



NIR Spectroscopic Techniques for Quality and Process Control in the Meat Industry

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Abstract: Near-infrared spectroscopy (NIRS) is widely used for quality and process control in the food industry. In the meat industry, the method is still used mainly for determination of fat, water, and protein content, while new applications are emerging. In this paper, we report on how in-line NIRS can be used to detect and sort chicken breast fillets with the myopathies wooden breast and spaghetti meat from normal fillets. A total of 270 fillets were measured with 2 different near-infrared systems. The near-infrared spectra contained information about protein content and water-binding properties that showed systematic differences between the 3 quality classes. Wooden breast could be well separated from normal fillets (96% correct classification), while spaghetti meat was slightly more difficult to separate from both normal and wooden breast because properties measured by NIRS were overlapping between the 3 groups. Two quite similar NIRS instruments had quite different classification performance, which emphasizes the importance of optimizing spectroscopic instrumentation for different applications.

Key words: chicken, wooden breast, spaghetti meat, near-infrared spectroscopy, classification

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Introduction

Near-infrared spectroscopy (NIRS) is a potent method for industrial quality control and process monitoring, and the potential for process optimization is large (Grassi and Alamprese, 2018). NIRS is an excellent tool for rapid and nondestructive determination of fat, water, and protein in ground meat, by measuring molecular vibrations involving hydrogen bonds (e.g. C-H, O-H, and N-H). This application is established for both at-line and in-line monitoring, and different types of commercial near-infrared (NIR) instruments are used for this. The method has been reviewed for other potential meat applications (Porep et al., 2015; Dixit et al., 2017) and appears to be a promising method for rapid and nondestructive determination of quality features

such as water-holding capacity, tenderness, pH, and specific fatty acids. However, none of these potential applications has reached the meat industry as a commercial tool.

The main applied research and development questions related to NIRS and meat are (1) what kind of quality features are possible to measure and (2) how these can be measured in process with satisfactory accuracy. New developments can therefore rely on novel instrumentation that enables, e.g., better and more representative sampling, or new applications for which there are strong needs in the industry. There are at least 3 reasons why reported novel NIRS applications for meat do not reach the market as commercial solutions:

1. The actual feasibility of the application is questionable (causality is not clear and application is developed and tested to a very limited extent).

2. There is a lack of instrumentation for certain applications that require particular performance with regard to, e.g., speed, resolution, or sampling of heterogeneous materials.
3. There is a lack of profitable business ideas. The application must be economically viable for both the meat processing company and the instrument vendor.

Representative sampling is often a limitation with spectroscopic methods regarding heterogeneous foods. NIR reflection was introduced as an on-line method for fat determination of batches of ground meat (Tøgersen, 1999), and it works well since an average value can be recorded over a large volume at the grinder outlet. NIRS technology based on high-speed hyperspectral imaging in combination with so called interaction measurements enables sampling across the entire width of a conveyor belt as well as about 10- to 15-mm depth into the meat. This produces good estimates of fat in boxes of meat trimmings (O'Farrell et al., 2010) and portions of trimmings on a conveyor belt (Wold et al., 2011). This technology enables automatic sorting of trimmings according to fat content (Wold et al., 2016) and potentially an improved utilization of the meat raw material. Recently, there has been a focus on measuring quality features related to the chemical state of water in meat. Shifts occur in the absorption peaks of water, and these are related to (1) how the water is bound to macromolecules like proteins (Chung et al., 2008) and (2) sample temperature (Buning-Pfaue, 2003). The former phenomenon is being industrially utilized today for in-line detection of the woody breast (WB) syndrome in chicken fillets based on NIRS ability to quantify protein and water binding in muscle tissue (Wold et al., 2017; Wold et al 2019). Another important process feature to monitor is the core temperature during heat treatment of meat products. For food safety reasons, the core temperature must exceed 72°C, whereas to avoid unnecessary drip loss affecting quality and profitability it should not be too high. Since the absorption of water in NIRS is sensitive to temperature, it is possible to measure temperature in biomaterials. Studies indicate that is possible to in-line monitor the core temperature of liver pate and sausages when the optical penetration depth is sufficient (O'Farrell et al, 2011; Wold et al., 2020).

In this article, we present a feasibility study on how NIRS can be used for in-line detection of so-called spaghetti meat (SM), a myopathy in chicken breasts. NIRS is already being used for detection of WB, but the growing incidences of SM creates a need for automatic detection and sorting of this quality defect as well. The

objective of the study was to clarify whether it is possible to distinguish and classify the muscle myopathy SM from WB and normal chicken fillets under industrial conditions by the use of NIRS.

