

Perspective of NIRS measurements early post mortem for prediction of pork quality

A.H. Hoving-Bolink ^{a,*}, H.W. Vedder ^b, J.W.M. Merks ^c, W.J.H. de Klein ^d,
H.G.M. Reimert ^a, R. Frankhuizen ^e, W.H.A.M. van den Broek ^b, en E. Lambooi ^a

^a Animal Sciences Group Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands

^b Agrotechnology and Food Innovations Wageningen UR, P.O. Box 17, 6700 AA Wageningen, The Netherlands

^c IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA Beuningen, The Netherlands

^d Nutreco Agriculture Research and Development, P.O. Box 240, 5830 AE Boxmeer, The Netherlands

^e RIKILT – Institute of Food Safety Wageningen UR, P.O. Box 230, 6700 AA Wageningen, The Netherlands

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Abstract

The potential of near-infrared spectroscopy (NIRS) measurements early post mortem was investigated to predict ultimate drip loss, colour, tenderness and intra-muscular fat of pork. Three locations (*M. longissimus thoracis*, *M. longissimus lumborum* and *M. semimembranosus*) in 102 pig carcasses were tested at the end of the slaughter line. A priori variation in pork quality was introduced using an experimental design covering: genotype, lairage time, pre-slaughter handling and day of slaughter. At 1 h post mortem a diode array VIS/NIR instrument (Zeiss MCS 511/522, 380–1700 nm) equipped with a surface fibre optic probe was used and at 1 day post mortem ultimate pH, drip loss, colour and shear force was measured on similar locations. Results indicated that it was possible to predict intra-muscular fat content (correlation (R^2) of 0.35 with multiple linear regression), standard error of prediction (SEP) = 3.6 g/kg, but the configuration has to be refined for on-line application (bigger aperture). For drip loss no correlation was achieved with the PLS method. Even extremes (low drip loss (<2.5%) or high drip loss (>4.5%)) in drip loss were not discriminated. Predicting drip loss with NIRS early post mortem is not successful, although NIRS in the slaughter line has potential as a fast predictor of intra-muscular fat. Possibilities for using the NIRS technique to get to know more about muscle metabolism and post mortem changes are promising.

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1. Introduction

Rapid screening techniques to determine quality characteristics of meat are of great interest for both industry and consumers. Pork that is aberrant in colour and/or water holding properties, in its most extreme form called PSE, is a big problem for the industry. Poor water hold-

ing capacity during storage results in reduced technological yield. From a logistic point of view, it is attractive to do the sorting of carcasses on the day of slaughter. Forrest et al. (2000) suggested that near-infrared spectroscopy (NIRS) is a valuable technique for predicting drip loss at day of slaughter. They constructed a PLSR model on a wide range of drip loss, based on early post mortem NIR spectra. The main objective of present experiment was to study the predictive value of early post mortem NIRS measurements in a normal meat quality range with an instrument which was suitable for use in a slaughter

* Corresponding author. Tel.: +31 320 238251; fax: +31 320 238094.
E-mail address: rita.hoving@wur.nl (A.H. Hoving-Bolink).



Fig. 1. Measurement with the probe of the Zeiss MCS 511/512 instrument.

house. The emphasis was on such quality aspects as drip loss, intra-muscular fat and colour. During the conversion of muscle to meat the metabolic process of rigor development and protein denaturation influence the water holding capacity of meat. From other studies, it is clear that post rigor drip loss during storage can be predicted adequately by NIRS (Geesink et al., 2003 & Brøndum et al., 2000). And it is proven that NIRS is a rapid tool for quality attribute determinations in food and feeds. An example is the prediction of intra-muscular fat and tenderness in beef, the first parameter could successfully be predicted, the second not (Rødbotten, Nilssen, & Hildrum, 2000). In our study, the potential of NIRS to predict pork quality attributes of early post mortem samples by using a diode array VIS/NIR instrument for measurements on intact carcasses at a commercial slaughterhouse is investigated (Fig. 1).

2. Materials and methods

2.1. Experimental design

A total of 130 pigs were slaughtered in 4 days at a commercial slaughter plant. In order to obtain variation in meat quality an experimental design was made with two genotypes (Yorkshire and Piétrain crossbreds), two lairage times (delivering the evening before and in the morning before slaughter) and two treatments before slaughter (relaxed or 5 min stress (Hambrecht, Eissen, & Versteegen, 2003)). The plant used an automatic head-to-heart electrical stunning system and the line speed was 600 pigs/h. The pigs came from the experimental farm of IPG, had an average carcass weight of 86 kg (SD 8) and a meat percentage of 57.1% (SD 2.6). NIRS measurements were taken about 1 h after exsanguination. The day after slaughter, the carcasses were dissected for the meat quality measurements.

