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Development of Optimal Protocol for Visible and Near-Infrared Reflectance Spectroscopic Evaluation of Meat Quality

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Development of optimal protocol for visible and near-infrared reflectance spectroscopic evaluation of meat quality ☆

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Abstract

The present experiments were conducted to develop an optimal protocol for visible and near-infrared reflectance spectroscopic evaluation of meat quality. It was determined that spectra were more repeatable using a 35 mm-diameter high-intensity reflectance probe rather than a 3 mm-diameter reflectance probe. Using the high-intensity reflectance probe, spectra were generally very repeatable (e.g., repeatability at 1080 nm ranged from 0.94 to 0.99) regardless of the number (50, 40, 30, 20, or 10) of spectra averaged per observation. At each wavelength (350–2500 nm), the highest repeatability was obtained when 20 spectra were averaged. It was determined that spectra were greatly different when the length of time that the muscle was exposed to air (bloomed) before spectroscopy was increased from 2 to 60 min. However, regardless of bloom time, the repeatability of reflectance values was >0.90 at each wavelength between 462 and 1371 nm. The protocol developed in this experiment should facilitate future experiments to determine if visible and near-infrared spectroscopy can be used to accurately predict meat quality.
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Keywords: Beef; Lamb; Meat; Near-infrared spectroscopy; Quality

1. Introduction

The meat industry has long sought non-destructive, objective techniques to predict meat quality. Several studies have shown that near-infrared reflectance spectroscopy can be used to predict beef tenderness (Byrne, Downey, Troy, & Buckley, 1998; Hildrum et al., 1995; Hildrum, Nilsen, Mielnik, & Naes, 1994; Liu et al., 2003; Mitsumoto, Maeda, Mitsuhashi, & Ozawa, 1991; Naes & Hildrum, 1997; Park, Chen, Hruschka, Shackelford, & Koohmaraie, 1998). However, the procedures used in those studies were either destructive in that they required excision of a muscle sample for spectroscopy (Hildrum et al., 1994, 1995; Liu et al., 2003; Naes & Hildrum, 1997; Park et al., 1998) or they were limited to sampling a very small area (4 cm^2) and thus, would be highly subject to error induced by non-representative sampling of the target muscle or interference from intramuscular fat (Byrne et al., 1998; Mitsumoto et al., 1991). Because it is unclear if reflectance in the visible or near-infrared range holds the most promise for prediction of meat quality, techniques should be developed to simultaneously evaluate visible and near-infrared spectroscopy of meat. Fortunately, advances in instrumentation now allow a single instrument to scan the entire visible and near-infrared spectrum (350–2500 nm). Therefore, the present experiments were conducted to develop a repeatable, non-destructive technique for visible and near-infrared reflectance (VISNIR) spectroscopic evaluation of meat quality.

2. Materials and methods

2.1. Experiment 1. Comparison of reflectance probes

The Roman L. Hruska US Meat Animal Research Center (MARC) Animal Care and Use Committee

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of other products that may also be suitable.

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approved the use of animals in this study. Lambs ($n = 12$) were grain-fed, slaughtered, dressed (hot carcass weight ranged from 25.5 to 32.3 kg), and chilled (48 h at 1 °C). The entire dorsal section (loin and rack) was removed from each carcass, vacuum packaged, aged (2 °C) until 14 d postmortem, and frozen (−30 °C). Using a band saw, a 2.54 cm-thick chop was obtained from the 12th rib region of the right side of each frozen dorsal section.

Vacuum-packaged chops were thawed and a transverse cut was made through the center of each chop to expose two fresh surfaces, which were mirror images of each other, for spectroscopic evaluation.

Spectroscopy was conducted using a Model A108310 LabSpec Pro portable spectrophotometer (ASD; Analytical Spectral Devices, Inc.; Boulder, CO) which was equipped to collect spectra from 350 to 2500 nm. Two different types of fiber optic reflectance probes were compared. The first probe was a 3 mm-diameter ASD Model 135680 bifurcated reflectance probe. A limitation to the bifurcated reflectance probe was that it only covered a very small proportion of a muscle cross-section, which is often very heterogeneous. Therefore, a second probe was tested that was capable of sampling a much larger area. The second probe was an ASD Model A122000 high-intensity reflectance probe that served as an external light source (2900 K color temperature quartz halogen light) to illuminate the object of interest. This probe can be used to collect reflectance spectra on an area as large as 55 mm in diameter. In the present experiment, the field of view was restricted to a circle that was 35 mm in diameter using a ASD Model A122040 field of view limiter and plate. Reflected light was collected through a ASD Model 135090 2-m long fiber optic jumper cable that consisted of a bundle of forty-four 200-μm fibers.

For each probe, a spectrum was collected on each of the two freshly exposed *longissimus* muscle surfaces. Spectra were collected with the LabSpec Pro software's "sample spectrum count" option set to 50. That is, 50 spectra were collected and averaged per observation.

2.1.1. Statistical analysis

Data were discarded for 350–449 nm due to a high level of spectral noise. To facilitate computations and to reduce spectral noise, spectra were reduced by averaging groups of nine consecutive wavelengths using Unscrambler® (Version 7.5; Camo, Inc.; Corvallis, OR). To assess the effect of probe type on the sharpness of reflectance peaks, the SD of reflectance was calculated across all wavelengths for each observation. The effect of probe type on the SD of reflectance was determined by one-way analysis of variance using the GLM procedure of SAS® (Version 7; SAS Inst.; Cary, NC). Repeatability of the reflectance measured at each wavelength was calculated using the VARCOMP procedure of SAS®. For each wavelength, repeatability was calculated as $\sigma^2_{\text{carcass}}/(\sigma^2_{\text{carcass}} + \sigma^2_{\text{error}})$.