Materials and Methods

Samples

Skinless and boneless chicken breast fillets (*Musculus pectoralis major*) were sampled directly from the line in a commercial poultry processing plant (Nortura Hærland, Hærland, Norway) 3 h after CO₂ stunning, bleeding, and slaughter of the birds. The birds were as hatched, 32–34 d old and of the strain Ross 308. Average standard broiler live weight at the processing plant is 2.1 kg. Samples were collected over 3 d in order to obtain fillets from different flocks, which spanned a relevant bio-variability. A total of 90 normal, 90 WB, and 90 SM fillets were collected. All samples were graded for WB and SM as well as white striping (WS) by an experienced veterinarian based on visual inspection and palpation of consistency. WB were classified according to a scale from 0 (normal) to 3 (severe) based on criteria defined by Bailey et al. (2015). The WB sample group consisted of 1 fillet at level 1, 45 fillets at level 2, and 44 at level 3 (severe). SM were classified according to a scale from 0 (normal) to 2 (severe). Level 1 was mild SM, in which limited parts of the fillet were loose/stringy. At level 2, large parts or the entire fillet was stringy/spongy. The SM sample group consisted of 35 fillets at level 1 and 55 at level 2. WS was scored from 0 (no WS) to 3 (severe) according to Bailey et al. (2015). WS of levels 1 and 2 occurred frequently in both the WB and SM group. Moreover, in the SM group, there were 14 samples with WB scores of 1 and 2, and in the WB group there were 7 samples with an SM score of 2 and 30 samples with an SM score of 1. Average weights (with standard deviations in brackets) for the normal, WB, and SM fillets were 197.4 g (25.6), 253.8 g (31.6), and 227.3 g (33.1), respectively. The fillets were measured in movement at high speed with an on-line NIR scanner in the production hall. They were then packed in plastic bags and transported to Nofima research lab and stored at 4°C for further analyses the next day. Each fillet was then measured with another NIR instrument in steady state for comparison with the industrial measurements. Nuclear magnetic resonance (NMR) relaxation curves were measured on 30 samples from each of the 3 fillet classes.

NIR measurements

NIR instrument 1. The on-line NIR system used in the processing plant was a QVision500 (TOMRA Sorting Solutions, Leuven, Belgium), an industrial hyperspectral imaging scanner designed for on-line measurement of fat in meat on conveyor belts. The instrument is based on interreflectance measurements in which the light is transmitted into the meat and then back scattered to the surface. Optical sampling depth in the chicken fillets is approximately 15 mm. The skin sides of the fillets were scanned on a moving conveyor belt, and each NIR measurement took less than 1 s. The scanner was placed 30 cm above the conveyor belt so there was no physical contact between samples and the instrument. The scanner collected hyperspectral images of 15 wavelengths between 760 and 1,040 nm with a spectral resolution of 20 nm. The output per sample scan was an image of the entire fillet with a rather coarse spatial resolution as seen in Figure 1B–1C. Each pixel represented a spatial area of about 7 mm × 5 mm across and along, respectively, the conveyor direction. The imaging capability of the used system was, in this work, used mainly to obtain one average spectrum from the whole fillet, but also for inspection of protein distribution within the fillets.

NIR instrument 2. The other NIR instrument was originally designed to measure fat in the muscle of live salmon (Folkestad et al., 2008). Two halogen light sources of 50 W each illuminate the sample in 2 rectangular regions of 5 mm × 20 mm size. Distance between the 2 illuminated regions is 10 mm. The system collects the light that has penetrated down into the sample and comes up again in a small area of 4 × 4 mm between the

2 illuminated rectangles. The system measures in the exact same spectral region and has the same spectral resolution as NIR instrument 1. The measurement principle is also the same, but system 2 is not imaging but measures deeper into the samples (about 20 mm). The measurements were done in steady state on the skin side of the fillets at the location indicated in Figure 1A (while NIR system 1 obtained an average spectrum from the entire skin side). The system is thoroughly described in O’Farrell et al. (2011). Each measurement took 1 s. The temperature of fillets was 4°C.