2.2. Measuring locations

Per carcass, three anatomic locations were measured, so information about variation between muscles was

gathered. The locations were *M. longissimus thoracis* (LDT) (13th thoracic vertebrae) and *M. longissimus lumborum* (LDL) (third to fourth lumbar vertebrae) and *M. semimembranosus* (SM) (Fig. 2). A piece of back fat was removed from the carcass, to reach the LDT and LDL location for surface measurements. The day after slaughter, at these locations pieces of meat about 12 cm in length were taken (less from the SM, because no tenderness measurements were made on this muscle) for meat quality measurements and another set of NIRS measurements.

2.3. Meat quality measurements

Temperature and pH measurements were determined with an Ebro TFN 1093 SK thermometer and a Schott CG 818 pH equipment with Xerolyt® electrode. The day after slaughter the muscles were removed from the carcass and meat quality measurements were carried out. Conductivity (LFu) was measured by an LF-STAR. Meat quality attributes drip loss, colour and tenderness were determined the day after slaughter under practical circumstances according to the internationally accepted reference methods for water holding capacity, tenderness and colour of meat (Honikel, 1997). Drip loss was measured after 5 days of chilled storage on a slice of 1 cm (100–150 g) placed on a grid in a container. The sides of the slices for drip loss were not totally free from surrounding connective tissue. Heating loss was measured after heating for 1 h at 75 °C a piece of meat 5 cm in length. Tenderness was assessed by the Warner-Bratzler shear test on six sample strips of 10 × 10 mm cross-section. Meat colour was determined in triplicate

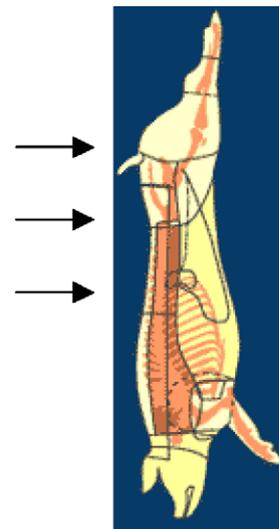


Fig. 2. Anatomic measuring locations: *M. longissimus thoracis* (about 13th thoracic vertebrae, LDT), *M. longissimus lumborum* (third to fourth lumbar vertebrae, LDL) and *M. semimembranosus* (SM), starting cranial.

after blooming for 30 min by measuring L^* , a^* and b^* values with a Minolta Chromometer CM525i, D65 lightsource, 10° observer and 25 mm measuring aperture. As an additional water holding technique the filter paper method (FP) was carried out (Kauffman, Eikelenboom, van der Wal, Merkus, & Zaar, 1986). Intra-muscular fat was determined on representative samples according ISO 1444 (Soxhlet extraction procedure for determination of free fat content (with petroleum ether, without acid pre-treatment)). On these data, a NIRS model (1206/1610 nm) was made for all the samples (IMVET).

2.4. NIRS measurements

About 1 h post mortem for the NIRS measurements a diode array VIS/NIR instrument equipped with a surface fibre optic probe for measurements on intact carcasses was used. The visible range (380–1000 nm) was scanned using a 256 pixel photodiode array (Zeiss MCS 521). The near-infrared range (1000–1700 nm) was scanned using a 128 pixel multiplexed InGaAs detector (Zeiss MCS 511 NIR 1.7). The instrument was equipped with a 20 W halogen light source and a diffuse reflection probe with 35° geometry with an effective measuring area of 1 mm². For transport of the light to the probe and back to the detectors a glass fibre cable of 3 m was used. A 20% reflection standard (USRS-20-010) was used as reflectance standard. Every spectrum consisted of 200 scans averaged; every scan took 45 ms so collecting a spectrum including data transfer took 10 s. During these 10 s the probe was moved 3 cm across the muscle surface, to enlarge the sampling area (30 mm²). The total volume of sample analysed varies with the spectral range. In the visible part (400–750 nm), meat is relatively opaque and information can be collected up to proximally 1 cm. In the NIR part, especially at the first overtone of the OH-band (± 1450 nm) penetration depth is at most 1 mm, due to the high water content.