2.2. Experiment 2. Optimal number of spectra per observation

Beef carcasses ($n = 72$) representing diverse levels of marbling and lean color were off-railed at a commercial United States fed-beef packing plant immediately after USDA grading. Carcasses were from grain-finished steers and heifers and were graded US Prime, US Choice, US Select or were not graded (qualified for US Standard). To guard against the possibility that a substantial amount of blooming of the *longissimus* (conversion of deoxymyoglobin to oxymyoglobin) would occur during the collection of repeatability data, the 12th rib cross-section was bloomed at least 60 min before data collection was initiated. Reflectance spectra were collected on the exposed 12th rib (i.e., forequarter) cross-section of *longissimus* of the left side of each carcass averaging 50, 40, 30, 20, and 10 spectra per observation. This process was repeated on the same muscle cross-section to provide duplicate observations for calculation of repeatability. To give independence to each replicate of data, the spectrophotometer was calibrated, the first replicate of data was collected for an entire rail of 38 or 34 carcasses, the spectrophotometer was recalibrated, and the second replicate of data was collected. This process was sequentially repeated for averaging 50, 40, 30, 20, and 10 spectra per observation.

Spectra were collected with the same spectrophotometer as in Experiment 1 using the high-intensity reflectance probe. The field of view limiter plate was modified to allow sampling of a greater proportion of the beef *longissimus* cross-section. Specifically, the diameter of the hole in the limiter plate was increased from 35 to 50 mm.

Time-in-motion data were collected for each replicate to estimate the effect of the number of spectra averaged per observation on the number of beef carcasses that could be tested per hour. In addition to including the time required to position the light source on the *longissimus* cross-section and to collect the spectra, our estimates of time-in-motion included the time required to recalibrate the system at 10 min intervals because ASD recommends that the spectrophotometer be recalibrated every 10 min.

2.2.1. Statistical analysis

Data were reduced and repeatability was calculated as in Experiment 1.

2.3. Experiment 3. Repeatability of spectra within and between beef carcass sides

At the MARC abattoir, beef carcasses ($n = 54$) were ribbed between the 12th and 13th ribs at 48 h post-

mortem. These carcasses were from 13 to 17 month old grain-finished steers. Marbling scores ranged from practically devoid to modest. After the 12th rib cross-section of the *longissimus* had bloomed 60 min, reflectance spectra were collected on the exposed 12th rib (i.e., fore-quarter) cross-section of *longissimus* of each carcass side averaging 20 spectra per observation. This process was repeated on the same muscle cross-section of each side to provide duplicate observations for calculation of repeatability.

Spectra were collected using the same spectrophotometer and probe as in Experiment 2. As in Experiment 2, the spectrophotometer was calibrated before each replicate of data was collected on each rail of carcasses. In this case, each rail consisted of 10 or 11 carcasses.

2.3.1. Statistical analysis

Data were reduced as in Experiment 1. Variance component analysis was conducted using the VARCOMP procedure of SAS. Variance in the reflectance at each wavelength was partitioned into carcass, side, carcass X side, and error effects.

2.4. Experiment 4. Effect of bloom time on repeatability of spectra of beef longissimus

At the MARC abattoir, beef carcasses ($n = 131$) were ribbed between the 12th and 13th ribs at 48 h post-mortem. These carcasses were from 13 to 17 month old grain-finished steers. Marbling scores ranged from practically devoid to modest. After the 12th rib cross-section of the *longissimus* had bloomed 2 and 60 min, reflectance spectra were collected on the exposed 12th rib (i.e., fore-quarter) cross-section of *longissimus* of the right side of each carcass averaging 20 spectra per observation. This process was repeated on the same muscle cross-section to provide duplicate observations for calculation of repeatability. Spectra were collected using the same spectrophotometer and probe as in Experiment 2. As in Experiments 2 and 3, the spectrophotometer was calibrated before each replicate of data was collected on each rail of carcasses. In this case, each rail consisted of 10 or 11 carcasses. Collection of the first and second replicate of data were separated by approximately 2 min (time required to collect data for one rail of carcasses and recalibrate).

2.4.1. Statistical analysis

Data were reduced as in Experiment 1. Variance component analysis was conducted using the VARCOMP procedure of SAS. Variance in the reflectance at each wavelength was partitioned into carcass, bloom time, carcass × bloom time, and error effects.

3. Results

3.1. Experiment 1. Comparison of reflectance probes

Fig. 1 is a plot of the mean reflectance spectra of lamb *longissimus* samples as measured with the contact reflectance probe and the high-intensity reflectance probe. To our knowledge, these data represent the first report of meat spectra spanning the entire range from 450 to 2500 nm. Fig. 1 shows that the high-intensity reflectance probe detects a peak in reflectance at 804 nm

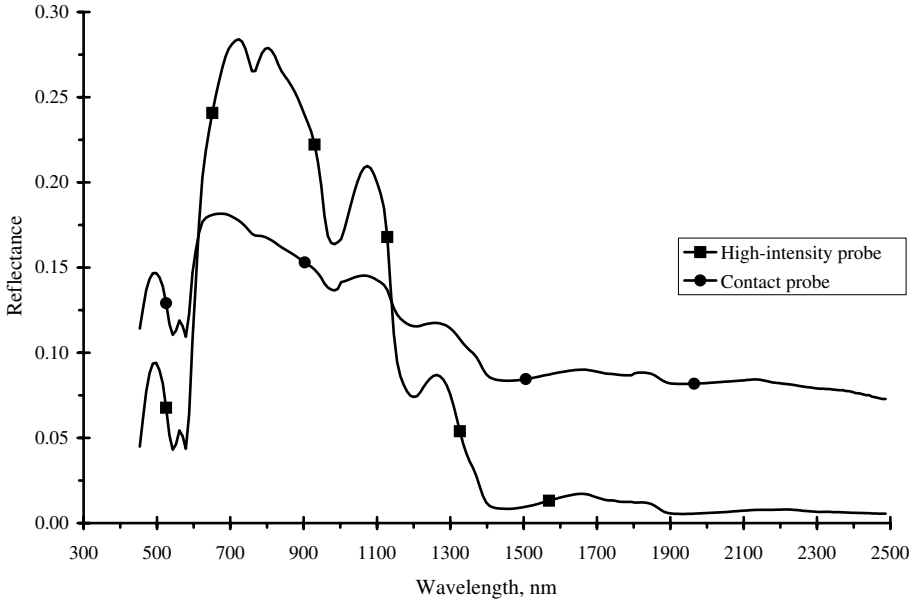

Fig. 1. Mean spectra obtained from lamb *longissimus* samples ($n = 12$) using the contact reflectance probe and the high-intensity reflectance probe (Experiment 1).

that the contact reflectance probe does not distinguish. Most peaks were more distinct for the high-intensity reflectance probe. Consequently, the SD of reflectance across wavelengths for a given sample was 2–5 times higher ($P < 0.001$) when spectra were collected with the high-intensity reflectance probe as compared with the contact reflectance probe.

