Nondestructive measurement of intramuscular fat content of fresh beef meat by a hand-held magnetic resonance sensor

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**ABSTRACT**
To realize nondestructive in-situ quantification of intramuscular fat in fresh beef meat, a lightweight (5.0-kg) hand-held sensor that consists of a planar radio-frequency coil and a unilateral (i.e., single-sided) magnetic circuit was constructed as a subunit of a time-domain proton magnetic resonance (MR) scanner system. The MR scanner based on proton relaxometry enables the nondestructive quantification of the fat content of meat by using the difference in the spin-spin relaxation times (T2) of water molecules in muscle and of fat molecules. The investigation depth of the sensor unit was 7 mm, which is sufficient to probe the meat beneath a subcutaneous fat layer less than a few millimeters thick. The scanner was successfully applied in a laboratory at a meat temperature of 30.5°C to quantify the fat content at 30 locations in fresh beef samples. The required measurement time was 22 s for each location. Reasonable agreement with a root-mean-square error as small as 5.4 wt% was obtained for fat quantification compared with the conventional destructive food analysis (Soxhlet extraction method). Thus, the portable MR scanner with a hand-held sensor unit is a promising nondestructive and noninvasive tool for the in-situ fat quantification of fresh beef (e.g., carcasses) with thin subcutaneous fat layers at meat processing factories.

**INTRODUCTION**

Intramuscular fat is an important beef meat quality, and it affects consumers’ sense of taste and ultimately determines price.\(^{[1-6]}\) Thus, a nondestructive mapping technique\(^{[7-11]}\) for the fat content of fresh beef using lightweight hand-held sensors is needed to accurately assess the value and price of large meat bodies (e.g., carcasses) at meat processing facilities and food factories. A portable surface scanner that employs low-field unilateral (i.e., single-sided) magnetic resonance (MR) relaxometry\(^{[12-16]}\) is one of the most promising techniques for the in-situ nondestructive quantification of the fat content of foods.\(^{[17-22]}\) The advantages of unilateral MR scanning over other nondestructive methods, such as near-infrared spectroscopy, microwave attenuation, ultrasound imaging, and electrical impedance,\(^{[23-26]}\) are that (i) the sensed region is compact and its location is accurately known and (ii) the undesirable effects of packages, bones, skin, and subcutaneous fat layers can be eliminated if the investigation depth (distance from the sensor to the center of the sensed region) is carefully designed. Petrov et al.\(^{[27]}\) successfully applied a unilateral MR surface scanner with a permanent magnet (weighing approximately 5 kg)\(^{[28]}\) to the fat quantification of four ground beef samples (fat content of 7.95 to 24.3 wt%) at room temperature.

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One of the two objectives of the present study\textsuperscript{[29]} is to extend the work by Petrov et al.\textsuperscript{[27]} as follows to evaluate the applicability of the low-field, time-domain unilateral MR scanner to the intramuscular fat quantification of beef samples. A lightweight hand-held sensor unit with a magnet weighing 3.7 kg was constructed. Its intended use is for the in-situ nondestructive fat mapping of meat blocks and beef carcasses at meat processing factories. Its investigation depth is 7 mm, which is sufficient to probe meat sections beneath subcutaneous fat layers less than a few millimeters thick. The detailed properties of the sensor unit, such as the spatial distribution of the magnetic field and MR sensitivity, were examined. An MR surface scanner system with the hand-held sensor unit was applied to the fat quantification of fresh beef meat samples in a laboratory. As many as 30 locations in meat samples with various fat contents (1.1 to 53.7 wt\%) were measured. Reasonable agreement in terms of fat content was found between the nondestructive MR measurements and destructive conventional food analysis.

Another objective of the present study is to obtain fundamental data on the temperature-dependent proton (\(^1\)H nuclei) relaxation of beef samples. Unilateral MR scanning of beef samples was performed at 30.5°C in the present study. The principle of the fat quantification by proton relaxometry is based on the detectable difference in the proton relaxation times for water molecules in muscle and for fat molecules.\textsuperscript{[22,30,31]} The spin-lattice relaxation time (\(T1\)) and the spin-spin relaxation time (\(T2\)) for such molecules can be sensitive to temperature.\textsuperscript{[31,32]} Thus, \(T1\) and \(T2\) for almost pure fat and lean meat samples were measured extensively at various temperatures (8.0 to 39.8°C) using a conventional bilateral MR apparatus, for which sample temperature control is much easier than that for the unilateral MR scanner. The \(T1\) and \(T2\) data obtained by the bilateral MR experiments were then evaluated to determine the applicability of the relaxometry-based MR fat quantification method to temperatures other than 30.5°C.

**MATERIALS AND METHODS**

This section consists of four subsections: *Unilateral sensor unit, Sample description, Unilateral MR scanning of meat samples, and Bilateral MR measurements of meat samples*. The property of the hand-held sensor unit constructed for this study, the feature and preparation of beef samples, the experimental procedure using the unilateral MR scanner, and that using the bilateral MR apparatus are described in the subsections, respectively.