2.5. Data analysis

The experiment is analysed with two goals.

1. To analyse the experimental design, an analysis of variance for unbalanced data (Wald test after REML analysis) in GenStat Release 6.1 (2002) was used. The experimental design covered genotype, lairage time, pre-slaughter handling, day of slaughter and sex (all fixed in the model).
2. To determine relationships between NIRS measurements in the slaughter line and final meat quality attributes multivariate regression techniques were used. Spectral data were corrected for scattering effects by transforming them to the second derivate

and calibration models were produced with multiple linear regression (MLR) using WinISI version 1.04 (Foss NIRSystems/Tecator) and partial least squares regression (PLS) techniques using Unscrambler version 6.0 (CAMO A/S, Norway). The MLR-models were not validated because of the limited number of samples, the PLS-models were validated using cross validation (five random subsets).

3. Results

3.1. Results experimental design

In Table 1, the summary statistics of the meat quality parameters are presented. The ultimate pH in the LDT ranged from 5.36 to 5.82 and drip loss varied between 0.7% and 6.8%. The colour L^* values ranged between 49.5 and 63.5. In Table 2, for anatomic location LDL, the main effects of genotype, lairage time, pre-slaughter handling are given. The Yorkshire crossbreds had a little higher pH 24 and a better water holding capacity than the Piëtrain crossbreds. The time in lairage had no effect. Rough pre-slaughter handling a few minutes before slaughter resulted in deterioration of meat quality. It gave a fast decline in pH soon after slaughter and a somewhat higher ultimate pH. The muscles were higher in drip loss than after a gentle treatment, but the differences were smaller than the genotype differences. Significant two-way interactions were recorded, these effects were difficult to interpret.

There is an obvious relationship in most meat quality characteristics between anatomic locations. For water holding capacity, intra-muscular fat and shear force the correlation coefficient is between 0.6 and 0.7, for

Table 1
Statistics for the meat quality attributes

Attribute (<i>n</i> = 130)	Mean	Minimum	Maximum	Standard deviation
pHu LDT	5.52	5.36	5.82	0.09
pHu LDL	5.59	5.36	6.10	0.13
pHu SM	5.62	5.40	6.18	0.16
FP LDT (mg)	60	10	120	22
FP LDL (mg)	69	20	130	25
FP SM (mg)	74	20	150	24
Minolta L^* LDT	55.6	49.5	63.5	2.4
Minolta L^* LDL	56.3	49.9	67.0	2.8
Minolta L^* SM	52.0	42.9	59.2	3.0
Heating loss LDT (%)	30.9	20.2	41.3	3.5
Heating loss LDL (%)	30.2	20.1	40.7	3.9
Shear force LDT (N)	48	27	72	11
Shear force LDL (N)	48	24	81	11
Drip loss LDT (%)	3.2	0.7	6.8	1.3
Drip loss LDL (%)	3.2	0.6	7.0	1.5
Drip loss SM (%)	3.7	0.7	8.6	1.6

Table 2
Main effects ($P < 0.005$) of the analysis of variance for genotype, lairage time, pre-slaughter handling for anatomic location LDL