Fig. 2 shows the repeatability of reflectance spectra of lamb *longissimus* samples as measured with the contact reflectance probe and the high-intensity reflectance probe. When spectra were collected using the contact reflectance probe, the repeatability (the proportion of the total variation attributable to differences among animals) of reflectance measurements was less than 0.55 at all wavelengths. In contrast, when spectra were collected using the high-intensity reflectance probe, the repeatability of reflectance measurements was >0.88 at each wavelength from 660 to 1326 nm. From 1700 to 2500 nm, spectra were not highly repeatable for either probe. The higher repeatability of spectra obtained in the range from 453 to 1326 nm with the high-intensity reflectance probe as compared to the contact reflectance probe was likely a function of the ability of the high-intensity reflectance probe to sample a greater area of *longissimus*. Sampling a greater area likely minimized the impact of uneven distribution of marbling on the spectra.

For the spectra that were collected with the high-intensity reflectance probe, there was substantial variation in reflectance among carcasses. For example, compared to the least reflective lamb *longissimus* sample, the most reflective lamb *longissimus* sample reflected 12%, 47%, 41%, 45%, 75%, 162%, and 231% more light at the 498, 561, 723, 804, 1074, 1263, and 1659 nm peaks, respectively (Fig. 3).

3.2. Experiment 2. Optimal number of spectra per observation

The VISNIR system used in this study allows the user to collect an infinite number of spectra for each observation. Theoretically, up to a certain point, collecting and averaging more spectra per observation should result in a reduction in the level of noise in the spectra. However, the more spectra collected, the more time is required per observation. Collecting more spectra than are needed to obtain a repeatable spectra is wasteful and may become an impediment to on-line application of VISNIR spectroscopy. Therefore, the present experiment was conducted to determine the optimal number of spectra per observation for VISNIR spectroscopic evaluation of the 12th rib cross-section of beef *longissimus*.

Within the range in values tested in this experiment, there did not appear to be any logical effect of the number of spectra averaged per observation on the repeatability of reflectance measurements. At each wavelength, the highest repeatability was obtained when 20 spectra were averaged and the lowest repeatability was obtained when 50 spectra were averaged (Fig. 4). Therefore, it appears that averaging more than 20 spectra per observation is unnecessary and may possibly result in less repeatable data. Spectra were generally very repeatable regardless of the number of spectra averaged

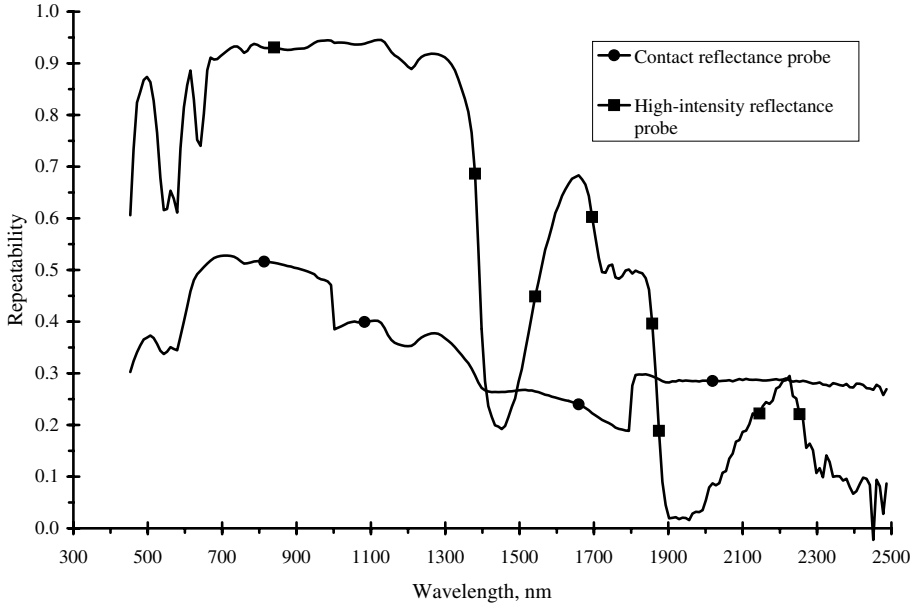

Fig. 2. Repeatability of reflectance spectra of lamb *longissimus* samples ($n = 12$) as measured with the contact reflectance probe and the high-intensity reflectance probe (Experiment 1).

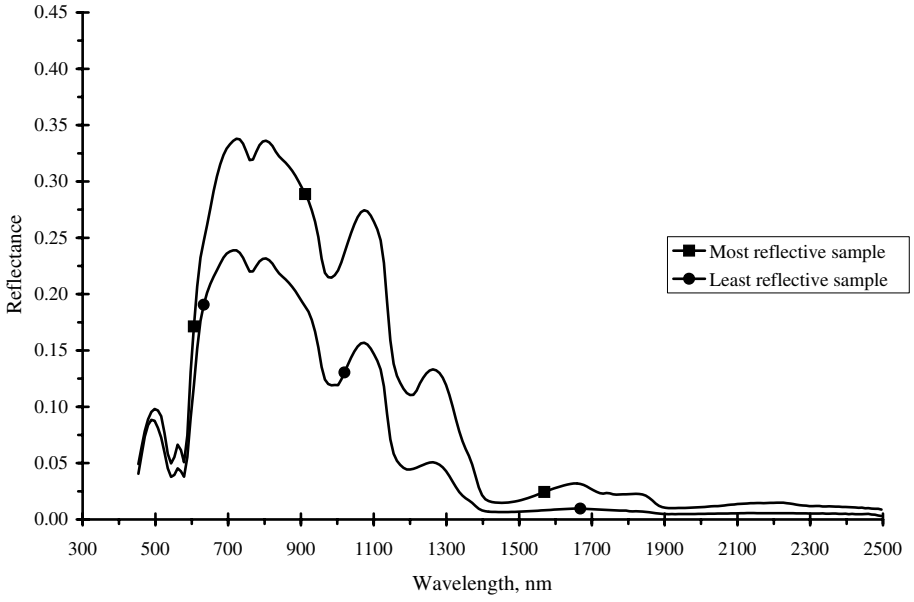

Fig. 3. Reflectance spectra of the most and least reflective lamb *longissimus* samples. Spectra were obtained using the high-intensity reflectance probe (Experiment 1).