**Unilateral sensor unit**

The hand-held MR sensor unit constructed in the present study is shown in Figure 1. It consists of a unilateral magnetic circuit (3.7 kg) and a double D-shaped planar radio-frequency (RF) coil with an aluminum tuning/matching (T/M) box with handles (1.3 kg). Thus, the total weight is 3.7 + 1.3 = 5.0 kg. Due to the unilateral magnet geometry,\textsuperscript{[12,13]} one side of the planar RF coil is exposed to free space (i.e., \(z > 0\) in Figure 1a), enabling the nondestructive surface scanning of large objects, such as a carcass.

The unilateral magnetic circuit employs Nd-Fe-B permanent magnets (Electronic Supplementary Material, Fig. ESM1). This is a copy of the magnetic circuit described in Utsuzawa and Fukushima.\textsuperscript{[33]} The magnetic circuit is axisymmetric and consists of an outer part (stacked ring magnets) and an inner part (center bar magnet). The outer and inner magnets, which have the same polarity, are magnetized in the \(z\)-direction as shown in Figure 1a to produce strong homogeneous magnetic fields (i.e., a “sweet spot”) far from their end surfaces. The spatial distribution of these magnetic fields measured by a Tesla meter is shown in Fig. ESM2.

The double D-shaped design\textsuperscript{[33,34]} was employed for the coil because the direction of the RF magnetic field oscillating at the Larmor frequency should be normal to that of the static magnetic field in the sensed region. As a result, the direction of the RF magnetic field is in the \(x\)-direction while that of the static field is in the \(z\)-direction. The details of the RF circuit are schematically shown in Fig. ESM3. The Larmor frequency tuning and 50 \(\Omega\) matching operation are performed by nonmagnetic trimmer capacitors. A fiber-reinforced plastic spacer (3 mm thick, Fig. ESM4a) was inserted between
the copper coil and the magnetic circuit. A transparent poly methyl methacrylate (PMMA) plate (1 mm thick) was placed on the RF coil (Fig. ESM4a) to mechanically protect the coil and electromagnetically decouple the coil and samples with various impedance values.

Although the exact shape of the sensed region of the unilateral MR sensor is generally rather irregular,\textsuperscript{35,36} this shape was approximated to be a simple cuboid in the present study. The dimensions and location of the cuboid as a sensed region of the sensor unit were measured using silicon rubber sheets\textsuperscript{32,31,37} before the beef measurements. The procedure and results are described in Fig. ESM4. As a result, the investigation depth (distance from the RF coil to the center of the sensed region) was 7 mm; the sensed region corresponds to the homogeneous region of the static magnetic field, and the dimensions of the sensed region as a cuboid were 14, 15, and 11 mm along the x-, y-, and z-axes, respectively. The RF coil and the magnetic circuit were carefully designed such that the layer within \( \approx 4 \) mm from the coil surface does not significantly contribute to the MR signals (Fig. ESM4c). Thus, MR signals from the subcutaneous fat layer less than a few millimeters thick could be eliminated, leaving those from the region away from the subcutaneous fat layer, which composed of only meat.

**Sample description**

A fresh round beef meat block (weight of approximately 3 kg) of a Japanese Black cow partly covered with a thin subcutaneous fat layer was purchased from a local store as a carcass analogue (Figure 2). The ID number of the cow was 1576954674, for which detailed information (e.g., date of birth and gender) is freely available from the National Livestock Breeding Center.\textsuperscript{38} An X-ray computed tomography (CT) image of the fatty meat block is shown in Figure 3, depicting the heterogeneous spatial distribution of intramuscular fat and muscle. The meat block was left in the laboratory overnight to allow it to equilibrate to ambient room temperature. Because the room temperature slightly fluctuated between 30 and 31°C, the meat temperature was approximated as 30.5°C. Then the four locations (\( L_A \) to \( L_D \), separated by 4 cm) marked in Figure 2a were scanned by the MR sensor (Figure 2b and ESM5). The unilateral MR scanner was developed for intramuscular fat mapping of a beef carcass immediately after slaughter. The temperature of a cooling carcass should be between that
of live cattle (approximately 40°C) and room temperature (typically 10 to 20°C). This is why the laboratory room temperature was maintained at 30 to 31°C with an air conditioner in the present study.

The number of measurement locations in Figure 2a is as small as four, and may be insufficient for statistically evaluating the performance of the MR scanner. Thus, additional samples, namely groups (i) and (ii) below, whose meat quality ranged broadly from lean meat to fatty meat, were prepared and measured by the same MR scanner. These additional samples were all boneless meat samples, typically 100 × 100 × 50 mm, large enough to completely encompass the sensed region of 14 × 15 × 11 mm. Each sample was vacuum-sealed in a thin plastic film package to prevent undesired water evaporation and fat alteration during the MR measurements. Proton relaxation times for expelled water (i.e., drips) are significantly different from those for water trapped within the intact myofibrillar network,[39,40] suggesting that the use of ground meat samples[27] may lead to incorrect MR data interpretation and that intact meat, such as a carcass analogue, is needed. Thus, grinding and mincing were not applied to the meat samples during the MR measurements.

