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Measurement of Glycogen Content in Wagyu Beef Using Near-infrared Spectroscopy with Multiple-reflection Attenuated Total Reflectance

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In this paper, we discuss and utilize the multiple-reflection attenuated total reflectance (ATR) method of near-infrared spectroscopy (NIRS) for the determination of glycogen content in Japanese wagyu beef. We have also conducted experiments on diffuse reflection to compare the experimental results. Glycogen, as a reservoir of glucose units, has a positive effect on the palatability of wagyu beef. The obtained nontreated spectra were preprocessed by several different methods, then a calibration and prediction model between spectral data and glycogen content was developed by partial least squares regression (PLSR). We found that when the multiple-reflection ATR method and the diffuse reflection method are used for NIR spectroscopy methods, there is a significant difference in their ability to predict the glycogen content in wagyu beef. The determination coefficient of the best prediction model for the spectra obtained by the multiple-reflection ATR method was 0.72 for the measurement of glycogen content in wagyu beef, whereas that obtained by the diffuse reflection method was only 0.29. Through experiments and data analysis, we found that the multiple-reflection ATR method is promising for predicting glycogen content in wagyu beef. The results of these experiments have important implications for the development of future small portable beef glycogen sensors.

1. Introduction

Japanese wagyu beef, a unique livestock product in Japan, has long been appreciated by consumers worldwide for its exceptional taste, beautiful marbling, and rich nutrition. The high quality of wagyu beef cannot be separated from the strict grade differentiation standards. Currently, beef trade is graded on the basis of meat quality and yield, which is based on the

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"Beef Trade Standards" approved by the Japanese Ministry of Agriculture, Forestry and Fisheries and established by the Japan Meat Grading Association.[1] Some researchers have shown that the most distinctive feature of wagyu beef is its marbling, which indicates high fat content.[2] Within the acceptable range, a higher intramuscular fat (IMF) content results in better tenderness and juiciness, thus improving the overall palatability.[3]

It is undeniable that tenderness is the most important factor affecting the palatability of beef.[4] However, when tenderness is in the acceptable range, flavor becomes the most important factor affecting consumer preference for beef.[5,6] It has been found that monosaccharides such as glucose can promote the formation of meat flavor through the Maillard reaction when heated.[7] Glycogen, a stored form of sugar in animals, is related to the amount of monosaccharides after slaughter.[8] The higher the glycogen content, the higher the monosaccharide content in the beef after slaughter, and the more intense the Maillard reaction of the beef. In our previous work, a sensory panel test was used to determine the relationship between sensory attributes (e.g., sweetness, fattiness, aroma, tenderness, flavor, and overall evaluation) and chemical composition characteristics (e.g., moisture, protein, free amino acid, glycogen, and fatty acid content) of Japanese wagyu beef prepared by the simmering method.[9] According to the results of multiple regression analysis, there was a positive correlation between the sensory attributes and the glycogen content. Therefore, studying the glycogen content, as one of the important factors affecting beef flavor, is important.

One of the common methods used to determine glycogen content in beef is the iodine binding method. However, this method not only requires many samples, but the preparation of solutions also requires much time,[10] making it difficult to complete the determination at the slaughter site.

The method proposed and used in this study is Fourier transform near-infrared spectroscopy (FT-NIR). As a simple, nondestructive, fast, and accurate technique, FT-NIR has been widely used for the measurement of various food components.[11,12] This includes the assessment of meat quality.[13] NIR spectroscopy is an analytical method that uses the near-infrared region of the electromagnetic spectrum (4000–12500 cm^{-1}). In NIR spectroscopy, the absorption of light is measured at different wavelengths in the near-infrared region of the sample. The recorded NIR spectra include overtones and combined vibrations of molecules containing C–H (5590 and 5917 cm^{-1}), N–H (5330 and 6443 cm^{-1}), or O–H (5128 cm^{-1}) groups.[14] Because these groups are widely present in various organic materials, this makes NIR spectroscopy the preferred choice for the analysis of organic materials in the food and agricultural industries. Previously, our team used FT-NIR spectroscopy coupled with multivariable data processing to predict the glycogen content in Japanese wagyu beef samples. Our study showed that the performance of predictive models for glycogen content predictive was not very strong, with the determination coefficient being only 0.42.[15]

In this study, our main objective is to verify whether the multiple-reflection attenuated total reflectance (ATR) method can be used to rapidly analyze the glycogen content in Japanese wagyu beef. This is a technique that is important for building an environment consistent with well-being. This technology enabled us to develop small portable beef glycogen determination sensors. The sensor allows for a more scientific and appropriate grading of beef quality based on

the relationship between glycogen content and beef palatability. In this way, people can better understand the quality of beef and choose the best quality of beef for their cooking needs. We also used the diffuse reflection method and compared the results of the two methods. To accomplish our goal, we conducted two sets of experiments. In the first set, we measured the spectra of samples enriched with glycogen using the two methods and developed predictive models to verify which method was more effective. In the second set of experiments, we measured 21 wagyu beef samples also using both methods to determine the feasibility of the multiple-reflection ATR method for measuring the glycogen content in wagyu beef. Both sets of experiments were conducted to determine the relationship between glycogen content and NIR spectra on the basis of partial least squares regression (PLSR).