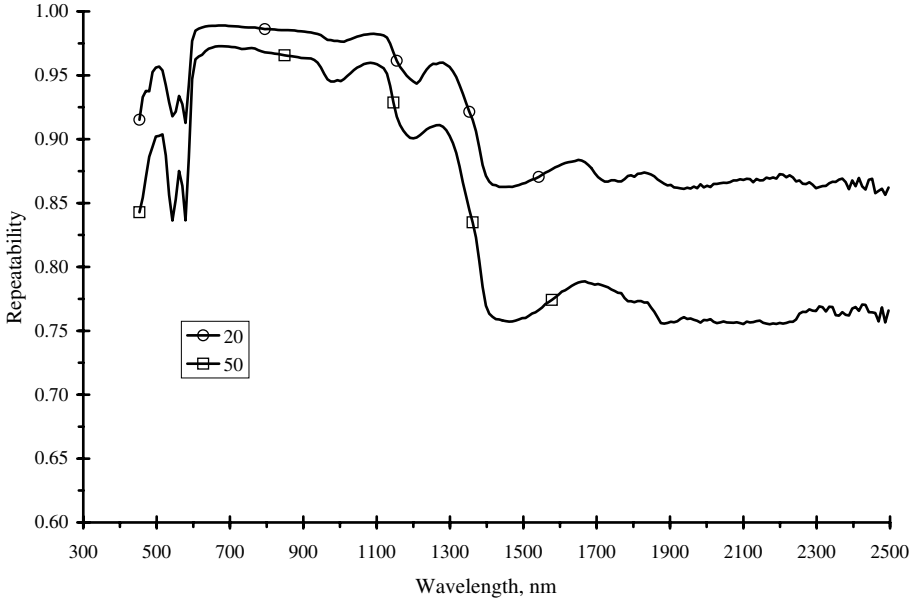

Fig. 4. Effect of the number of spectra averaged per observation on the repeatability of reflectance spectra of the 12th rib cross-section of *longissimus* of beef carcasses ($n = 72$). At each wavelength, the highest repeatability was obtained when 20 spectra were averaged and the lowest repeatability was obtained when 50 spectra were averaged. For clarity, repeatability is not shown for the cases where 10, 30, and 40 spectra were averaged. All spectra were obtained using the high-intensity reflectance probe (Experiment 2).

per observation. At 669 nm repeatability ranged from 0.972 to 0.989, depending on the number of spectra averaged. Whereas in Experiment 1 repeatability of lamb *longissimus* reflectance was very low at 1900–2500 nm, in this experiment repeatability of beef *longissimus* reflectance was >0.75 at all wavelengths. There were differences in the protocols of these experiments that preclude determination of the cause of the greater repeatability in beef. These protocol differences include: (1) the lamb had been aged 14 d, frozen, and thawed before evaluation whereas the beef was evaluated fresh at 36 h postmortem; (2) the field of view limiter was changed to account for the muscle size difference between lamb and beef; and (3) the lamb was evaluated immediately after cutting whereas the beef was exposed to air for 60 min before evaluation. It is likely that all of

these factors affect the spectra observed. For instance, Hildrum et al. (1994) observed differences in the shape of spectra between fresh and frozen beef.

Compared to the least reflective beef *longissimus* sample, the most reflective beef *longissimus* sample reflected 89%, 118%, 100%, 94%, 124%, 180%, and 118% more light at the 498, 561, 723, 804, 1074, 1263, and 1659 nm peaks, respectively (Fig. 5). There was a small amount of autocorrelation between reflectance in the visible and near-infrared ranges (Fig. 6). For example, the correlation between reflectance at 1400–2500 nm and reflectance at 723 nm was 0.30–0.45 ($P < 0.001$). The combination of greater variation in reflectance in beef and the autocorrelation between visible and near-infrared reflectance may have resulted in the greater repeatability of reflectance at 1700–2500 nm in beef relative to lamb.

Time-in-motion data indicated that 348 carcass sides could be tested per hour with this system, averaging 20 spectra per observation (Table 1).

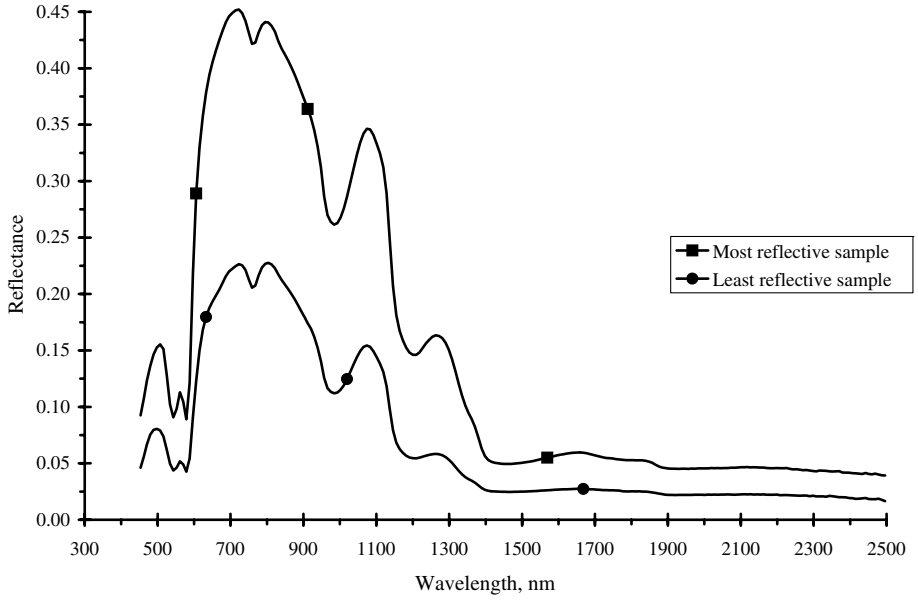

Fig. 5. Reflectance spectra of the most and least reflective beef *longissimus*. Twenty spectra were averaged per observation (Experiment 2).