NMR relaxation measurements

For 30 fillets of each fillet class, a cylindrical sample of diameter 8 mm and height approximately 20 mm was excised from the same spot at which the second NIR measurement was performed (Figure 1A). The entire cylinder was weighted and placed in a sealed Teflon container, which was inserted in the NMR probe. Before measurement, the samples were thermostated to 25°C. The transverse (spin-spin) relaxation time T_2 was measured with a Maran Ultra Resonance 0.5 T (Oxford Instruments, Oxfordshire, UK). T_2 was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958). The T_2 measurements were done with a tau value of 150 μ s. Data from 8,000 echoes were collected during 16 scans. The Carr-Purcell-Meiboom-Gill pulse sequence data were analyzed using WinDXP software (Resonance instruments, Oxfordshire, UK). To show the distribution of water binding among the chicken samples, we calculated a loosely bound water index: the area under the T_2 curve in the region 0.18–0.50 s divided by the area in the region 0.02–0.1 s.

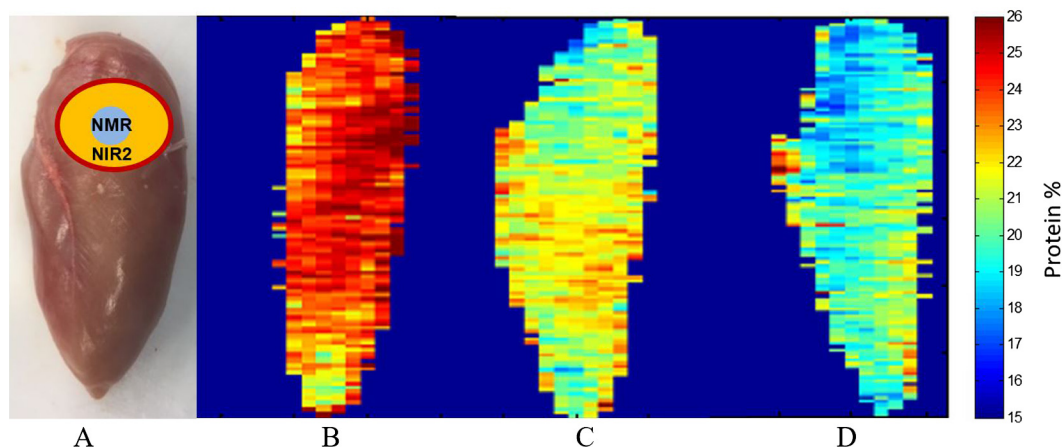


Figure 1. (A) Chicken breast with indicated measurement location for NIR instrument 2 (yellow region) and excised cylinder for NMR (blue region). Chemical maps based on NIR instrument 1 showing estimated distribution of protein concentration in (B) normal, (C) moderate WB, and (D) severe WB. NIR = near infrared; NMR = nuclear magnetic resonance; WB = wooden breast.

Data analysis

Spectral preprocessing. The NIR spectra were linearized using the logarithm of the inverse of the intercalance spectrum (T), $\log_{10}(1/T)$. To reduce the effects of light scattering and varying distance to sample, the spectra were normalized by standard normal variate (Barnes et al., 1989): for each spectrum, the mean value was subtracted, and the spectrum was then divided by the standard deviation of the spectrum.

Estimation of protein. From prior reported work we had available a protein calibration for chicken fillets based on data from NIR instrument 1 (Wold et al., 2017). This calibration was based on partial least-squares regression (Martens and Næs, 1993). The calibration could be applied on average spectra from the hyperspectral images or on single pixels to obtain chemical images showing the protein distribution. Another protein calibration for chicken fillets had been obtained from NIRS instrument 2 (Wold et al., 2019). This model was used to estimate protein in the 270 fillets based on the NIR measurements at the rostral location (Figure 1A).

Linear discriminant analysis (LDA; Duda and Hart, 1973) was used to test how well NIR spectra could discriminate the different classes (normal, SM, and WB) from each other. Since the variables in NIR spectra are generally highly correlated, we used the score values from a principal component analysis (Martens and Næs, 1993) of these data. These score values are orthogonal to each other and well suited as input variables in LDA. The functions were validated by full cross-validation (leave one sample out).

The software The Unscrambler version 9.8 (CAMO Software AS, Oslo, Norway) was used for principal component analysis and protein predictions. Calculation of LDA, image processing, and spectral preprocessing was carried out in MATLAB version 7.10 (MathWorks Inc., Natic, MA).