2. Materials and Methods

2.1 Preparation of beef samples enriched with glycogen

The samples used were thawed and frozen beef sold within the consumption period. They were purchased in block form, and the blocks were subsequently ground into minced meat in the laboratory using a mincer at room temperature (25 °C) to facilitate the subsequent addition of glycogen to them. Glycogen powder (from Oyster, FUJIFILM Wako Pure Chemical Corporation) was used in the experiments. The delineated glycogen content in the beef is shown in Table 1. These values are added glycogen concentrations and do not take into account the glycogen naturally present in the beef.

Ten samples were prepared for each concentration. We added the glycogen powder to the beef samples in accurate amounts. A glass stirring rod was used to mix thoroughly the glycogen powder with the beef. In this way, 100 beef samples were obtained. They were used in the experiments to compare the results of both diffuse reflection and multiple-reflection ATR methods. The only difference in sample handling between the two methods is the difference in the number of grams per small sample. Diffuse reflection requires 3 g of beef per experiment. The multiple-reflection ATR, on the other hand, has a larger contact plane because of its multiple-reflection principle, so each experiment requires 4 g of beef to cover the entire plane.

In summary, each method has 10 glycogen concentrations, each with 10 samples, for a total of 100 beef samples. They were applied to the experiments to evaluate the ability of both methods to measure glycogen content.

2.2 Preparation of wagyu beef samples with known glycogen content

The wagyu beef samples analyzed in this study were from Tottori Prefecture, Japan. Carcass characteristics of meat located between the sixth and seventh ribs (the longissimus thoracis

Table 1
Glycogen concentration in beef samples obtained by adding glycogen powder. The fraction ratios are mass percents.

Glycogen (wt%)	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55

muscle) were evaluated following the standards of the Japan Meat Grading Association,[1] and the quality grades of meat were higher than Grade 4 (4 or 5). A total of 21 samples were taken. The common substances and their contents in wagyu beef are shown in Table 2. It can be seen that the glycogen content is only a small part of the total material composition of beef. The average concentration of glycogen in wagyu beef is only 0.12%. This also proves that the analysis of glycogen content is difficult. One sample of the longissimus thoracis muscle was collected from each of the 21 carcasses, minced, and stored at −30 ℃ until analysis. Each meat sample had an identification number. After being frozen and transported to our laboratory, we stored the samples in a freezer at −24 ℃. Before the experiment, the samples were thawed at room temperature (25 ℃) for 2 h, and then 12 g of each of the 21 wagyu samples were taken for the experiment. The wagyu samples were evenly divided into three portions of 4 g each. A total of 63 samples were used for each method in this experiment.

2.3 Determination of glycogen content

The glycogen content was determined by the iodine binding method according to Dreiling *et al.*[16] About 0.5 g of meat sample was homogenized with 5 ml of cold 7% perchloric acid for 30 s at 30000 rpm. The samples were subsequently left for 10 min at room temperature and then filtered with filter paper (Advantec, No.1, Japan). The fraction including glycogen was collected and used for glycogen measurement. The glycogen content was determined using a spectrophotometer (Shimadzu, UV-1200V, Japan) at 460 nm and calculated as milligrams per gram of raw meat.

2.4 NIR instruments and accessories

The spectrometer used was an FT-NIR spectrometer (PerkinElmer, Frontier NIR Spectrometer, USA). It was equipped with two near-infrared reflectance accessories: a diffuse reflection accessory (PerkinElmer, NIRA, USA), and a multiple-reflection ATR accessory (Horizontal Attenuated Total Reflectance Optics Division for Perkin Frontier, PIKE TECHNOLOGIES) for the measurements. Figure 1 shows an illustration of the two accessories. The wall surface of the diffuse reflection integrating sphere is a circular plane of 7 mm radius. The wall surface of the multiple-reflection ATR is a rectangular plane of 10 mm × 80 mm area. During the experiment, the sample was evenly and tightly adhered to the wall surface to ensure that no light was exposed and to reduce the interference of bubbles. For spectra with high

Table 2
Reference measurements of the contents of various components of 21 wagyu beef samples.

	Moisture (%)	Protein (%)	Fat (%)	Glycogen (%)	Monosaccharides (%)		
					Mannose	Glucose	Ribose
Mean	38.37	10.75	50.52	0.12	0.02	0.07	0.07
SD	7.00	1.96	9.03	0.05	0.01	0.03	0.03
Max.	52.61	14.74	70.11	0.23	0.03	0.14	0.14
Min.	23.18	6.49	32.15	0.04	0.00	0.03	0.00