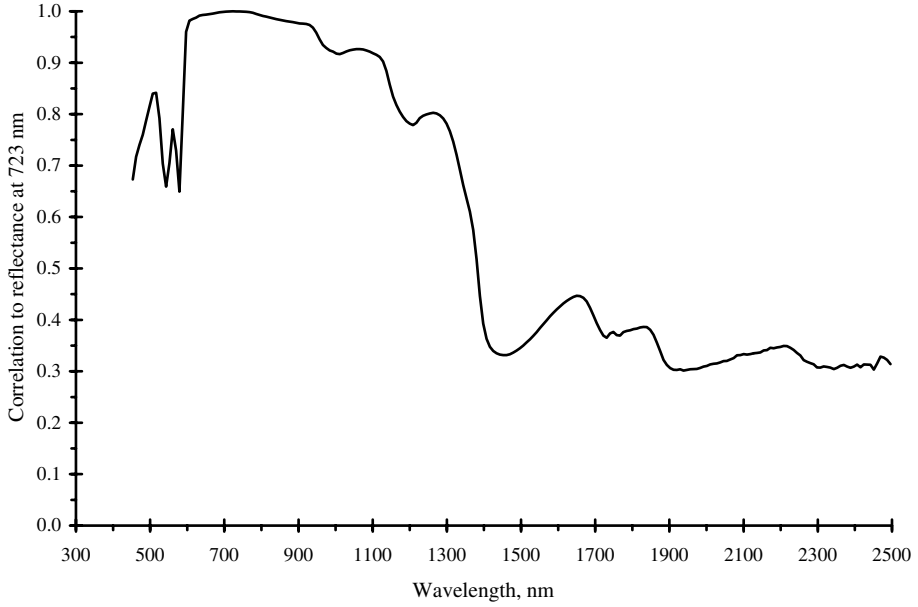

Fig. 6. Correlation of reflectance of beef *longissimus* at each wavelength to reflectance at 723 nm. Twenty spectra were averaged per observation (Experiment 2).

Table 1
Effect of the number of spectra averaged per observation on the number of beef carcasses that could be tested per hour

Number of spectra averaged per observation	Number of carcasses that could be tested per hour[a]
50	219[f]
40	258[e]
30	300[d]
20	348[c]
10	428[b]
SEM	7

[a] Estimates include the assumption that only one side of each carcass is to be measured. The number of carcasses that could be tested per hour would be half if both sides need to be tested.
[b–f] Means that do not share a common superscript letter differ ($P < 0.01$).

3.3. Experiment 3. Repeatability of spectra within and between beef carcass sides

In Experiment 2, we determined that repeatable reflectance spectra could be obtained on the 12th rib cross-section of beef *longissimus* using the high-intensity reflectance probe and averaging 20 spectra per observation. While Experiment 2 indicated that the operation of the spectrophotometer and light source were very repeatable, it did not provide an indication of how representative the spectra obtained were of the *longissimus* of that carcass. Clearly, marbling and lean color are not homogeneous within *longissimus*. Therefore, the present experiment was conducted to determine how repeatable spectra were between and within beef carcass sides.

Variance component analysis is shown in Fig. 7. At 597–1128 nm greater than 70% of the total variation in reflectance was due to variation among carcasses. In that range, most of the remaining variation was attributable to the interaction of carcass and side (i.e., random differences among carcass sides).

At 1300–2500 nm, there was very little repeatable variation in reflectance among carcasses. In that range, approximately 40% of the variation in reflectance was attributable to fixed differences among sides. Specifically, reflectance was higher ($P < 0.001$) for the *longissimus* of left carcass sides than the *longissimus* of right sides. It is unclear why reflectance was higher for the *longissimus* of left carcass sides. This likely was an anomaly of the data set. This result suggests that reflectance at 1300–2500 nm should be avoided in model development and that if those wavelengths are used, the model may not be robust.

In this experiment, within-side repeatability, which is denoted by the top line of the portion of variance attributable to the interaction of carcass and side, was very high ($\geqslant 0.88$) at all wavelengths.

Although less than 82% of the total variance at any single wavelength could be attributed to variation among carcasses, as much as 94% of the variation in the difference in reflectance at certain wavelengths was attributed to variation among carcasses. Variance component analysis of the differences between the amount of reflectance at 1173 nm and the amount of reflectance at

