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Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle

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ABSTRACT

Analysis of the quality of highly marbled beef during dry aging for 60 days after slaughter showed that the changes in some qualities differed from those of conventional meat.
The tenderness of these meats did not change during aging for 50 days but then gradually increased until day 60. The juiciness of these meats, as determined by sensory evaluation, did not change during aging for 60 days, except for a decrease on day 20. The *umami* intensity of these meats in the sensory evaluation and the value calculated by Glu and IMP quantification were highest on day 40. This high *umami* intensity was induced by the synergistic effect of *umami* compounds such as Glu and IMP. These results for tenderness, juiciness, *umami* intensity, and flavor intensity suggested that the best duration of dry aging for highly marbled beef was 40 days.

1. Introduction

Marbled beef from Japanese black cattle is very palatable and has recently become very popular, both in Japan and overseas. It is more tender than conventional lean beef (without marbling meat of around10% fat content) from Holstein cattle and has a strong, sweet aroma (Matsuishi et al., 2004). It is well known that beef should generally be stored for about 2 to 3 weeks at 2 to 4 °C to improve its tenderness and flavor after the post-slaughter rigor mortis has subsided (Campbell, Hunt, Levis, & Chambers, 2001, Dixon et al., 2012; Nishimura, Rhue, Okitani, & Kato, 1988, Shimada, Watanuki, Tanisawa, & Hatae, 1992). However, highly marbled beef is sometimes stored longer, under specific regulated conditions, than conventional beef. For example, one brand of marbled beef, Tajima beef, is conditioned for several weeks at about 1 °C and 80% humidity, with an air stream over the meat surface. Such regulated conditioning improves the quality of this highly marbled beef.

Although there have been many reports of the changes in the quality of lean meat over short periods of 2 to 3 weeks, there have been few reports of the changes in quality of marbled beef during conditioning for long periods. The tenderness of beef is improved by postmortem aging in vacuum packaging (i.e. by wet aging; Brewer & Novakofski, 2008, Jones, Jeremiah, Tong, Lutz, & Robertson, 1991, Smith, Culp, & Carpenter, 1978). In wet aging for short periods, meat toughening from rigor mortis after slaughter has been improved by the actions of calpain, cathepsins B, D, and L (Okitani & Fujimaki, 1968, Okitani, Nakamura, & Fujimaki, 1968; Okitani, Otsuka, Sugitani, & Fujimaki, 1974; Okitani et al., 1988), or calcium (Takahashi, 1992). In the case of flavor improvement, Nishimura et al. (1988) reported that the free amino acid (FAA) content in round from Holstein cattle increased during postmortem aging from 4 to 12 days after slaughter. These increases were shown to be caused by the actions of aminopeptidases C and H. Moreover, Shimada et al. (1992) showed that the tenderness of lean meat from Holsteins improved for 14 days after slaughter. On the other hand, taste was not improved during aging, despite the increase in FAA content.

There have been reports of the improvement of meat quality during long-term wet or dry aging. Campbell et al. (2001) reported that the tenderness, flavor intensity, and juiciness of lean beef stored for 16 or 21 days by dry aging were higher than those of beef stored for a few days. The softness of lean beef has been improved by wet aging for 28 days (Dixon et al., 2012). Nishimura et al. (1988) have reported that the intramuscular connective tissue content decreases progressively for 28 days after slaughter. Furthermore, Yanagihara, Yano, Nakamura, Nakai, and Tanabe (1995) showed in meat from Holsteins that qualities such as taste and tenderness improved during postmortem aging for 32 to 56 days; the taste worsened with further aging. Despite these studies, there are still few reports on quality improvement of highly marbled beef during aging.

Here, we examined the changes in taste compounds, breaking properties, and sensory attributes of highly marbled beef during dry aging

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for 60 days. We also measured the activity of aminopeptidases involved in the increase in FAA content.

2. Materials and methods

2.1. Samples

On day 3 postmortem, we collected carcasses from five 30-month-old Tajima Japanese black cattle (Kobe, Japan) with Japan Meat Grading Association marbling scores ranging from 5 to 8 (according to grades A3 to A5, which in the US corresponds to meat that is extremely abundant in marbling). The five carcasses were selected and aged at 1 to 4 °C and a relative humidity of 80% to 90% until 60 days after slaughter. On days 4, 11, 20, 30, 40, 50, and 60 postmortem during aging, a single, 5.0-cm-thick *longissimus thoracis* steak was taken from the right side of each carcass at a level corresponding the 7th to 13th ribs (US National Association of Meat Purveyors [NAMP] items 174 and 103). Steaks were transported under refrigeration (at 2 °C) to the Japan Women's University within 24 h after being cut, without being vacuum packed. After removal of the surfaces of these steaks, they were cut into five slices (1 cm thick) with a Ritter Multischneider meat slicing machine (E26 Vario Perfekt Diagonal, Berlin, Germany). One slice was used immediately for analysis, and the remaining four slices were vacuum packed in an air-impermeable plastic bag (made from polypropylene–polyamide nylon; FLAME NUOVA, Milano, Italy) at a pressure of 79 kPa (Magic Vac Alice V952S, Dairei, Tokyo, Japan), frozen, and stored at −40 °C until sensory evaluation and evaluation of stress–strain properties. Analyses other than the sensory evaluation and texture measurement were performed immediately after the samples arrived at the university.

2.2. Chemical analysis

2.2.1. Measurement of pH, moisture and crude fat content

Ribeye meat 1 cm thick was minced with a Bamix food processor (BM0101, Mettlen, Switzerland) at 17,000 rpm for 1 min. A pH meter (IQ Scientific Instruments IQ170, Hach, Loveland, CO, US) was inserted into about 50 to 60 g of the minced meat to measure the pH. The moisture content of the *longissimus thoracis* muscle was measured by weighing minced muscle before and after it had been heated at 105 °C for 180 min (DRA330 DA ELWCTRIC DRYING OVEN: ADVANTEC, Tokyo, Japan).