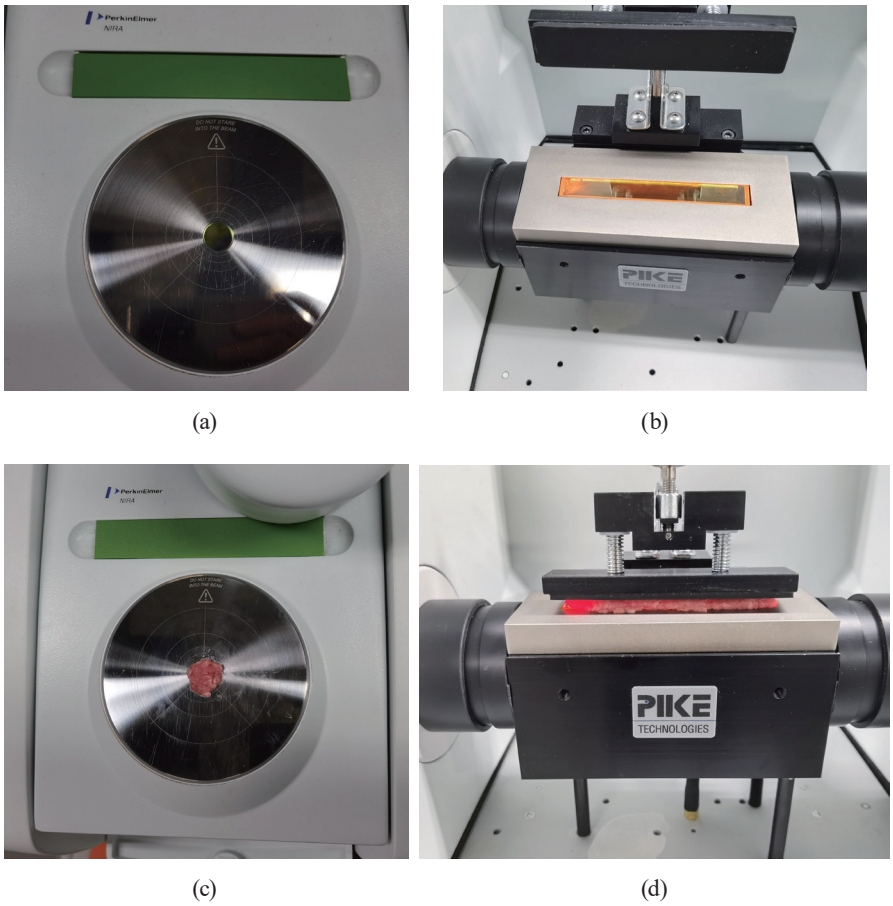

Fig. 1. (Color online) Pictures of the two near-infrared reflectance accessories. (a) Diffuse reflection accessory without samples. (b) Multiple-reflection ATR accessory without samples. (c) Diffuse reflection accessory with samples. (d) Multiple-reflection ATR accessory with samples.

interference, we adjusted the sample state and re-examined the spectrum until the results were satisfactory. The measurement environment was room temperature (25 °C). Each spectrum was the average of 16 measurements at 8 cm^{-1} resolution over the range from 12500 to 4000 cm^{-1}. With 16 measurements, the calibration and prediction model was robust, and only a short test time was needed. The absorbance spectra were acquired as logarithmically transformed reflectance $\log(1/R)$.

2.5 Data analysis

2.5.1 Spectral preprocessing

Preprocessing is important for the spectra before modeling the data. It can reduce the sample-to-sample variability due to scattering and optical interference and improve the predictive capability of models. We tried to use several preprocessing methods before modeling, including smoothing, normalization, and determination of standard normal variable (SNV).

The purpose of smoothing is to eliminate the random noise in the spectral signal and improve the signal-to-noise ratio of the sample signal. Firstly, the SNV was used to normalize the data. The SNV is obtained by subtracting the mean value of the spectrum from each wavelength in the spectrum and dividing it by the standard deviation of the spectrum.[17] Next is normalization. The main purpose of data normalization is to minimize or even exclude duplicated data. Normalization is similar to SNV, with the difference that the former averages the columns of the spectra and the latter averages the rows.[18]

The preprocessed spectra are used for modeling. The goodness of the model is determined by looking at the coefficients of determination of the prediction and calibration sets.

2.5.2 PLSR

PLSR generalizes and combines the features of principal component analysis and multiple regression. PLSR is particularly useful when we need to predict a set of dependent variables from a set of a large number of independent variables.[19] The PLSR modeling process is divided into two steps. The first step is to build a calibration model based on the calibration data. To ensure that the obtained calibration model is not overfitted, the leave-one-out method is generally used for cross-validation. The second step is to build a predictive model based on the validation method. The predictive model is used to check the reliability of the calibration model and to ensure that the relevant calibration model works properly.[20] In this study, owing to the small number of samples, the calibration model was validated using the leave-one-out cross-validation method after the calibration model was established, and a prediction model for the glycogen content in beef was established.

In this experiment, we used the determination coefficient of calibration (R_c^2) and prediction (R_p^2) set, and the root mean square errors of calibration (RMSEC) and prediction (RMSEP) to evaluate the performance of the prediction model. It is worth mentioning that good models should have high R_c^2 and R_p^2 values and low RMSEC and RMSEP values. The small difference between RMSEC and RMSEP indicates that the model is stable.[21] In addition, the predictive performance of the model can be evaluated using the value of the residual predictive deviation (RPD). When the value of RPD is greater than or equal to 3, it means that the model is excellent; when the value of RPD is greater than 2 and less than 3, it means that the model is average; when the value of RPD is less than 2, it means that the model is poor or its predictive ability is unreliable.[22]

2.5.3 Feature spectral selection

For quantitative analysis, it is very important to choose the best feature spectra. On the one hand, feature spectra can eliminate irrelevant wavelengths and make the spectral analysis more effective, and on the other hand, a smaller wavelength range with the same effect makes the subsequent spectral analysis instruments easier to develop. In this study, we used the loading coefficients in the PLSR model to select the wavelengths that have the greatest effect on the

prediction of glycogen content in Japanese wagyu beef. All data analysis programs used throughout the work were implemented using The Unscrambler X software (CAMO software, X 10.5, Norway).