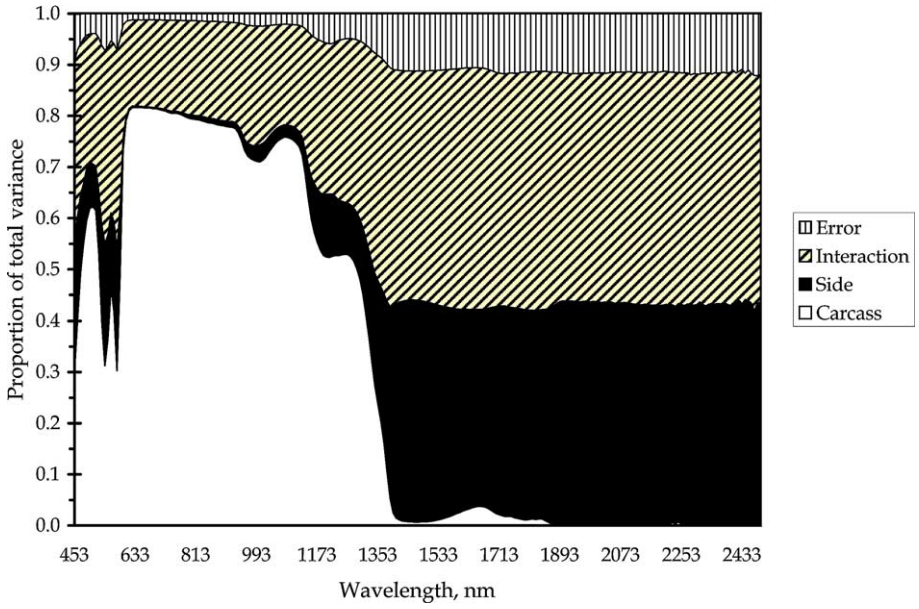

Fig. 7. Variance component analysis for reflectance spectra of the 12th rib cross-section of *longissimus* of beef carcasses ($n = 54$; Experiment 3). Interaction indicates the interaction of carcass and side. Repeatability among carcasses is equal to the proportion of the total variance attributable to variation among carcasses. Repeatability within sides is equal to the proportion of the total variance attributable to variation among carcasses plus that proportion attributable to sides plus that proportion attributable to the interaction of carcass and side.

516 nm is shown in Fig. 8. For that trait, 94% of the variance was attributable to variation among carcasses. It remains to be seen if that trait or another of the traits that were highly repeatable among carcasses is related to meat quality.

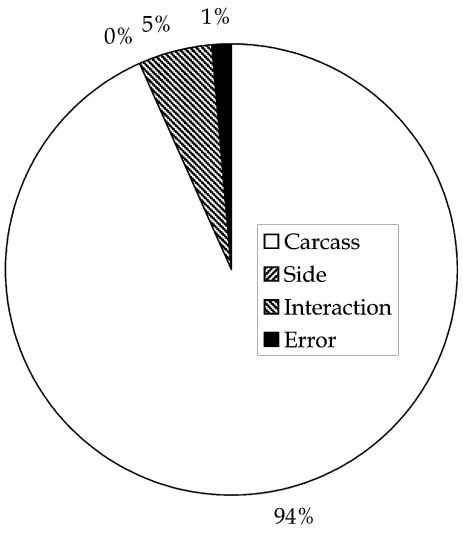

Fig. 8. Variance component analysis for the difference in the amount of reflectance at 1173 nm and the amount of reflectance at 516 nm for the 12th rib cross-section of beef *longissimus* ($n = 54$; Experiment 3). Interaction indicates the interaction of carcass and side. Repeatability among carcasses is equal to the proportion of the total variance attributable to variation among carcasses. Repeatability within sides is equal to the proportion of the total variance attributable to variation among carcasses plus that proportion attributable to sides plus that proportion attributable to the interaction of carcass and side.

3.4. Experiment 4. Effect of bloom time on repeatability of spectra of beef longissimus

At 453–1326 nm, repeatability of reflectance was not affected by bloom time (Fig. 9). At 1334–2496 nm, reflectance measurements were more repeatable when the *longissimus* was exposed to air for 60 min before spectroscopy as compared to when the *longissimus* was exposed to air for 2 min before spectroscopy. When samples were bloomed 2 min, repeatability estimates were greater than 0.75 at all wavelengths. When samples were bloomed 60 min, repeatability estimates were greater than 0.88 at all wavelengths. Thus, in research applications, it may be possible to acquire more repeatable spectra by allowing the *longissimus* to bloom for 60 min before spectroscopy. On the other hand, spectroscopy can be conducted after only 2 min of bloom with little loss of information.

Reflectance was affected ($P < 0.05$) by bloom time at each wavelength except 570, 804, and 1713–1767 nm (Fig. 10). Reflectance was higher for samples bloomed 60 min at 516, 561, and 597–795 nm. Reflectance was lower for samples bloomed 60 min at 453–507, 525–552, 579, 588, 813–1704, and 1776–2496 nm. At 606 nm, reflectance was 33% higher for samples bloomed 60 min ($P < 0.001$; F-ratio = 2732). At 471 nm, reflectance was 23% lower for samples bloomed 60 min ($P < 0.001$; F-ratio = 975). For most carcasses, *longissimus* reflectance was greater at 804 nm than 723 nm after 2 min of exposure to air. However, for most carcasses, *longissimus* reflectance was greater at 723 nm than 804 nm after 60 min of exposure to air.

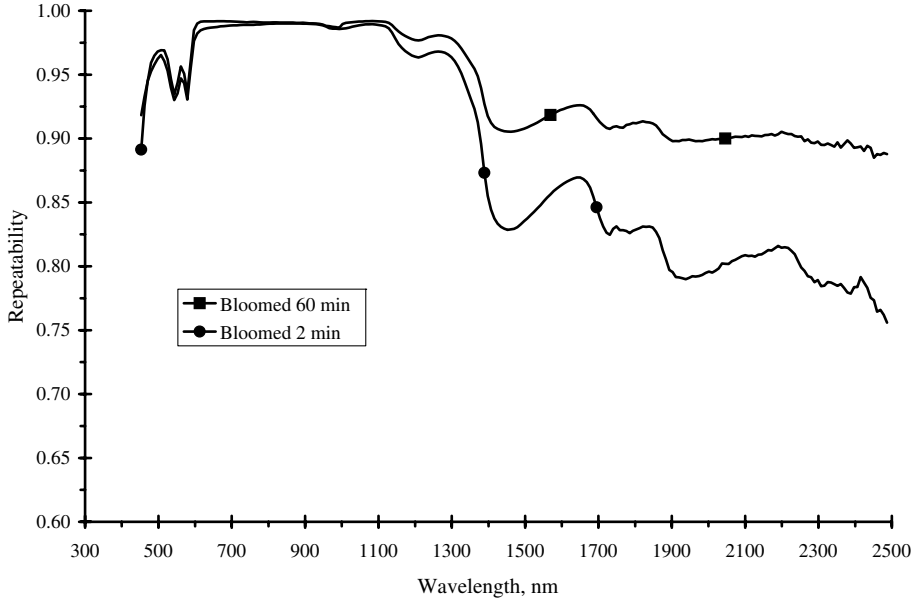

Fig. 9. Effect of the amount of time the *longissimus* muscle is exposed to air (bloomed) on the repeatability of reflectance spectra of the 12th rib cross-section of *longissimus* of beef carcasses ($n = 131$; Experiment 4). All spectra were obtained using the high-intensity reflectance probe and 20 spectra were averaged per observation.