Figure 2. Photograph of a round meat block (weighing approximately 3 kg) measured in the present study. (a) Top view. Four MR scanning locations are marked along a dotted baseline (Lₐ to Lₜ) separated by 4 cm along the curved sample surface. The sample is partly covered with a thin layer of subcutaneous fat. An X-ray CT image taken along the dotted line is shown in Figure 3. (b) Side view with the sensor unit focused on location Lₜ. A wide-angle photograph of this scene is shown in Fig. ESM5. The RF shield cloth provides an electric ground via connection to the outer shield of the BNC cable at the position indicated by the yellow arrow (see also Fig. ESM3).

Figure 3. Two-dimensional X-ray CT image of the round meat block (shown in Figure 2) obtained by a medical CT scanner. The density affects the CT image gray scale; bright, gray, and black regions are muscle, fat, and gas (air), respectively. The slice thickness was 0.63 mm, and the acceleration voltage of the X-ray tube was 120 kV. The approximate locations of Lₐ to Lₜ in Fig. 2a and the corresponding sensed regions (11 × 15 mm) are marked by yellow dots and dotted rectangles, respectively.
Group i: Seven cattle (Japanese Black) were raised and slaughtered in a different research project (Shiba et al., unpublished work) under the approval of the Committee of Animal Experiments, Tohoku Agricultural Research Center, National Agriculture and Food Research Organization (NARO). Ten round fatty meat samples were taken from the cattle and vacuum-sealed. Three of the ten samples were relatively large, and thus, two locations were measured by the MR scanner in each of these samples. The setup for the MR measurement of the NARO samples is shown in Fig. ESM6a with the corresponding CT image (Fig. ESM6b).

Group ii: Thirteen fresh meat samples of beef raised in Japan (Japanese Black and crossbreed) and Australia (the breed information is not available) were purchased from local stores, and then vacuum-sealed in a thin plastic film package. The meat portions of the 13 samples were round and chuck flap.

Between sample groups (i) and (ii), 23 vacuum-sealed samples were prepared, and the fat content of a total of 26 locations was quantified by the unilateral MR scanner. According to the conventional Soxhlet extraction method, the fat content of the 26 locations ranged from 1.1 to 53.7 wt%, which is much broader than the previous study of four samples\textsuperscript{[27]} (i.e., 7.95 to 24.3 wt%).

Furthermore, four almost pure fat samples without muscle (e.g., subcutaneous fat) and three almost pure lean meat samples with only a small amount of fat, end members of marbled beef, were purchased in local stores. The food properties of the samples are listed in Table ESM1. The six samples (F1 to F3 and LM1 to LM3) were individually inserted into six small glass tubes (volume of 1 mL) for bilateral MR relaxometry at various temperatures (8.0 to 39.8°C); two relatively large (\(100 \times 100 \times 50 \) mm) vacuum-sealed samples (an almost pure fat sample F1 and an almost pure lean meat sample LM2) were also prepared for the unilateral MR experiments at 4.5 MHz and 30.5°C.

**Unilateral MR scanning of meat samples**

The transient transverse relaxation of protons in the beef samples was measured using the MR console system (MRTechnology Inc., Tsukuba, Japan) shown in Fig. ESM5. The proton Larmor frequency corresponding to the magnetic flux density in the sensed region (Fig. ESM2) was 4.5 MHz at the room temperature range of 30 to 31°C. The center (i.e., \(x = y = 0\)) of the RF coil shown in Figure 1 was placed on one of the four locations marked in Figure 2a and adjusted for T/M by the capacitors shown in Fig. ESM3 using the Smith chart displayed on a network analyzer, and then the transient proton relaxation was measured. The resultant quality factor Q for the RF coil after the T/M adjustment ranged from 28 to 29 depending on the measurement location.

A phase-alternated pair-stacking (PAPS) Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence\textsuperscript{[41]} was employed to acquire the proton transverse relaxation data. The CPMG sequence comprises a 90° RF pulse followed by many 180° RF pulses that are equally separated at a specific interval (i.e., echo spacing), and a series of decaying echo signals refocused at the midpoint of each interval is acquired as CPMG “train” data. This CPMG method was modified in the present study by employing the phase-cycling technique, PAPS, to reduce the undesirable ringing artifact.\textsuperscript{[22]} The parameters for the CPMG sequence were as follows. The duration of the 90° and 180° RF pulses was 0.06 ms, the echo spacing was 0.5 ms, the sequence repetition time was 2 s (i.e., the T1 full relaxation condition), and the number of echoes acquired was 1000. As for the number of stacked signals \(N_{stack}\), two values (10 and 18) were employed for the round block sample (Figure 2) and the 23 vacuum-sealed samples to evaluate the effect of \(N_{stack}\) on measurement accuracy. The number of dummy scans without the CPMG train data acquisition was unity. Thus, the required MR measurement time for each location was 2 s \(\times (10 + 1)\) times = 22 s for \(N_{stack} = 10\), and 2 s \(\times (18 + 1)\) times = 38 s for \(N_{stack} = 18\). As for the four locations in Figure 2a, MR scanning with \(N_{stack} = 18\) was also performed to preliminarily evaluate the effect of a very small number of stacked signals on the fat estimation accuracy.