Parameter ($n = 130$)	Significant two-way interaction ^a	Prob. main effect of lairage time	Predicted mean evening delivery	Predicted mean morning delivery	s.e.d.	Prob. main effect of pre-slaughter handling	Predicted mean stress handling	Predicted mean gentle treatment	s.e.d.	Prob. main effect of genotype	Predicted mean Piétrain genotype	Predicted mean Yorkshire genotype	s.e.d.	Prob. day of slaughter	Prob. sex
Carcass weight (kg)	Lairage ^a slaughter day	Ns	86.2	86.5	1.3	Ns	85.9	86.8	1.2	0.000	83.6	89.1	1.2	0.000	Ns
HGP-meat (%)		Ns	57.0	57.1	0.4	Ns	57.2	56.9	0.4	0.000	58.0	56.1	0.4	Ns	0.000
pH 4	Lairage ^a slaughter day	0.005	5.70	5.81	0.04	0.000	5.67	5.84	0.04	Ns	5.73	5.78	0.04	0.002	Ns
pHu		Ns	5.59	5.58	0.02	0.007	5.61	5.55	0.02	0.002	5.55	5.62	0.02	Ns	Ns
Temp 4 (°C)		Ns	20.6	20.5	0.3	Ns	20.7	20.4	0.3	0.04	20.2	20.9	0.3	0.000	Ns
IMVET (%)		Ns	1.46	1.33	0.1	Ns	1.43	1.36	0.1	Ns	1.41	1.38	0.1	Ns	0.000
LFu (mS)		Ns	10.1	9.5	0.4	0.000	11.3	8.3	0.4	Ns	9.9	9.8	0.4	0.000	Ns
FP (mg)		Ns	69	69	4	0.001	76	63	4	0.001	76	63	0.4	Ns	0.02
Minolta <i>L</i> *		Ns	56.4	56.2	0.5	Ns	56.6	56.0	0.5	Ns	56.2	56.4	0.5	Ns	0.01
Minolta <i>a</i> *	Lairage ^a slaughter day	Ns	6.3	6.3	0.2	Ns	6.4	6.2	0.2	0.02	6.5	6.1	0.2	0.02	0.03
Minolta <i>b</i> *	Lairage and handling ^a slaughter day	Ns	13.4	13.4	0.2	Ns	13.3	13.4	0.2	Ns	13.4	13.2	0.2	0.04	Ns
Heating loss (%)	Lairage and handling ^a slaughter day	Ns	29.9	30.3	0.6	0.000	31.6	28.6	0.5	0.04	30.7	29.5	0.6	0.000	Ns
Shear force (N)		Ns	49	46	2	Ns	48	48	2	Ns	46	49	2	0.001	0.04
Drip loss (5 days) (%)		Ns	3.2	3.2	0.3	Ns	3.4	3.0	0.2	0.000	3.8	2.5	0.2	Ns	Ns

Ns means not significant.

^a Significant two-way interactions are written down, the predicted means are then italic.

colour this was lower 0.4–0.56 (Table 3). The water holding characteristic of drip loss and FP had a correlation coefficient of about 0.7 and the drip loss correlation with ultimate pH was about -0.5 (not in the table).

3.2. Results NIRS

The spectra determined on the first day of the experiment (the first 28 animals) were measured against a

Table 3
Correlation coefficients for the meat quality attributes within an animal

	LDL	SM
pHu LDT	0.67	0.58
FP LDT (mg)	-0.22	0.53
Minolta L^* LDT	0.56	0.39
Heating loss LDT (%)	0.71	^a
Shear force LDT (N)	0.70	^a
Drip loss LDT (%)	0.70	0.69
IMVET LDT (%)	0.64	^a

^a No information, for the *M. semimembranosus* no heating loss, tenderness and intra-muscular fat is determined.

100% reflection standard. Due to the high water absorbance of the fresh meat cuts the odd–even characteristic of the diode array detector was dominating the spectral signal (saw tooth shaped spectra). By using a 20% reflectance standard for the additional experiments (102 of the 130 animals) this effect was reduced effectively. In Fig. 3 from the spectra measured early post-mortem transformed to the second derivatives are given. This figure shows a lot of variation for some colours (400–750 nm), water (OH second overtone around 960 nm and first overtone around 1440 nm) and fat (CH_2 second overtone around 1200 nm). In Table 4, the results for the predictive models are given. For intra-muscular fat a calibration model was derived using MLR, with a primary wavelength of 1210 nm (R^2 of 0.35). This wavelength can be assigned to fat. The other constituents did not show a correlation with a wavelength (area). Therefore, PLS models seem to perform better. For colour a weak relationship was found with the Minolta L^* value (R^2 of 0.25, not in Table 4) and for the Minolta a^* value (R^2 of 0.31). For drip loss no correlation with information from the NIR spectra was found. It appeared not to be possible to develop a model for drip loss at the day of slaughter.

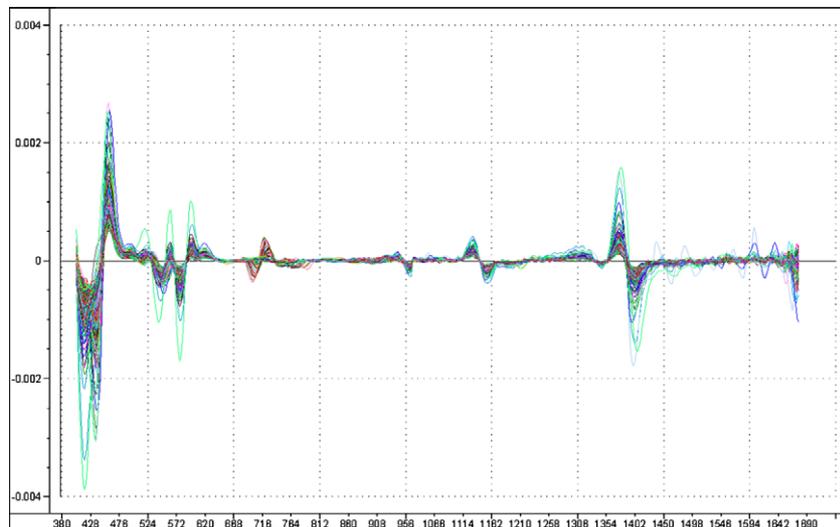


Fig. 3. Second derivate spectra of the Zeiss MCS 511/522 measured early post mortem.