3. Results and Discussion

3.1 Experimental measurement of beef samples with added glycogen using FT-NIR

3.1.1 Experimental results of diffuse reflection

Figures 2(a) and 2(b) show the original absorption spectra of 100 beef samples enriched with glycogen obtained by diffuse reflection and multiple-reflection ATR methods in the region of 12500–4000 cm^{-1}, expressed in absorbance, respectively. The absorption band in this region is mainly generated by the low-energy electron leap and stretching vibrations of hydrogen-containing atomic groups (e.g., O–H, N–H, C–H).[23] It can be seen in the figure that there are two large absorption peaks at 6900 and 5170 cm^{-1}. They are formed due to the first and second overtones generated by the O–H stretching vibration in the water molecule. Because the beef samples contain a lot of water, these two peaks appear to be large.[24] Additionally, the wave numbers at 7040, 6330, 5710, and 5920 cm^{-1} were found to be the most useful for developing the calibrations in the known studies on glycogen.[25] Except for 6330 cm^{-1}, the peaks of the remaining wave numbers could be observed in this experiment. These data show consistency in the NIR region when the same functional group is present in different substances.

After obtaining the original spectra, a preprocessing method was used to process the spectra in order to reduce the effects of sample preparation and light scattering. The experimental results are shown in Table 3. PLSR models for glycogen prediction were developed in the range of 12500–4000 cm^{-1}. The results show that the normalization-smoothing model predicts glycogen with the highest R_p^2 value of 0.04 and RPD of 1.01. The low value of R^2 and the value of RPD < 2 indicate that the model is not robust. It also indicates that the diffuse reflection method is not strong in predicting glycogen content in beef samples enriched with glycogen.

3.1.2 Experimental results of multiple-reflection ATR

Figure 2(c) shows that there is a large difference in absorbance between the spectrograms obtained by the two methods, with the absorbance of diffuse reflection being about ten times higher than that of multiple-reflection ATR. However, there is not much difference between the two in terms of waveform. This indicates that the type of functional group has little effect on the two methods, but there are differences in the absorbance of the functional groups when using different methods. This may be related to the fact that the multiple-reflection ATR method can expand the absorption signal of low-concentration substances by several times through several reflections. After obtaining the original spectra, they were processed with multiple preprocessing methods.

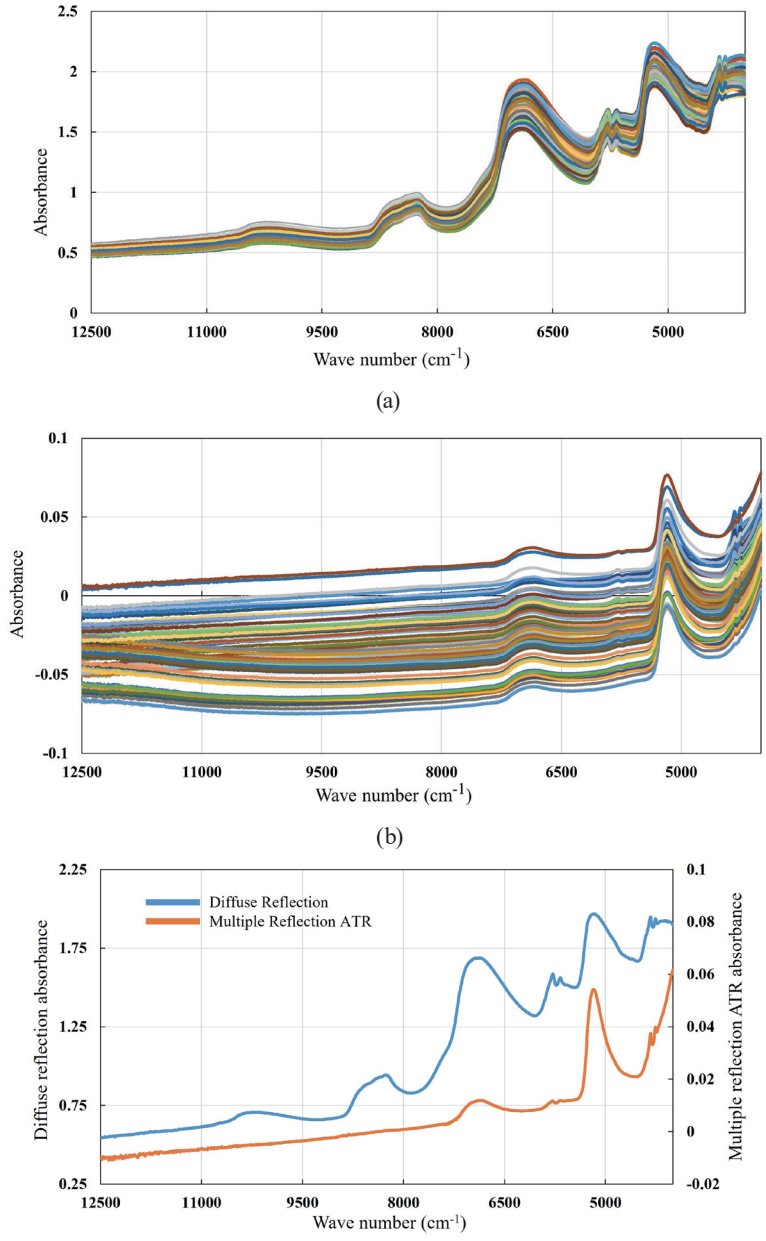

Fig. 2. (Color online) Nontreated spectra of 100 beef samples. (a) Diffuse reflection method. (b) Multiple-reflection ATR method. (c) Comparison graph, where the selected samples are all beef samples with glycogen content of 0.35 mg/g.