After moisture content measurement, the crude fat content was measured by using the Soxhlet extraction method after mincing and heating as described above. The crude fat content was measured by using the *Official methods of analysis* (Association of Official Analytical Chemists (AOAC), 1990).

2.2.2. 5′-IMP measurement

The method reported by Suzuki et al. (1994) was used in the following experiment. Ten grams of raw minced meat was homogenized with 25 mL of 1 M $HClO_4$ (Bamix BM0101 blender, Mettlen, Switzerland) for 1 min. After the homogenate had been centrifuged at 11,500 ×*g* for 10 min at 4 °C, the supernatant was passed through a filter paper (Advantec 5B; Toyo, Tokyo, Japan). Its pH was adjusted to 6.5 to 6.8 with 1 or 5 M KOH and 1 M HCl, and it was left overnight at 4 °C. The supernatant was then passed through a membrane filter (0.45 μm, Advantec, Toyo) and diluted 10 times with distilled water to determine the 5′-IMP (inosine monophosphate) content by using HPLC (Shimadzu SPD-10AV UV–VIS Detector with LC-10AD pump, Kyoto, Japan) with a column (Senshu Pak PEGASIL-B ODS 4.6 φ × 250 mm, Tokyo, Japan) equilibrated with 20 mM phosphoric acid–22 mM diethylaminoethanol. The flow rate was 1.0 mL/min, and the IMP was detected at a wavelength of 250 nm.

2.2.3. Free amino acid measurement

The method reported by Nishimura et al. (1988) was used in the following experiment. Ten grams of raw minced meat was homogenized with 25 mL of distilled water for 1 min. The homogenate was centrifuged at 11,500 ×*g* for 10 min at 4 °C and the supernatant was collected. The supernatant was then percolated through a filter paper (Advantec 5B; Toyo). After the proteins had been removed from this filtrate by the addition of trichloroacetic acid (5% final concentration) and the filtrate centrifuged as described above, the supernatant was passed through a membrane filter (0.45 μm, Advantec; Toyo) and analyzed for free amino acids with an amino acid analyzer (Jasco, Tokyo, Japan LC-NETII/ADC Analyzer).

2.2.4. Assay of aminopeptidase C and H activity

The method reported by Nishimura et al. (1990, 1991, 1994) was used in the following experiment. Ten grams of raw minced meat was homogenized with 30 mL of 40 mM Tris–HCL (pH 7.2) (Bamix BM0101) for 1 min. The homogenate was centrifuged at 11,500 ×*g* for 10 min at 4 °C.

The protein assay was performed by the method of Bradford (1976), using a protein assay kit (Bio Rad, CA, USA) with bovine serum albumin as the standard (absorbance at a wavelength of 595 nm).

Enzyme activity against the β-naphthylamide derivatives of Glu-NA and Leu-NA was measured. After 0.2 mL of enzyme solution had been incubated at 37 °C for 5 to 60 min with 0.1 mL of 1 mM Glu-NA and Leu-NA in 100 mM Tris chloride (pH 7.2) containing 2 mM DTT, we used 0.4 mL of 0.23 N HCL in ethanol and 0.4 mL of 0.06% *p*-dimethylaminocinnamaldehyde in ethanol to halt the enzyme reaction. The redness that developed was measured at 540 nm, and the β-naphthylamines released from Glu-NA and Leu-NA were measured.

The measurement was performed four times, and enzyme activity was expressed as the average of the four measurements.

2.2.4.1. Sensory evaluation and measurement of cooking loss. The frozen steaks were moved to a refrigerator, where they were thawed at 2 °C for 24 h. They were then taken out of the refrigerator, left at room temperature for 0.5 h, and grilled on a hot plate (Zojirushi Corporation, Osaka, Japan) preheated to 200 °C. One side of the steak was grilled for 1 min and the other side was grilled for 1.5 min, so that the internal temperature reached 60 ± 1 °C (AD-5604 thermocouple, AND Tokyo, Japan). We used these conditions in accordance with a report by Iida, Horie, and Nishimura (2014).

Cooking loss of the steaks (around 150 to 180 g weight) was measured by weighing before cooking and again 5 min after removal from the hot plate. The following equation was used: Cooking loss (%) = (weight before cooking (g) − weight after cooking (g)) / weight before cooking (g) × 100. The cooked meat was cut parallel to the muscle fiber orientation to a size of 3 × 2 cm. Because 10 min was needed for the above-described sample preparation, the temperature of the steaks had by then dropped to 33 to 35 °C. The steaks were served to panelists for sensory evaluation at room temperature (20–23 °C). In consideration of the potential order effect in tasting, the order of the samples was randomized (except in the case of the day 4 samples). We prepared a total of 63 samples per animal from day 4 to day 60. Panelists therefore simultaneously compared different combinations of one of six slices (sampled on day 11, 20, 30, 40, 50, or 60) with the sample taken on day 4. Samples were offered under room (200 lx) and natural light.

The sensory attributes of marbled beef aged for 11, 20, 30, 40, 50, or 60 days were evaluated by nine panelists and compared with those of the samples taken on day 4 as the standard. Tenderness, juiciness, intensity of pleasant beef flavor (orthonasal aroma), and *umami* intensity were evaluated by using relative scores on an 8-point scale compared with the 4.5 score of the sample taken on day 4. The highest score of 8 was given to samples that were very tender and very juicy, with very strong beef flavor intensity and *umami* intensity. The lowest score of 1