A tent made of RF shield cloth (MS-PY*, Microwave Absorbers Inc., Tokyo, Japan)\textsuperscript{[22]} to fully cover the samples was not employed. Rather, the RF shield cloth was placed beneath the sample (Figure 2b and ESM5) because it would be very difficult to fully cover each large carcass body using a shield cloth.
at an actual food processing factory. The open structure of the RF coil, however, inevitably allows the environmental RF noise to contaminate the MR signal, yielding a low signal-to-noise ratio. Thus, the conductive RF shield cloth was connected to the outer shield of the BNC cable (i.e., electric ground) in the present study (Figure 2b) to cancel the environmental RF noise as much as possible.

The principle of the CPMG data processing for the fat quantification is described here based on T2 relaxometry. The raw time-series data obtained using the CPMG pulse sequence consisted of 1000 echo data points. The first four echoes were truncated and discarded because the initial echoes are inevitably transient and distorted due to the grossly inhomogeneous magnetic and RF fields. The RF coil quality factor Q is an important quantity to affect the magnitude of the CPMG signal. The Q value depends on the electromagnetic property of the sample placed on the RF coil. Actually, Q varied in the range of 28 to 29 among the measured beef samples. Because the magnitude of the CPMG signal increases with $Q^{0.5}$, raw CPMG signals were divided by $Q^{0.5}$ to quantitatively compare CPMG datasets obtained in cases with different Q values.

Although the target nuclei are the protons of hydrogen atoms in fat molecules, the acquired CPMG data are contaminated by MR signals from water molecules in muscle. Thus, it is necessary to distinguish the signals from the fat molecules from those from the water molecules. It was assumed that boneless fatty meat is a mechanical mixture of lean meat (water + protein) and fat. This assumption has been validated by previous studies on beef and tuna meat. It is difficult to obtain proton relaxation signals from protein macromolecules using a low-field, time-domain MR scanner with an echo spacing as long as 0.5 ms. Thus, after the Q-value correction mentioned above, the time-series data $f(t)$ are a mixture of proton relaxation signals from the two end members, fat and water, written as

$$f(t) = A_{\text{fat}} \exp(-t/T_{2,\text{fat}}) + A_{\text{lean}} \exp(-t/T_{2,\text{lean}})$$  \hspace{1cm} (1)

where $t$ is time; $A_{\text{fat}}$ and $A_{\text{lean}}$ are the MR signal amplitudes for fat molecules and water molecules in lean meat, respectively; and $T_{2,\text{fat}}$ and $T_{2,\text{lean}}$ are the T2 values for fat and lean meat, respectively.\(^{22,30,31}\) The values of $A_{\text{fat}}$ and $A_{\text{lean}}$ were determined from the bi-exponential model, Eq. (1), using the method of least squares.

The constants $T_{2,\text{fat}}$ and $T_{2,\text{lean}}$ essential for the fitting of $A_{\text{fat}}$ and $A_{\text{lean}}$ were determined by MR scanning under the same conditions (i.e., 4.5 MHz and 30.5°C) for an almost pure fat sample without muscle ($F_4$) and an almost pure lean meat sample (LM2), as listed in Table ESM1. The CPMG pulse parameters (e.g., repetition time, echo spacing, and RF pulse duration) were the same as those employed for the 30 locations in the meat block sample (Figure 2) and 23 vacuum-sealed samples (e.g., Fig. ESM6), but the number of stacked signals ($N_{\text{stack}}$) was increased to be 160 (neither 10 nor 18) to obtain more accurate T2 values. The conventional saturation recovery measurement\(^{13,44}\) with $N_{\text{stack}} = 100$ was also employed to measure the TI relaxation for the same fat and lean meat samples. The "summation of echoes"\(^{44}\) was calculated by stacking the signal intensity from the 4th to the 200th CPMG echo trains, and was plotted as a function of the recovery time of the nuclear magnetization. The three-parameter fitting method\(^{45}\) was applied to the recovery curve to obtain TI values.

After the MR measurements, a small piece of meat with dimensions of approximately 40, 40, and 20 mm (volume of 32 cm$^3$) along the x-, y-, and z-axes, respectively, was cut from each section using a knife, with care taken to completely include the sensed region ($14 \times 15 \times 11$ mm = 2.3 cm$^3$). The fat content of these meat pieces was then analyzed using a conventional food chemical analysis technique (Soxhlet extraction method) to obtain ground truth values. The analytical technique followed the official method determined by the Ministry of Education, Culture, Sports, Science and Technology, Japan.\(^{46}\) A sample volume of 32 cm$^3$, significantly larger than the sensed region of 2.3 cm$^3$, was needed for reliable food chemical analysis.
**Bilateral MR measurements of meat samples**

Unilateral MR scanning for the fat quantification was performed at 30.5°C in the present study. The principle of the fat quantification by proton relaxometry is based on the detectable difference in the proton relaxation times for water molecules in muscle and for fat molecules.\(^{[22,30,31]}\) The proton relaxation times, \(T1\) and \(T2\), for such molecules strongly depend on temperature.\(^{[31,32]}\) Thus, \(T1\) and \(T2\) for almost pure fat and lean meat samples were measured in a broad temperature range using a conventional bilateral MR apparatus, for which sample temperature control is much easier than that for the unilateral MR scanner.