The experimental results are shown in Table 4. As shown, the highest R_p^2 value of 0.82 was obtained for the normalization-smoothing-treated model. The value of RDP was 2.37. This can be considered a good predictive result. It indicates that the multiple-reflection ATR method has the potential to correctly predict the glycogen content in wagyu beef and is worth using in experiments with real wagyu beef samples.

Table 3
PLSR results obtained by diffuse reflection method for predicting glycogen content in beef samples enriched with glycogen.

	Preprocessing	Spectral region (cm^{-1})	Calibration		Prediction		RPD
			R_c^2	RMSE	R_p^2	RMSE	
Glycogen (mg/g)	Nontreated	12500–4000	0.0567	0.1395	0.0362	0.1424	1.0085
	Smoothing	12500–4000	0.0572	0.1395	0.0390	0.1420	1.0114
	Normalization	12500–4000	0.0574	0.1394	0.0398	0.1421	1.0107
	Normalization-smoothing	12500–4000	0.0575	0.1394	0.0399	0.1421	1.0107

R^2, coefficient of determination.
RMSE, root mean square error.
RPD, ratio of standard deviation to root mean square error of cross-validation.

Table 4
PLSR results obtained by multiple-reflection ATR method for predicting glycogen content in beef samples enriched with glycogen.

	Preprocessing	Spectral region (cm^{-1})	Calibration		Prediction		RPD
			R_c^2	RMSE	R_p^2	RMSE	
Glycogen (mg/g)	Nontreated	12500–4000	0.9156	0.0417	0.7986	0.0651	2.2061
	Smoothing	12500–4000	0.9146	0.0420	0.8084	0.0638	2.2510
	Normalization	12500–4000	0.9155	0.0413	0.8165	0.0624	2.3015
	Normalization-smoothing	12500–4000	0.9225	0.0400	0.8241	0.0607	2.3660

R^2, coefficient of determination.
RMSE, root mean square error.
RPD, ratio of standard deviation to root mean square error of cross-validation.

3.2 Measurement of Tottori wagyu beef samples using FT-NIR

3.2.1 Experimental results of diffuse reflection

The original absorption spectra obtained by diffuse reflection and multiple-reflection ATR methods for a wagyu sample in the region of 12500–4000 cm^{-1} are shown in Fig. 3. After preprocessing, a new spectrum with low noise and high signal-to-noise ratio was obtained.

The reference results of glycogen content in Japanese wagyu beef analyzed in this study are presented in Table 2. The glycogen content in 21 samples ranged between 0.43 and 2.29 mg/g. The experimental results are shown in Table 5. The results show that with different pretreatments, the SNV-treated spectrum obtained has the best predictive ability, and the model predicts the highest R_p^2 value of glycogen at 0.29. The RPD value is 1.20, which is also close to the results reported in the existing experiments for the measurement of glycogen content in wagyu.[15] It was further found that the PLSR model obtained using the diffuse reflection method was not very reliable in predicting glycogen content; further studies are needed to obtain a more robust model for the analysis.

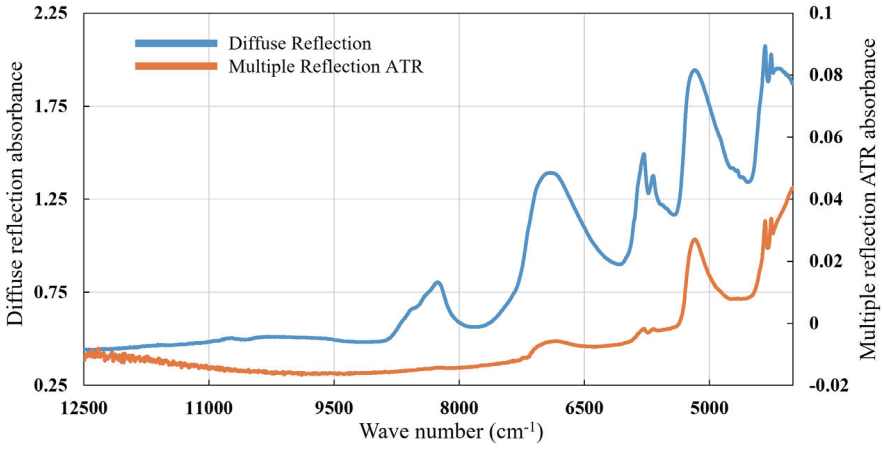

Fig. 3. (Color online) Comparison of wagyu spectra of the two methods.

Table 5
PLSR results obtained by diffuse reflection method for predicting glycogen content in wagyu beef.

	Preprocessing	Spectral region (cm^{-1})	Calibration		Prediction		RPD
			R_c^2	RMSE	R_p^2	RMSE	
Glycogen (mg/g)	Nontreated	12500–4000	0.3928	0.3893	0.2170	0.4492	1.1354
	Normalization	12500–4000	0.4335	0.3761	0.2778	0.4315	1.1819
	SNV	12500–4000	0.4288	0.3776	0.2935	0.4267	1.1952

R^2, coefficient of determination.
RMSE, root mean square error.
RPD, ratio of standard deviation to root mean square error of cross-validation.
SNV, standard normal variate.

