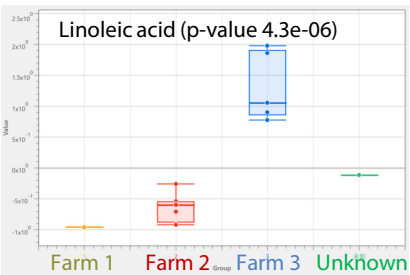
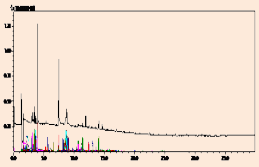
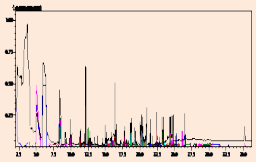


Fig. 9 Examples of compounds used in the RF regression model

Fresh aroma compounds of the unknown sample
Scan/MRM Simultaneous Measurement Chromatogram



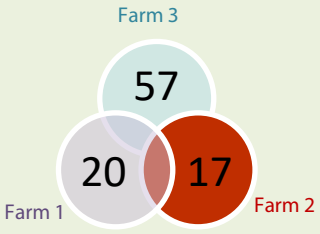
Primary metabolites of the unknown sample
Scan/MRM Simultaneous Measurement Chromatogram



UV, microwave-drying moisture meter, meat lipid measuring device, and visual evaluation for the unknown sample

Random forest discriminant model

Total of about 600 inspection items



The number of items that are characteristically high in each farmer (ANOVA p-value less than 5 %) are as follows.
Farm 1: 20
Farm 2: 17
Farm 3: 57

Select	☐
Name	Unknown (Farmer 2)
Group	Farmer 2
Score	70

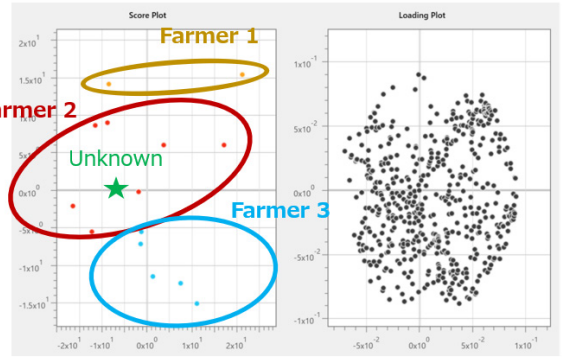
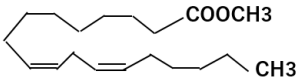
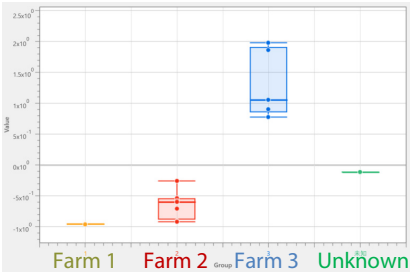


Fig. 10 Farmer identification results of unknown samples by RF regression model

Primary metabolite
Cholesterol-lowering linoleic acid (p value 4.3e-06)





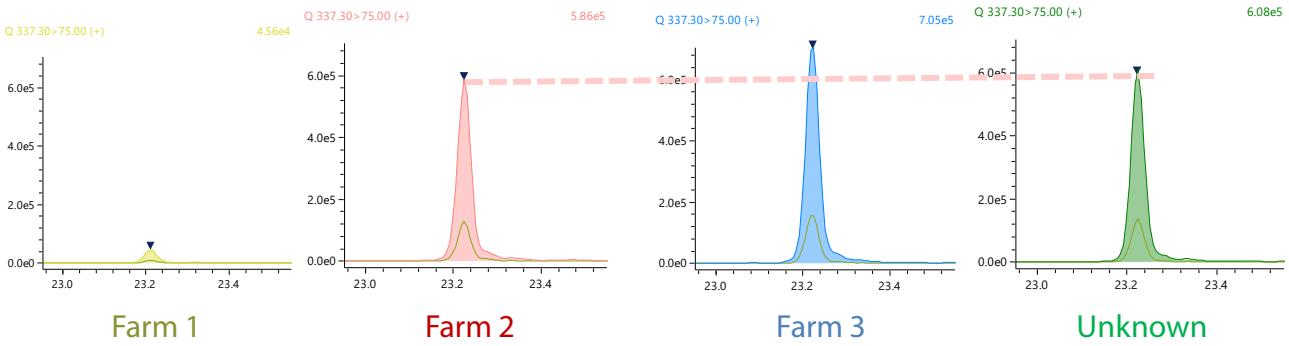


Fig. 11 Examples of useful compounds for discriminating unknown samples by RF regression model

8. Conclusion

In this report, 61 beef samples of Tottori Wagyu beef were evaluated using a gas chromatograph mass spectrometer (GCMS-TQ8040 NX), an ultraviolet/visible spectrophotometer (UV-1280), a meat lipid measuring device, a microwave-drying moisture meter, and visual evaluation, and compounds that could serve as brand indicators were searched. Based on the 603 items, eMSTAT Solution performed an integrated analysis using U-test, ANOVA, PLS-DA, SVM, and random forest. As a result, items that differed by pedigree or farmer were identified.

<References>

1) 2023年5月の輸出実績（概要） [Export Results for May 2023 (Overview)], accessed on July 18th, 2023

2) 和牛大国 米国で続く「和牛」人気、50年で独自進化？　すし・和食と違う「流行の秘訣」 [Wagyu beef continues to be popular in the U.S., but has evolved independently since 50? "Fashion secret" that is different from sushi and Japanese food accessed on July 18th, 2023

3) Dreiling CE, Brown DE, Casale L, Kelly L. 1987. Muscle glycogen : Comparison of iodine binding and enzyme digestion assays and application to meat samples. Meat Science 20, 167-177

4) 鳥取県和牛振興計画 [Tottori Wagyu Beef Promotion Program], accessed on July 18th, 2023

5) 鳥取県水産部＞とっとりの農業＞畜産試験場＞試験研究結果＞肥育 [Tottori Prefectural Fisheries Department > Totori Agriculture > Livestock Experiment Station > Results of Study > Fattening], accessed on July 18th, 2023

6) Sensory Properties and Main Differential Metabolites Influencing the Taste Quality of Dry-Cured Beef during Processing, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8870990/

First Edition: Mar. 2024

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/