The samples (\(F_1\) to \(F_3\) and \(LM_1\) to \(LM_3\)) listed in Table ESM1 were stored in a 1-mL glass tube and measured using a bilateral proton MR apparatus (NMS120, Bruker, Karlsruhe, Germany) at a Larmor frequency of 20 MHz (470 mT). A water-circulating temperature control unit was installed in the apparatus\(^{[47]}\) to enable relaxometry to be performed at various temperatures. The sample temperatures ranged from 8.0 to 39.5°C for \(F_1\), \(F_2\), \(LM_1\), and \(LM_2\) and from 8.7 to 39.8°C for \(F_3\) and \(LM_3\). The conventional inversion recovery method\(^{[13,44]}\) and the CPMG method were employed for the \(T1\) and \(T2\) relaxometry, respectively. The pulse parameters were as follows: the typical duration of the 90° and 180° RF pulses were 3.2 and 5.7 μs, respectively; the echo spacing was 0.8 ms with a single dummy echo inserted; the sequence repetition time was 4 s (i.e., the \(T1\) full relaxation condition); and \(N_{\text{stack}}\) was 8.

Because the seven samples in Table ESM1 are end members (almost pure fat or lean), a mono-exponential model (not bi-exponential model) assuming a single value of \(T1\) or \(T2\) was employed. The conventional three-parameter fitting method\(^{[45]}\) was applied to the inversion recovery data to obtain the \(T1\) value. For the CPMG time-series data \(f(t)\), the following mono-exponential model was fitted using the method of least squares to obtain the \(T2\) value:

\[
f(t) = A \exp(-t/T2) \quad (2)
\]

where \(A\) is \(A_{\text{fat}}\) and \(T2\) is \(T2_{\text{fat}}\) for the fat samples, and \(A\) is \(A_{\text{lean}}\) and \(T2\) is \(T2_{\text{lean}}\) for the lean meat samples.

**RESULTS**

**Bilateral MR measurements**

Examples of the raw data from bilateral MR measurements of meat samples at 20 MHz are shown in Fig. ESM7 for \(T1\) relaxometry and in Fig. ESM8 for \(T2\) relaxometry. The effects of the sample temperature on the MR signal\(^{[48]}\) were corrected by multiplying the signal intensity by the absolute temperature (high-temperature approximation). The degree of fitting using the mono-exponential model (not the bi-exponential model) is reasonable. This is a consequence of the sample properties (Table ESM1) being almost pure fat or pure lean meat. While the signal intensity extrapolated as \(t \rightarrow 0\) is almost temperature independent for the lean meat sample (Fig. ESM8d), it decreases with temperature for the fat sample (Fig. ESM8b). Probably this decrease is due to the partial solidification or crystallization of fat molecules.\(^{[49,50]}\)

The results of the bilateral 20-MHz experiments for the six samples are summarized in Figure 4 as an Arrhenius plot. The results of the conventional food analyses (e.g. Soxhlet extraction method) of the samples are listed in Table ESM1. Although there is a slight difference in composition, as noted in Table ESM1, the three fat samples (\(F_1\) to \(F_3\)) and the three lean meat samples (\(LM_1\) to \(LM_3\)) show almost the same respective \(T1\) and \(T2\) values. A strong temperature dependence of \(T1\) and \(T2\) is observed for the fat samples (\(F_1\) to \(F_3\)) as reported elsewhere.\(^{[51]}\) It should be noted that the crossover of the \(T2\) values for fat and those for lean meat occurred at approximately 15°C. This crossover is a remarkable property of beef meat when compared with tuna meat.\(^{[31,32]}\)
**Unilateral MR scanning**

Raw data from the unilateral MR measurement of the two end members (an almost pure fat sample F4 and an almost pure lean meat sample LM2) at 4.5 MHz and 30.5°C are shown in Fig. ESM9. A mono-exponential model reasonably fitted both the $T1$ relaxometry (Fig. ESM9a) and $T2$ relaxometry data (Fig. ESM9b). As a result, the $T1$ values were determined to be 194 ms and 278 ms for the fat and lean meat samples, respectively. These values ensure that the unilateral MR scanning experiments performed with a sequence repetition time of 2 s fell sufficiently under the $T1$ full relaxation condition. Equation (2) was successfully fitted to the CPMG data (Fig. ESM9b) to determine $T2_{fat}$ and $T2_{lean}$ to be 94 ms and 43 ms, respectively. These $T2$ values were employed in Eq. (1) throughout the CPMG data analysis for fat quantification in the present study. It should be noted that $T2_{fat}$ and $T2_{lean}$ are significantly different (=2-fold difference). This large difference ensures that the fat and lean meat contained in the sensed region can be differentiated with reasonable accuracy.

