APPLICATION OF MASS SPECTROMETRY FOR DETERMINING THE GEOGRAPHIC PRODUCTION AREA OF WAGYU BEEF

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I. INTRODUCTION

Projected global population growth demands increased livestock production by 2050. Sustainable food production systems are essential, but enhancing productivity in local environments is difficult. Efficient cross-border trade can help reduce food-production challenges. International law requires tracking and certification of food ingredients and their processing regions. The EU has laws Protected Designation of Origin (PDO) to protect product names associated with specific regions [1]. Protected Geographical Indication (PGI) systems protect farmers from imitation and unfair competition (Figure 1). Japanese Black cattle beef (Japanese Wagyu) is attracting attention for its unique aroma and marbling [2]. GI tags are registered for Japanese Wagyu from specific Japanese prefectures. Genetic analysis can identify animal breeds but is not perfect for similar

Figure 1. Japan's GI system was launched in 2015 to protect the names of traditional agricultural, forestry, and fishery products.

DNA sequences. Here, we focused on the origin discrimination of Wagyu beef and analyzed the nutritional components of Japanese Wagyu, Hybrid Wagyu, and Australian Wagyu beef using mass spectrometry (MS) [3].
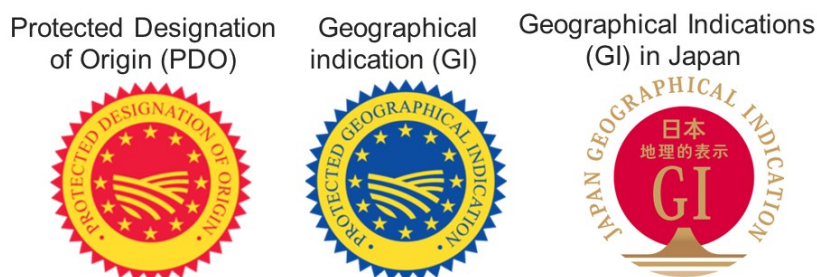
II. MATERIALS AND METHODS

1. Sample Collection

Japanese Wagyu, Hybrid Wagyu (a crossbreed of Angus and Wagyu born in Australia, transported to Japan as a calf and raised for 16 months in Japan), Australian Wagyu (a crossbreed of Angus and Wagyu), and New Zealand Wagyu beef were purchased commercially through a trading company.

2. Analysis of the Fatty Acid and triacylglycerides

The total lipid fraction was dried with nitrogen gas, redissolved in methanol, and subjected to liquid chromatography–tandem MS performed using DIONEX UtiMate 3000 with an L-column3 C18 metal-free column and Q Exactive Plus (Thermo Fisher Scientific) [3]. GC-2010 Plus (Shimadzu, Kyoto, Japan) determined fatty acid composition using a TC-70 capillary column after methyl esterification [4]. For the triacylglycerides analysis, samples dissolved in isopropyl alcohol were subjected to High-performance liquid chromatography (HPLC) using an Agilent Technologies 1260 Infinity equipped with Poroshell 120 EC-C18 LC column following a previously described method [3].

3. Multielement Analysis

Defatted samples were prepared by removing the lipid using organic solvent and washing with hexane. Next, after nitric acid digestion, the samples were digested until the organic compounds with 70% perchloric acid followed a previously described method [3]. We used different analytical instruments

depending on the element characteristics with inductively coupled plasma(ICP)–MS (Varian 820MS, Agilent Technologies) and ICP–emission spectrometry (Varian 725-ES, Agilent Technologies).

4. *Statistical Analyses*

Statistical processing, such as log transformation and scaling, and multivariate analysis (OPLS-DA and PCA) were performed using SIMCA 14.1 software (Infocom, Tokyo, Japan).

III.   RESULTS

To distinguish between the geographic origins of Wagyu beef via omics analysis, we compared the differences in the nutritional composition of Wagyu beef from different countries using MS. We identified 128 different triacylglycerides using a quadrupole Orbitrap MS system. Next, we compared the triacylglycerides content of Japanese, Hybrid, and Australian Wagyu beef using conventional HPLC. Triolein (C18:1/C18:1/C18:1) was significantly more abundant in Japanese Wagyu beef (Data not shown).

Next, we tried elemental analysis using ICP–MS. Japanese Wagyu beef contained higher levels of molybdenum and cadmium, whereas Australian Wagyu beef contained significantly more rubidium, cesium, and lithium (Figure 2).

IV.   CONCLUSION

These results of omics analyses revealed that the nutritional composition of Wagyu cattle differed with the country of production. This study suggests that combining multiple omics analyses enables the discrimination of the origin of genetically similar Wagyu cattle.

Figure 2. Comparison of Wagyu beef by elemental analysis. (**a**) The score plot of orthogonal least-square discriminant analysis reveals the relationships among Wagyu beef types. (**b**) Loading plot presenting the relationship between elements and each Wagyu type). (**c**) Box plot of the exclusive median and all plots. The cross marks indicate the mean (n = 5). Significant differences are presented as follows: * $p < 0.05$ and ** $p < 0.01$ (Tukey's test).

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4. Ueda, S., Sasaki, R., Nakabayashi, R., Yamanoue, M., Sirai, Y. & Iwamoto, E. (2021). Exploring the Lipids Involved in the Formation of Characteristic Lactones in Japa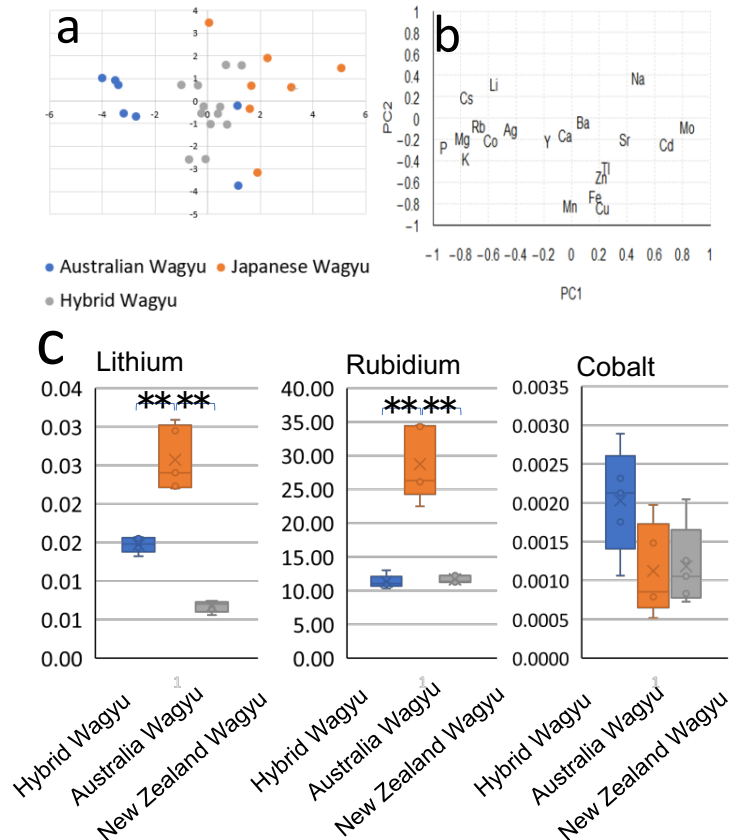nese Black Cattle. Metabolites 11: 203.