Results and Discussion

Analysis of spectral properties

Figure 2 shows typical normalized NIR spectra from chicken fillets in the relevant spectral region (from NIR instrument 2; spectra from instrument 1 were similar). The main peak at around 980 nm stems from the absorption of water. There are also contributions from fat (around 930 nm) and protein (around 1,020 nm), but these are difficult to discern. The spectra

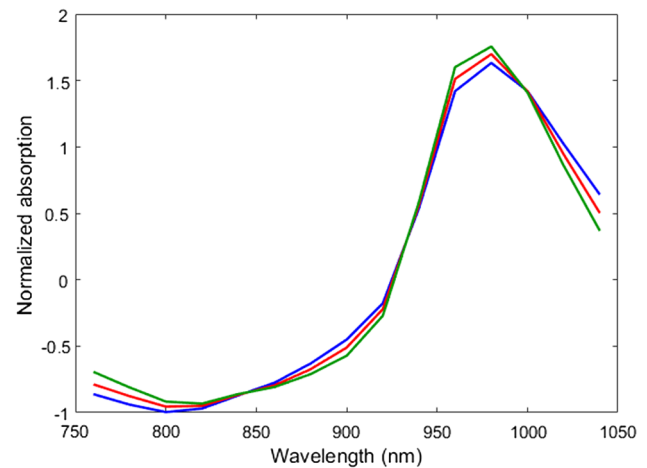


Figure 2. Typical NIR spectra from normal (blue), SM (red), and WB (green) chicken fillets. NIR = near infrared; SM = spaghetti meat; WB = wooden breast.

shown are from one normal fillet, one SM, and one severe WB. Note that the water peak in spectra from WB fillets was shifted towards shorter wavelengths compared with normal fillets, as previously reported (Wold et al. 2017; Wold et al., 2019). Furthermore, SM expressed this shift, although it was not as pronounced. The water absorption peak is affected by the water-binding properties. Hydrogen bonding between water and protein molecules causes a spectral shift towards longer wavelengths as well as a peak broadening (Chung et al., 2008). This indicates that WB as well as SM muscle contains more loosely bound water than normal muscle. The first principal component of the NIR spectra expressed this shift (92% of the total variation in the spectra), and the variations for all 270 samples are shown in Figure 3A (estimated for NIR instrument 2). Low score values indicate large shifts towards shorter wavelengths and consequently more loosely bound water in the muscle. Most of the WB samples had values below 0, whereas most of the normal samples were above 0. This difference alone was almost enough to fully separate the 2 classes. The SM samples, on the other side, were in between and overlapped the 2 other groups. Spectra from NIR instrument 1 gave a similar result, but with slightly more overlap between normal and WB.

Soglia et al. (2016) measured water mobility by the use of NMR and reported that there is a significantly higher share of loosely bound water in WB muscle than in the normal ones, probably due to muscle fiber degeneration. The same was found by Wold et al. (2019). Figure 4A shows the mean T_2 relaxation distribution curves for the 30 randomly picked samples from each of the groups normal, SM, and WB. The curves consist of 2 major components, which are characteristic for

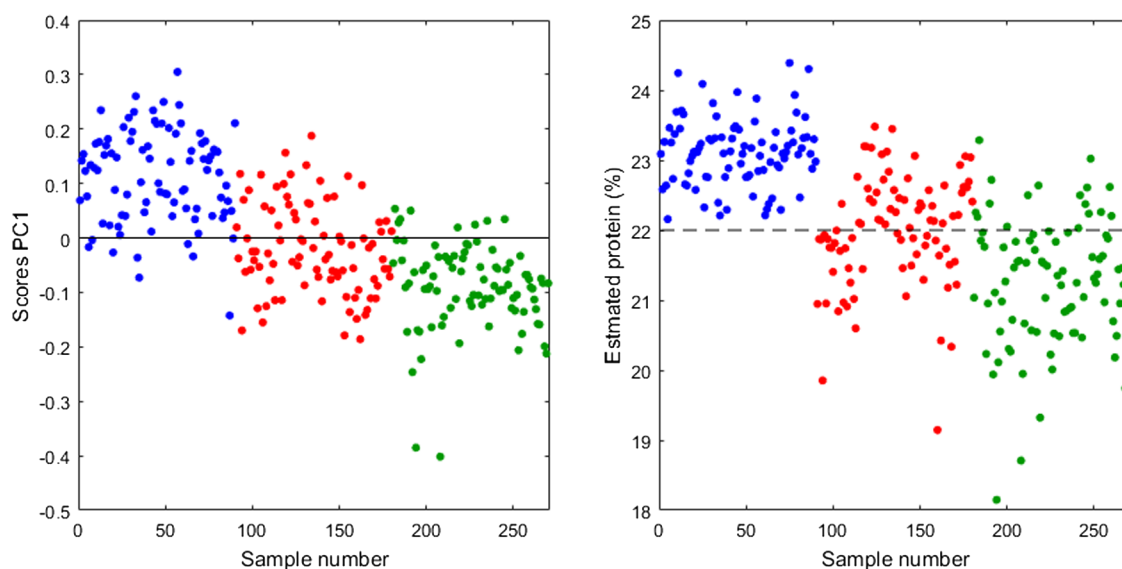


Figure 3. Left plot: PC1 score values for samples of normal (blue), SM (red), and WB (green) fillets. Right plot: Estimated protein content in the same samples as in panel A. PC1 = first principal component; SM = spaghetti meat; WB = wooden breast.