The $T1$ and $T2$ data measured at 4.5 MHz and 30.5°C are plotted in Figure 4. The $T2$ data at 4.5 MHz and 30.5°C agree well with those at 20 MHz and around 30°C. This is consistent with literature reporting $T2$ to be nearly independent of the Larmor frequency for biological samples.\(^{52,53}\) In contrast, the discrepancy is significant with respect to the $T1$ data between 4.5 and 20 MHz. This is probably due to the strong dependence of the $T1$ values for beef samples on the static magnetic field (i.e., Larmor frequency).\(^{22}\)

An example of the CPMG data obtained by the unilateral MR scanner (Fig. ESM5) is shown in Figure 5 for $N_{stack} = 10$ and in Fig. ESM10a for $N_{stack} = 18$. Although the signal-to-noise ratio is obviously lower for $N_{stack} = 10$ compared with that for $N_{stack} = 18$, the degree of fitting with Eq. (1) is
reasonable for both cases. The apparent decay rate for location $L_A$ falls between those for the pure fat and lean meat samples in Figure 5 and ESM10a, which is a reasonable consequence of the bi-exponential model, Eq. (1), where fatty meat (i.e., marbled beef) is a mechanical mixture of pure fat and lean meat.

Unknown quantities $A_{fat}$ and $A_{lean}$ for the 30 beef sample locations were determined by fitting using the bi-exponential model, Eq. (1), with known $T_2_{fat}$ and $T_2_{lean}$ values (i.e., 94 and 43 ms, respectively) to the CPMG relaxation data. The determined $A_{fat}$ values are plotted against the fat content values obtained by the conventional food analysis (i.e., Soxhlet extraction method) in Figure 6a for $N_{stack} = 10$ and in Fig. ESM10b for $N_{stack} = 18$. The 30 data points were fitted to a theoretical calibration curve written as:

$$A_{fat} = \frac{B_{fat} \rho_{lean} w_{fat}}{100 \rho_{fat} + (\rho_{lean} - \rho_{fat}) w_{fat}}$$  \hspace{1cm} (3)

where $B_{fat}$ is a constant; $w_{fat}$ is the weight fraction of fat (in wt%); and $\rho_{fat}$ and $\rho_{lean}$ are the bulk densities of fat and lean meat, respectively. In accordance with a previous study, $\rho_{fat}$ and $\rho_{lean}$ were taken to be 0.92 and 1.06 g/cm$^3$, respectively, in the present study.

The constant $B_{fat}$ in Eq. (3) was determined to be 8808 for Figures 6a and 15,874 for Fig. ESM10b by the method of least squares. Then, the vertical axes of Figure 6a and Fig. ESM10b were rewritten using the determined constant to obtain Figure 6b and ESM10c, respectively. The root-mean-square errors (RMSEs) for the 30 data points were 5.4 wt% for Figure 6b, and 4.8 wt% for Fig. ESM10c. The results for the four locations ($L_A$ to $L_D$) shown in Figure 2a are summarized in Table 1.

CPMG measurements for $N_{stack} = 4$ were performed only for the four locations in Figure 2a. An example of the raw CPMG data for $N_{stack} = 4$ is shown in Fig. ESM11a for location $L_A$. Equation (1) was fitted to the CPMG data to obtain $A_{fat}$ for $N_{stack} = 4$. Theoretically, the CPMG signal intensity for $N_{stack} = 4$ is as small as $4/10 = 40\%$ compared with that for $N_{stack} = 10$. Thus, the obtained $A_{fat}$ value for $N_{stack} = 4$ was multiplied by $10/4 = 2.5$, and the calibration curve in Figure 6a for $N_{stack} = 10$ was employed to convert the $A_{fat}$ value for $N_{stack} = 4$ into the $w_{fat}$ value. The results are summarized in Table 1, and plotted in Fig. ESM11b.
Figure 6. Summary of the unilateral MR measurements of meat samples for \( N_{\text{stack}} = 10 \). Four locations (L\(_A\) to L\(_D\)) in Fig. 2a and 26 locations of 23 vacuum-sealed samples (e.g., Fig. ESM6) were measured (total of \( 4 + 26 = 30 \) data points). (a) \( A_{\text{out}} \) of Eq. (1) plotted against the fat content obtained by conventional food analysis (Soxhlet extraction method) and fitted by Eq. (3). The horizontal axis is \( w_{\text{fat}} \) in Eq. (3). (b) Cross-plot of the fat content for the 30 data points in (a) measured by MR scanning and by conventional food analytical methods. Three error contours, corresponding to 0 and ±5 wt%, are indicated by solid and dotted lines, respectively. For the 30 data points in (b), the RMSE value was 5.4 wt% and the coefficient of determination \( R^2 \) was 0.84.