3.2.2 Experimental results of multiple-reflection ATR

3.2.2.1 12500–4000 cm^{-1} region

Wagyu beef samples from Tottori Prefecture in the spectral range of 12500–4000 cm^{-1} were used for glycogen content prediction using the PLSR model. The results of spectra nontreated and preprocessed by different methods are shown in Table 6. To graphically illustrate the performance of this model, the values of the calibration and prediction set of the model with the best results obtained by preprocessing are shown in Fig. 4. The results show that the best PLSR model for glycogen prediction was established using normalization-baseline-preprocessed spectra. The best PLSR model predicted glycogen with the highest R_p^2 value of 0.72, the lowest PMSE value of 0.27, and the RPD value of 1.8861. The relationship between the normalization-baseline-preprocessed spectra and the regression coefficients is plotted in Fig. 5, which shows that the large peaks at 6900 and 5170 cm^{-1} in the spectra do not have a significant effect on the regression coefficients. This is consistent with the previous report describing these two peaks as O–H in water molecules.[24] At the same time, the peaks at 7040, 5920, and 5710 cm^{-1} affect the variation of the regression coefficients. This is also consistent with the previous findings of

Table 6
PLSR results obtained by multiple-reflection ATR method for predicting glycogen content in wagyu beef (Region: 12500–4000 cm^{-1}).

Preprocessing			Calibration		Prediction		RPD
			R_c^2	RMSE	R_p^2	RMSE	
Nontreated			0.7396	0.2549	0.6015	0.3205	1.5913
		Parameters					
Smoothing	Moving average	11	0.6719	0.2862	0.5266	0.3493	1.4601
		31	0.7396	0.2549	0.5807	0.3290	1.5502
		101	0.7396	0.2549	0.5809	0.3216	1.5858
	Gaussian filter	11	0.6992	0.2740	0.5560	0.3383	1.5075
		31	0.6313	0.3034	0.4870	0.3636	1.4026
		101	0.6023	0.3151	0.4704	0.3695	1.3802
	Median filter	11	0.6860	0.2800	0.5401	0.3443	1.4813
		31	0.6350	0.3018	0.4932	0.3614	1.4112
		101	0.6120	0.3112	0.4743	0.3681	1.3855
Normalization	Area normalization		0.7820	0.2333	0.6422	0.3037	1.6793
	Maximum normalization		0.8191	0.2125	0.6627	0.2948	1.7300
	Range normalization		0.7582	0.2457	0.6187	0.3135	1.6268
	Mean normalization		0.6766	0.2841	0.3855	0.3980	1.2814
SNV	None		0.8618	0.1857	0.6474	0.3015	1.6915
Baseline			0.7864	0.2309	0.6493	0.3007	1.6960
Normalization-baseline			0.8248	0.2091	0.7164	0.2704	1.8861
Normalization-baseline-SNV			0.7356	0.2570	0.5930	0.3239	1.5746

R^2, coefficient of determination.
RMSE, root mean square error.
RPD, ratio of standard deviation to root mean square error of cross-validation.
SNV, standard normal variate.

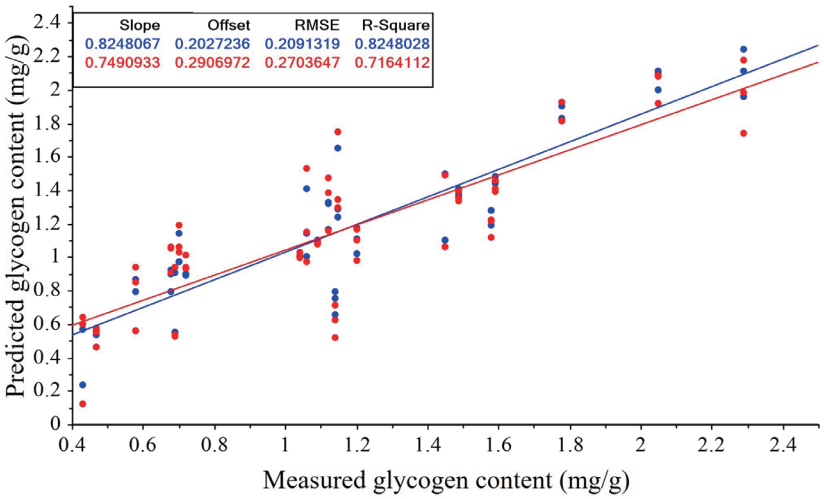

Fig. 4. (Color online) Best model for predicting glycogen content obtained by the multiple-reflection ATR method (12500–4000 cm^{-1} region).