meat. The peak between 0.02 and 0.1 s indicates the share of water bound in myofibrils, and the smaller peak in the region 0.18 to 0.50 s indicates share of more loosely bound water (Bertram et al, 2002). The curves clearly illustrate that there was a much larger share of loosely bound water with higher mobility in WB muscle. Furthermore, SM muscle tended to contain more loosely bound water, but not as much as WB. To show the distribution among the 30 samples from each group, the loosely bound water indexes are plotted in Figure 4B. All WB samples had high indexes, whereas normal samples had low values. The SM samples varied from normal values to quite high, which supports findings by

Baldi et al. (2018) that SM has a higher proportion of loosely bound water in the superficial section. The pattern in Figure 4B can, to a large extent, explain the shift variation in the NIR spectra (Figure 3A).

Based on an existing NIR calibration for protein in chicken breast meat, we estimated the protein in all 270 samples based on NIR instrument 2. Figure 3B shows the distribution of protein values in the 3 groups. Normal muscle had estimated protein concentrations above 22%, and most of the WB fillets were below 22%, whereas the SM samples were again distributed between the normal and WB population. It is well known that WB has lower total protein content compared with normal

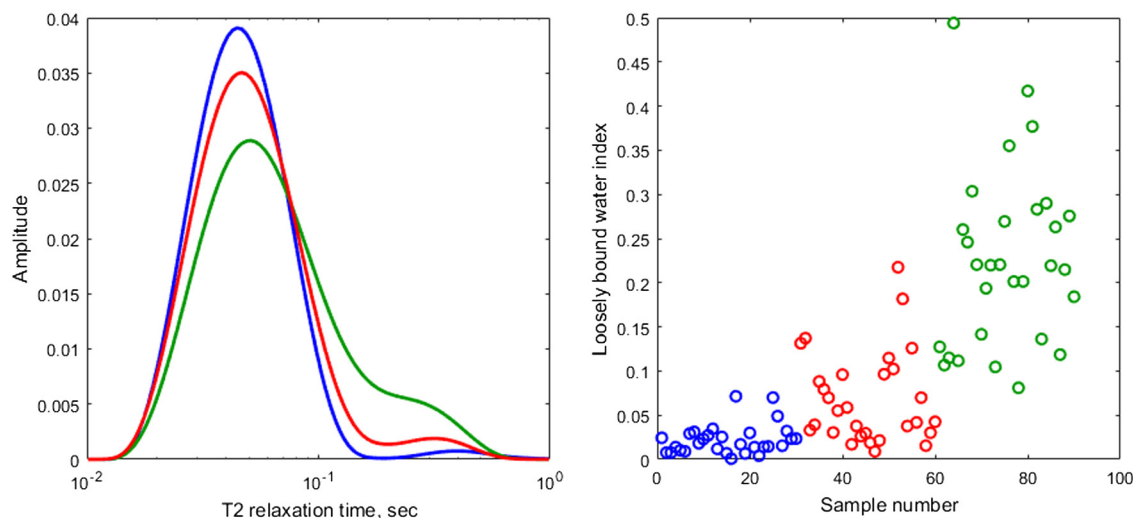


Figure 4. Left plot: Mean normalised NMR T_2 relaxation curves for the groups normal (blue), SM (red), and WB (green). Right plot: Loosely bound water index for samples of normal (blue), SM (red), and WB (green) chicken breasts. NMR = nuclear magnetic resonance; SM = spaghetti meat; T_2 = transverse relaxation time; WB = wooden breast.

muscle, and it is also reported that SM fillets have significantly lower protein content in the superficial regions (Baldi et al., 2018). Protein estimates from NIR instrument 1 followed the same trend.