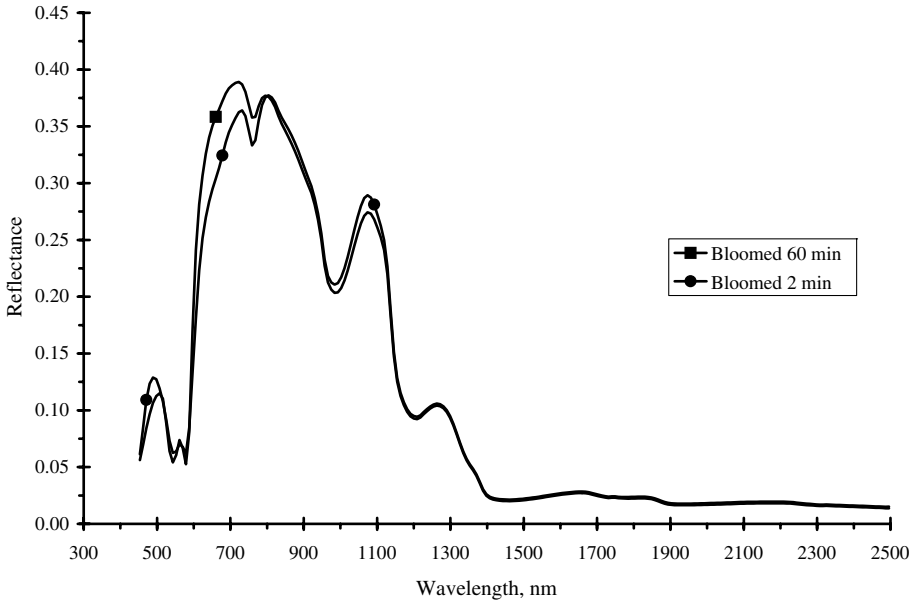

Fig. 10. Effect of the amount of time the *longissimus* muscle is exposed to air (bloomed) on the mean reflectance spectra of the 12th rib cross-section of *longissimus* of beef carcasses ($n = 131$; Experiment 4). All spectra were obtained using the high-intensity reflectance probe and 20 spectra were averaged per observation.

The trait that was most different between beef *longissimus* samples bloomed 2 and 60 min was the difference between reflectance at 498 and 516 nm (Fig. 11; $P < 0.001$; F-ratio = 4236). Whereas the difference between reflectance at 498 nm and reflectance at 516 nm was greater than 0.01 for all samples after 2 min of exposure to air, the difference between reflectance at 498 nm and reflectance at 516 nm was less than 0.01 for all samples after 60 min of exposure to air.

The American Meat Science Association (1991) indicated that the ratio of reflectance at 610 and 525 nm was an indicator of the percentage of myoglobin that was in the oxymyoglobin state. In the present study, that ratio was higher ($P < 0.001$) for beef *longissimus* exposed to air for 60 vs. 2 min. However, there was some overlap between bloom times in that trait. The range in the ratio of reflectance at 610 and 525 nm was 1.7–2.8 after 2 min of bloom and 2.2–4.1 after 60 min of bloom.

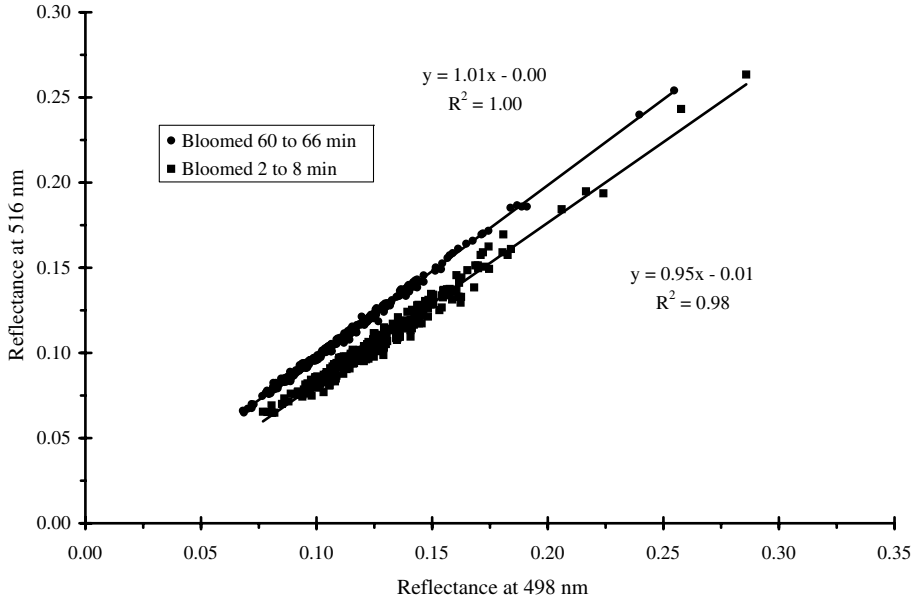

Fig. 11. Effect of the amount of time the *longissimus* muscle is exposed to air (bloomed) on the relationship between the reflectance at 498 nm and reflectance at 516 nm ($n = 131$; Experiment 4).

Variance component analysis showed that at 606 nm, less than half of the total variance was attributable to carcass because of the large effect of bloom time on reflectance at 606 nm (Fig. 12). Clearly, for VISNIR spectroscopy to be successfully used to predict meat quality, bloom time will have to be standardized. Because beef grading chains frequently stop for prolonged periods of time, the most practical way to standardize the length of bloom time in commercial beef processing plants is to conduct spectroscopy immediately after ribbing.