Brown.[25] Moreover, the two absorption peaks at 4300 and 4260 cm^{-1} also have a large effect on the variation of the regression coefficients. This may be related to the binding tone generated by the stretching and changing angle of the $–CH_2$ group in glycogen between 4310–4260 and 4350–

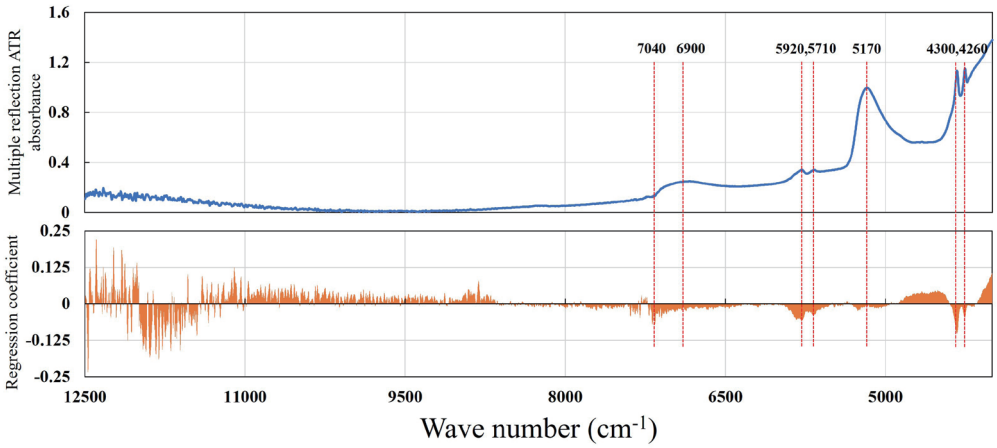

Fig. 5. (Color online) Plot of spectra vs regression coefficients (12500–4000 cm^{-1} region).

4260 wave numbers.[26] In addition, since the near-infrared absorption peaks are coupling peaks or overtone peaks of the fundamental vibration absorption peaks, it is difficult to determine the exact attribution. This means that one peak may represent multiple substances, and one substance may exist in several peaks.

Compared with the results of the experiments with the addition of glycogen powder shown in Sect. 3.1.2, the prediction model showed a decrease in R_p^2 and RPD values. The best prediction models in both experiments were obtained after normalization-baseline preprocessing. The RPD value of the former was 2.36 and that of the latter was 1.8861. According to available studies, the fat content in beef can be easily measured by NIR spectroscopy.[27] Owing to the consistency of the functional groups of glycogen and fat, it is possible that an interaction in the spectra occurs. It is inferred that the reduced predictive ability of the model may be related to the higher fat content in wagyu beef. In addition, the number of samples used for the predictive model construction was only 63. This is smaller than the 100 samples in the first set of experiments, which may also be one of the reasons why the model in the experiment is not very robust.

It is clear that the multiple-reflection ATR method is much better than the diffuse reflection method in predicting the glycogen content in wagyu beef, and there is much room for improvement.

3.2.2.2 8000–4000 cm^{-1} region

There are a large number of combination bands and overtones in the NIR spectrum, and they tend to affect the construction of predictive models. Therefore, it is important to select the wavelength of a certain spectral region. In this study, we selected the important spectra using the regression coefficients obtained from the PLSR model to build a simplified PLSR model.

After that, we chose to build a simplified PLSR model using spectra from the 8000–4000 cm^{-1} region. The performance of the PLSR model to predict the glycogen content is shown in Table 7. To graphically illustrate the performance of this model, the values of the calibration and prediction set of the model with the best results obtained by preprocessing are shown in Fig. 6.

Table 7
PLSR results obtained by multiple-reflection ATR method for predicting glycogen content in wagyu beef (Region: 8000–4000 cm^{-1}).

Preprocessing			Calibration		Prediction		RPD
			R_c^2	RMSE	R_p^2	RMSE	
Nontreated			0.5828	0.3227	0.4734	0.3684	1.3844
		Parameters					
Smoothing	Moving average	11	0.5810	0.3234	0.4722	0.3688	1.3829
		31	0.5811	0.3234	0.4702	0.3695	1.3802
		101	0.5759	0.3254	0.4581	0.3737	1.3647
	Gaussian filter	11	0.5815	0.3232	0.4759	0.3676	1.3874
		31	0.5801	0.3238	0.4684	0.3702	1.3776
		101	0.5771	0.3249	0.4618	0.3714	1.3732
	Median filter	11	0.5812	0.3233	0.4739	0.3683	1.3847
		31	0.5850	0.3219	0.4740	0.3682	1.3851
		101	0.5916	0.3193	0.4842	0.3646	1.3988
Normalization	Area normalization		0.7463	0.2514	0.5936	0.3258	1.5654
	Maximum normalization		0.8098	0.2179	0.6003	0.3210	1.5888
	Range normalization		0.5454	0.3369	0.4455	0.3781	1.3488
	Mean normalization		0.3429	0.4050	0.2830	0.4299	1.1863
SNV			0.7439	0.2529	0.5763	0.3723	1.3699
Baseline			0.7174	0.2977	0.5982	0.3305	1.5431
Normalization-baseline			0.8022	0.2222	0.6458	0.3025	1.6860
Normalization-baseline-SNV			0.8067	0.2197	0.6136	0.3137	1.6258

R2, coefficient of determination.
RMSE, root mean square error.
RPD, ratio of standard deviation to root mean square error of cross-validation.
SNV, standard normal variate.