The industrial NIR instrument 1 is imaging, which means that it is possible to apply an NIR calibration for protein at the pixel level. Figure 1B–1D illustrates the distribution of protein content in 1 normal and 2 WB fillets (1 moderate and 1 severe). The normal fillet had an overall high protein level, while the 2 others had lower levels. For the 2 WB fillets, there was also a tendency to have slightly less protein in the rostral region, which is the location where the WB myopathy usually develops first and is most pronounced. The images illustrate the heterogeneity in chicken breasts and underlines the importance of thoughtful sampling.

Discriminant analysis

In this section, we compare the ability of the 2 NIR systems to discriminate between the 3 classes of chicken fillets. Based on the results given earlier, it is clear that NIRS contains information about differences in both protein and water binding and

should therefore be a promising method for rapid quality classification. Tables 1–2 summarize the results that show how well different quality classes can be separated from each other and how well WB and SM together can be distinguished from normal fillets. NIR instrument 2 performed slightly better than the on-line NIR instrument 1, with overall higher rates of correct classifications. None of the cases were 100% correctly classified, which is reasonable because the myopathies vary in severity and because there will always be a gradual gradient from normal to affected muscle. WB was well separated from normal as reported by others (Wold et al., 2017; Wold et al., 2019). The SM samples were to a higher degree classified either as WB or normal. Severe SM could typically be classified as WB, and moderate SM classified as normal. This harmonizes with the aforementioned results, which suggest that the SM sample group overlapped both the normal and WB groups in terms of protein content and degree of loosely bound water. In addition, we observed symptoms of both WB and SM on several fillets. These fillets were in a grey zone between the 2 classes and illustrate that the classes were indeed not distinct but were also

Table 1. Classification results based on on-line NIR instrument 1

	Predict Normal	Predict WB		Predict Normal	Predict SM
True Normal	83	9	True Normal	73	23
True WB	7	81	True SM	17	67
	<i>91.1% correct</i>			<i>77.8% correct</i>	
	Predict SM	Predict WB		Predict Normal	Predict SM+WB
True SM	73	24	True Normal	78	36
True WB	17	66	True SM+WB	12	144
	<i>77.2% correct</i>			<i>82.2% correct</i>	

“Predict” indicates predicted class. “True” indicates the true class. % correct classified samples is given for each case.

NIR = near infrared; SM = spaghetti meat; WB = wooden breast.

Table 2. Classification results based on NIR instrument 2

	Predict Normal	Predict WB		Predict Normal	Predict SM
TrueNormal	87	3	TrueNormal	76	12
TrueWB	3	87	TrueSM	14	78
	<i>96.6% correct</i>			<i>85.5% correct</i>	
	Predict SM	Predict WB		Predict Normal	Predict SM+WB
TrueSM	70	20	TrueNormal	80	23
TrueWB	20	70	TrueSM+WB	10	157
	<i>77.8% correct</i>			<i>87.8% correct</i>	

“Predict” indicates predicted class. “True” indicates the true class. % correct classified samples is given for each case.

NIR = near infrared; SM = spaghetti meat; WB = wooden breast.

overlapping when judged by a trained veterinarian. The occurrence of WS did not much affect the NIR spectra in this study and most likely did not affect the classification results. WS is limited mainly to the surface of the fillets, while the NIR systems in this study measured in depth.

Better performance by NIR instrument 2 can be explained by the following factors.

1. Samples measured by NIR instrument 2 held a stable temperature of 4°C, whereas samples in the process measured by NIR instrument 1 varied in temperature from 2°C to 6°C. Temperature variations induce a shift in the water absorption peak, which might disturb classifications.
2. NIR instrument 2 measured on a limited part of the fillet but measured deeper into the samples and obtained signals with higher signal-to-noise ratio, which might be of importance to separate subtle differences in the sample groups.

NIR spectra with instrument 2 were recorded about 18 h after those of NIR instrument 1. With time post mortem, the hardness of the WB condition is known to decrease, and fillets might lose moisture via drip loss (to a higher degree in WB and SM fillets). These potential changes would most likely reduce the differences between the normal and the myopathy groups, making it more difficult to separate them with NIR. Satisfactory results were still obtained.

Conclusions

It is possible to use on-line NIRS to detect the myopathy WB in chicken fillets with good accuracy. SM was slightly more difficult to separate from both normal and WB because properties measured by NIRS were overlapping between the 3 quality groups. Two quite similar NIRS instruments performed differently, which emphasizes the importance of optimizing instrumentation, sampling, and process (e.g., temperature stability) for optimal performance of applications. Depending on the purpose of a potential industrial use of this method (sorting or registration), decision limits can be adjusted for optimal use.

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