Spectra of the least and most reflective beef *longissimus* samples are shown in Fig. 13. Data were plotted as absorbance rather than reflectance to allow comparison of these spectra to those reported by Hildrum et al. (1994), Hildrum et al. (1995), and Park et al. (1998). Within the range of wavelengths investigated in those

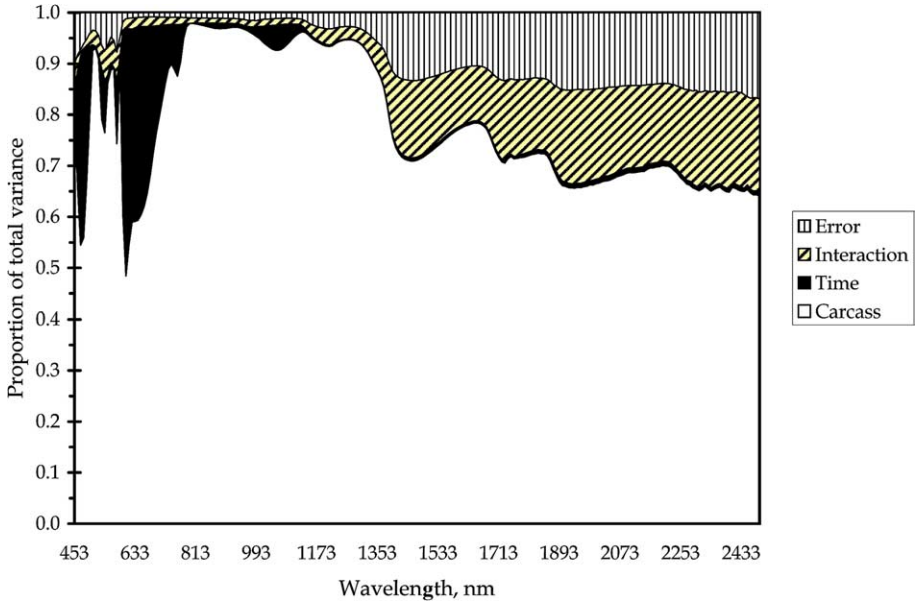

Fig. 12. Variance component analysis for reflectance spectra of the 12th rib cross-section of *longissimus* of beef carcasses ($n = 131$; Experiment 4). Interaction indicates the interaction of carcass and bloom time.

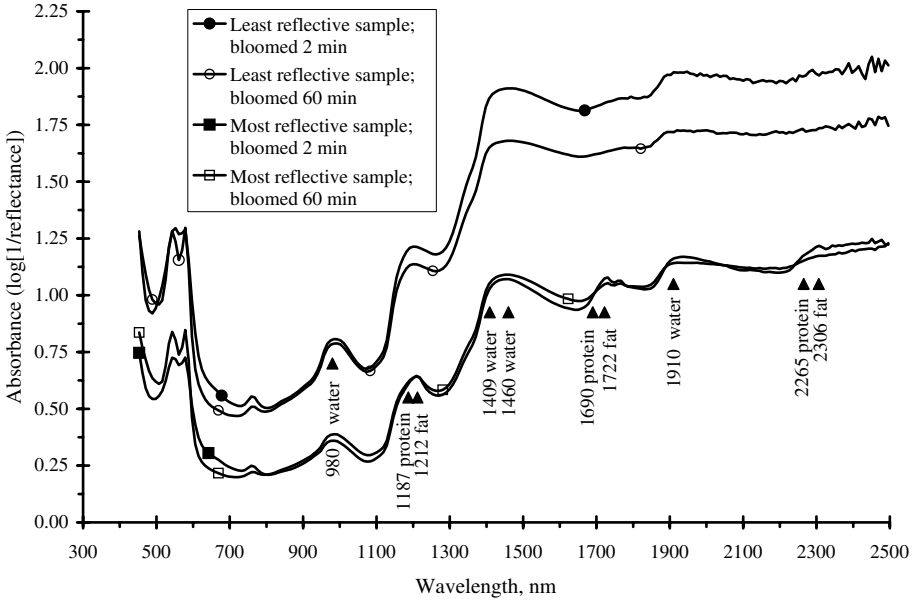

Fig. 13. Absorbance spectra of the most and least reflective beef *longissimus* (Experiment 4). Spectra of the same two carcasses are shown after 2 and 60 min of exposure to air. The wavelengths associated with chemical constituents are shown.

studies (1100–2500 nm), our spectra show the same features detected by Hildrum et al. (1994), Hildrum et al. (1995), and Park et al. (1998). The system used in this study has these advantages: (1) it covers the entire visible and near-infrared spectrums (350–2500 nm) and (2) it is non-destructive and, thus, would be amenable to on-line application. Within the range of wavelengths (750–1098 nm) investigated by Byrne et al. (1998), our spectra show the same features as those authors detected in beef *longissimus*. Mitsumoto et al. (1991) and Byrne et al. (1998) used a fiber optic approach to the prediction of beef quality. Whereas the high-intensity probe investigated in this study sampled an area of 19.6 cm^2, the technique used by Mitsumoto et al. (1991) and Byrne et al. (1998) was limited to sampling an area of 4 cm^2 and, thus, would be much more susceptible to the error-inducing effects of uneven marbling distribution.

4. Conclusions

The optimal protocol for on-line visible and near-infrared reflectance evaluation of *longissimus* quality traits of ribbed beef carcasses would include: use of the high-intensity probe with a 50 mm diameter hole in the limiter plate, averaging 20 spectra per observation, measurement of *longissimus* from one side per carcass, and obtaining spectra at a standardized bloom time (i.e., as soon as possible after ribbing). For off-line application to lamb and pork, which are not typically ribbed in commercial practice, or to smaller muscles, a reduction in the size of the opening in the limiter plate may be required. Repeatability data suggests that reflectance at 1300–2500 nm should be avoided in model development and that if those wavelengths are used, the model may not be robust. Extrapolation of the findings of this experiment to other instruments would require validation. The protocol developed in this experiment should facilitate future experiments to determine if near-infrared spectroscopy can be used to accurately predict meat quality.

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