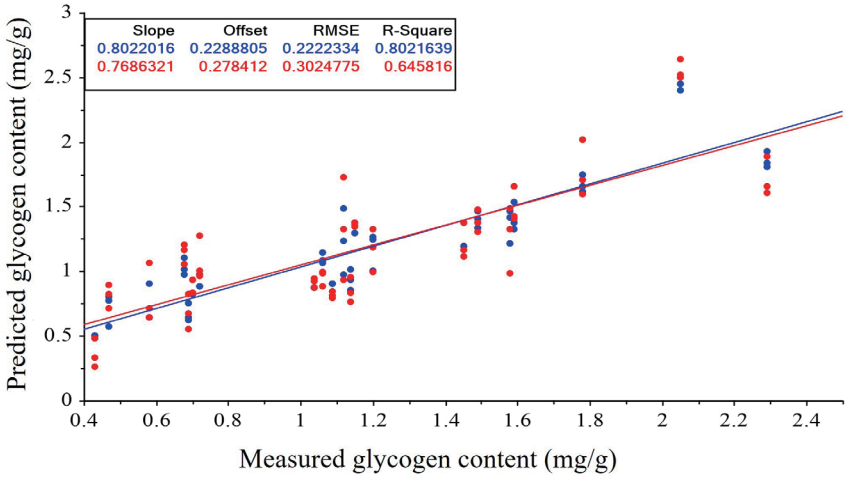

Fig. 6. (Color online) Best model for predicting glycogen content obtained using the multiple-reflection ATR method (8000–4000 cm^{-1} region).

The results show that the best PLSR model based on feature spectra with the highest R_p^2 value of 0.65, the lowest RMSEP value of 0.30, and an RPD value of 1.69 was obtained. These results are similar to those obtained using the best PLSR model based on full spectra. A narrow range reduces the difficulty of instrumentation and makes the collection of data more efficient.

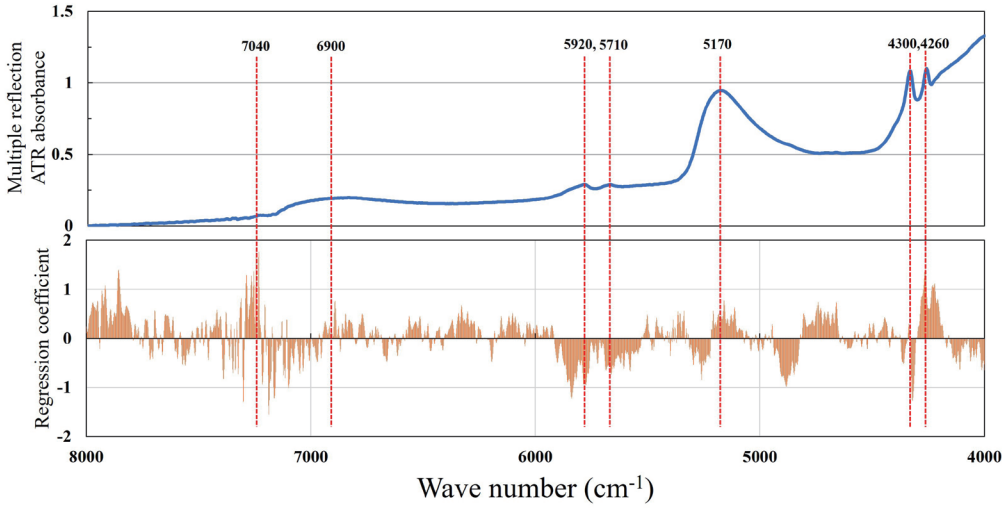

Fig. 7. (Color online) Plot of spectra vs regression coefficient (8000–4000 cm^{-1} region).

The relationship between the normalization-baseline-preprocessed spectra and the regression coefficients is plotted in Fig. 7. Compared with the results for the 125000–4000 cm^{-1} wave number interval, the effect of noise in the high wave number interval is no longer present, and the previously insignificant effect of the 8000–4000 cm^{-1} wave number interval is now magnified. As a result, in addition to the peak of the spectrum, high regression coefficients are also seen in the flat areas. This indicates that the effect of the same groups as glycogen in other substances has increased. This may be the reason why the model RPD values are lower than those of the 12500–4000 cm^{-1} region model. However, the peaks at 7040, 5920, and 4300 cm^{-1} can still be identified.

In summary, the wave number range in 12500–4000 or 8000–4000 cm^{-1} region does not have a significant effect on the accuracy of the predictive model. Among the predictive models built with 8000–4000 cm^{-1}, normalization-baseline has the best preprocessing effect with an RPD value of 1.69.

4. Conclusions

In this study, the glycogen content in Japanese wagyu beef samples was predicted using multiple-reflection ATR and diffuse reflection methods in FT-NIR spectroscopy combined with multivariable data processing. In the experiments for the prediction of glycogen content in beef samples enriched with glycogen, the PLSR model developed using the multiple-reflection ATR method with 100 samples was better for the prediction of glycogen content in beef than the diffuse reflection method. The PLSR model established using the diffuse reflection method was not satisfactory for predicting glycogen content in beef. In the experiments for predicting glycogen content in Japanese wagyu beef, the PLSR model for 63 samples developed using the multiple-reflection ATR method was satisfactory for the prediction of glycogen content in wagyu beef. The PLSR model established using the diffuse reflection method also did not

predict glycogen content in wagyu beef well. One of the reasons for this is that the concentration of glycogen in beef itself is very low, which makes the measurement very difficult. Moreover, the multiple-reflection ATR method enhanced the light signal of glycogen by multiple reflections, which reduced the difficulty of glycogen content measurement in beef. In the future, we will improve the experiment by optimizing the analysis method, replacing the instrument plug-in with a more flexible one, and increasing the number of samples. Overall, the multiple-reflection ATR method is promising for predicting glycogen content in wagyu beef, and this result has important implications for the development of small portable beef glycogen determination sensors.

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