Table 1. Fat content values (wt%) obtained by MR scanning (Fig. ESM5) and by conventional food analysis (Soxhlet extraction method) for the four locations (L\(_A\) to L\(_D\)) in Fig. 2a. These data are plotted in Figs. 6b, ESM10c, and ESM11b.

| Location | by MR scanning \( N_{\text{stack}} = 4 \) | by MR scanning \( N_{\text{stack}} = 10 \) | by MR scanning \( N_{\text{stack}} = 18 \) | by Soxhlet extraction method* |
|----------|-----------------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
| L\(_A\)  | 24.1                                    | 22.4                                   | 23.6                                   | 23.5                        |
| L\(_B\)  | 18                                      | 17.7                                   | 19.2                                   | 27.8                        |
| L\(_C\)  | 22.7                                    | 23.4                                   | 24.8                                   | 24.7                        |
| L\(_D\)  | 28.7                                    | 28.4                                   | 27.6                                   | 24.9                        |

Notes: *Conventional Soxhlet extraction method using diethyl ether as a solvent.[46]

DISCUSSION

The experimental results described in the previous section show that accurate fat quantification was performed successfully for the fresh beef samples using the constructed sensor unit. The RMSE and \( R^2 \) values were 5.4 wt% and 0.84, respectively, for \( N_{\text{stack}} = 10 \) (Figure 6b) and 4.8 wt% and 0.87, respectively, for \( N_{\text{stack}} = 18 \) (Fig. ESM10c). These RMSE values are much better than the RMSE value of \( \approx 1 \) wt% for 17 beef samples obtained using a different unilateral sensor unit.[22] The reasonable agreement in the present study with respect to fat content between MR scanning and conventional food analysis demonstrates the followings: (i) although the acquired CPMG data were contaminated by MR signals from water molecules in muscle, the discrimination of the signals from the fat molecules and those from the water molecules was successful by employing Eq. (1); (ii) unilateral MR scanning is thus a promising technique for intramuscular fat quantification of beef meat. The scanner system with the hand-held sensor unit (Fig. ESM5) is portable and works with an AC 100-V power supply. Thus, this system is promising for in-situ, accurate, rapid, and nondestructive fat mapping of a large meat body, such as a carcass at a meat processing factory, if the subcutaneous fat layer is less than a few millimeters thick. It also should be noted that unilateral MR scanning based on T2 relaxometry is applicable to various foods, and actually the same magnetic circuit as Fig. ESM1 is being applied to the fat quantification of bluefin tuna (Fig. ESM12).[54,55]
The RMSE and $R^2$ values for these 30 data points in Figure 6b and ESM10c noted above are slightly worse than those for four beef samples reported in a previous study (RMSE = 1.0 wt% and $R^2 = 0.96$)\textsuperscript{[27]} obtained using a different unilateral MR scanner. One possible reason for the slightly larger error in the present study is the heterogeneous meat structure of intact marbled beef. While homogeneous ground beef samples were analyzed by Petrov et al.,\textsuperscript{[27]} the beef samples in this study (Figures 2, 3, and ESM6b) contained millimeter- and centimeter-scale heterogeneity in terms of the spatial distribution of intramuscular fat and muscle. Although a simple cuboid approximation is made in Figure 3, ESM4, and ESM6b, the true shape of the sensed region is not a cuboid of $14 \times 15 \times 11$ mm but is rather irregular.\textsuperscript{[35,36]} The fat content on the vertical axis in Figure 6b and ESM10c represents that for a sensed region with an irregular shape. In contrast, the fat content on the horizontal axis in Figure 6b and ESM10c represents that for a cuboid meat piece ($\approx 40 \times 40 \times 20$ mm) cut for the Soxhlet extraction method. The difference in shape and volume among the vertical and horizontal axes are probably responsible for the data scatter, yielding worse RMSE and $R^2$ values compared with those obtained for the homogeneous ground samples.\textsuperscript{[27]}

The short measurement time would contribute to rapid fat mapping of a large carcass body at meat processing factories. There is no significant difference in the fat quantification accuracy between the measurements for $N_{\text{stack}} = 10$ and $N_{\text{stack}} = 18$ (Fig. ESM10c). Thus, $N_{\text{stack}} = 10$ is recommended for reducing the measurement time. The measurements for $N_{\text{stack}} = 10$ were performed with a sequence repetition time of 2 s and one dummy scan. Thus, the present study demonstrates that a unilateral MR scan as short as $2 \times (10 + 1)$ times = 22 s for each meat location is possible using the MR scanner built for this study (Figure 1 and ESM5). MR scanning with $N_{\text{stack}}$ as small as 4 was applied to the four locations in Figure 2, and the results are listed in Table 1 and shown in Fig. ESM11b. Although the signal intensity for $N_{\text{stack}} = 4$ is significantly smaller than those for $N_{\text{stack}} = 10$ and 18 (Fig. ESM11a), Table 1 and Fig. ESM11b show that the accuracy of fat quantification is reasonable. This suggests that a unilateral MR scan as short as $2 \times (4 + 1)$ times = 10 s for each meat location is promising and should be examined extensively using many more than four samples in the future.

The $T_{2\text{fat}}$ and $T_{2\text{lean}}$ values in Eq. (1) should be chosen appropriately for accurate fat quantification. Figure 4 shows that $T_{2\text{fat}}$ significantly depends on the sample temperature. The following three points (A to C) should be noted with respect to the effects of the temperature-dependent relaxation times on the fat quantification using CPMG measurements.

A: A significant difference in the $T_{2\text{fat}}$ and $T_{2\text{lean}}$ values is essential for accurate fat quantification based on the $T_2$ relaxometry employed in the present study. Fortunately, a large difference in the $T_{2\text{fat}}$ and $T_{2\text{lean}}$ values (2- or 3-fold difference) was observed for temperatures higher than 30°C (Figure 4). This would ensure accurate fat quantification of a cooling carcass immediately after slaughter.

B: Although measurements at a sample temperature of 30.5°C were performed in the present study, low-temperature measurement of carcasses is needed in refrigerated warehouses. Fortunately, again, a difference in the $T_{2\text{fat}}$ and $T_{2\text{lean}}$ values was observed at low temperatures (Figure 4). For example, at 8.0°C, $T_{2\text{fat}}$ = 38.0 ms and 31.1 ms for samples F$_1$ and F$_2$, respectively, and $T_{2\text{lean}}$ = 48.1 ms and 45.0 ms for samples LM$_1$ and LM$_2$, respectively. This observed difference in the $T_{2\text{fat}}$ and $T_{2\text{lean}}$ values is not so large compared with that observed at higher than 30°C, but significant nevertheless. Furthermore, according to the data trend in Figure 4, a great difference can be expected at temperatures lower than 8.0°C. Although a slight decrease in the MR signal from fat molecules occurs at low temperatures, probably due to the partial solidification of the fatty acid,\textsuperscript{[49,50]} Fig. ESM8b demonstrates that it is possible to acquire CPMG signals with a reasonable signal-to-noise ratio using the low-field, time-domain MR apparatus. This suggests that careful measurements with an increased $N_{\text{stack}}$ value would enable accurate fat quantification at low temperatures in refrigerated warehouses.

C: Undesirable agreement of the $T_2$ values for fat and lean meat occurs at approximately 15°C (Figure 4). This agreement could make the fat quantification of beef meat at this temperature less accurate because the bi-exponential model, Eq. (1), which uses the difference in the $T_2$ values, breaks
down. The following two solutions would be feasible: (i) The $T1$ values for 20 MHz are significantly different (=3-fold difference) at 15°C between the fat and lean meat samples (Figure 4). Thus, CPMG measurements with a small value of the sequence repetition time (not a full relaxation condition) would enhance the fat signal and suppress the lean meat signal, making fat quantification possible.[56] (ii) The self-diffusivity of fat molecules is approximately two orders of magnitude smaller than that of water molecules in muscle.[22] Thus, the use of the CPMG sequence with a diffusion-edited component would successfully quantify fat.[27,56]

Although the water quantification is not the subject of the present study, CPMG data analysis based on Eq. (1) would allow us to quantify water content as well as fat content. The quantity $A_{\text{lean}}$ obtained by fitting the CPMG data to Eq. (1) can be converted into water content using a calibration curve similar to that in Figure 6a and ESM10b.[31] Muscle consists of water and protein, and the weight ratio of protein to water is constant.[22] Thus, it is also possible to convert the water content value into the muscle content value.[31]

The sensed region of the sensor unit is located at $z = 4$ to 15 mm (Fig. ESM4c). Thus, the layer within =4 mm from the coil surface does not significantly contribute to the MR signals, which is sufficient for the sensor unit in Figure 1 to probe the meat beneath a subcutaneous fat layer less than a few millimeters thick (Figure 2a and ESM6b). However, the thickness of the subcutaneous fat layer could be much thicker than 4 mm (e.g., 10–20 mm) depending on which part of the carcass is measured. At such thicknesses, the sensor unit in Figure 1 cannot probe the meat beneath the subcutaneous fat layer. Three possible solutions are available for this problem: (i) Shaving the subcutaneous fat layer at the measurement location of the carcass to a few millimeters in thickness using a knife. (ii) Employing a larger sensor unit with a deeper investigation depth[57] (e.g., 30 mm[22,30]). It should be noted, however, that a larger sensor unit would require a Nd-Fe-B permanent magnetic circuit as heavy as =43 kg,[22] which is no longer “hand-held” but requires a mechanical support.[13,15,58] (iii) Developing an emerging bulk high-temperature superconducting magnet[59,60] that would be much lighter than the Nd-Fe-B permanent magnet.

CONCLUSION

A lightweight (5.0-kg) hand-held sensor unit that consists of a planar RF coil and a unilateral magnetic circuit was constructed for an MR surface scanner system to nondestructively quantify the intramuscular fat content of beef meat. The principle of the MR scanner is based on time-domain proton relaxometry using the difference in $T2$ values between the water molecules in muscle and the fat molecules. The investigation depth of the constructed sensor was 7 mm, which is sufficient to probe the meat beneath a subcutaneous fat layer less than a few millimeters thick. The unilateral MR scanner was applied in a laboratory to scan 30 locations in fresh beef samples at 30.5°C; the measurement time was 22 s for each location. The RMSE value, adopted as the measurement error, for the MR scanning was as small as 5.4 wt% when compared with ground truth values obtained by conventional destructive food analysis (Soxhlet extraction method). Thus, this portable MR scanner is a promising tool for the in-situ, rapid, accurate, and nondestructive quantification of fat in beef (e.g., carcasses) at meat processing factories. Proton relaxometry data on fat and lean meat samples were also measured in a sample temperature range of 8.0 to 39.8°C using a conventional (i.e., bilateral) MR apparatus. The obtained data suggest that the MR method presented in the present study using the difference in $T2$ between water and fat molecules is applicable within this temperature range except at around 15°C.
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Disclosure statement

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