Table 4
Model results for the NIRS predictive models

Attribute	Intra-muscular fat (g/kg)	Colour (a^*)	Drip loss (%)	Drip loss (%)
Model	MLR	PLS2	PLS2	PLS2
First selected wavelength	1210 nm (CH_2 -vibrations)	400–750 nm	1400 nm (OH-vibrations)	Model with meat quality parameters included
Coefficient of determination (R^2)	0.35	0.31	0.004	0.06
SEC(V)	3.6	1.0	–	1.6
n	102	102	102	102

4. Discussion

A reasonable variation in meat quality was realised through the experimental design, but no sample was classified as DFD meat (pH > 6.4 and dark) and only one sample was classified as nearly PSE (pH < 5.5 and pale and exudative).

The water holding characteristics of drip loss and FP have a correlation coefficient of about 0.7. Brøndum et al. (2000) found a correlation between the two water holding capacity methods of 0.61 and attributed this to the fact that the two methods give different information on the water holding capacity in meat. Drip loss reveals the amount of free water that exudes under the force of gravity from the muscle fibres, the FP measures the water that is extracted by capillary force.

For intra-muscular fat ($R^2 = 0.35$) two facts must be recognized; the variation in the intra-muscular content is limited (with a minimum of 5.1 and a maximum of 25.7 g/kg) and the measurements suffered from the small sampling area in a heterogeneous material (meat). Using a NIR-spectrometer with a larger sampling area (laboratory system with a sampling area of $\pm 5 \text{ cm}^2$) a R^2 of 0.7 can be achieved (data not reported). Thus, there are possibilities for measuring intra-muscular fat.

Colour predictions are poor as well, although the spectral range of the VIS/NIR spectrometer covers the wavelength used in the colour measurements (Minolta values). Colour developed during the rigor process is poorly related to colour immediately after slaughter. Cozzolino, Barlocco, Vadell, Ballesteros, and Gallieta (2003) found no satisfactory result on intact pork muscle samples the day after slaughter. Homogenisation of the sample gave a better correlation, the R^2 was >0.70.

The predictive ability of the NIR-models is poor. For drip loss, it was not possible to construct a useful NIRS with respect to the R^2 value. Although a standard error of prediction (SEP) of 1.0% is quite acceptable, compared with the SEP of 1.8% reported by Forrest et al. (2000). An explanation could be the limited variation in meat quality in Dutch pork in comparison with that studied by Forrest et al. (2000). The range drip loss in the Danish experiment was from 0.9% to 16.9% and in the present study was from 0.7% to 6.8%. In a second Danish experiment, with meat of normal quality, no useful NIRS model could be developed (personal communication Borggaard, 2003). In our experiment the residual standard deviation (RSD) of the reference method for drip loss was almost as big as the root mean squares (RMS) of the population (1.1% versus 1.4%). This implies that predicting drip loss with NIRS on the slaughter line with this method will be difficult, since the RMS/RSD ratio is too small.

In this study, as in the study of Geesink et al. (2003), the NIR-spectra of the meat cuts were recorded 2 days post mortem using the same Fourier transform NIR

spectrophotometer (unpublished data). The prediction errors for drip loss were similar (Geesink et al. 1.0% and this study 1.1%). Using the diode array VIS/NIR spectrophotometer 2 days post mortem for drip loss a prediction an error of 1.0% was found. From these results, it can be concluded that for drip loss the NIR-instrument was not a limiting factor. It is more likely the forming of drip loss itself, during the conversion of muscle to meat. Differences are not seen 1 h after slaughter. So, it is questionable if NIR at that time can determine whether the meat is going to develop high or low drip loss. Pedersen, Morel, Andersen, and Engelsens (2003) published results of spectral information determined on the slaughter line with a correlation of 0.79–0.89 with water holding capacity. They used information from the IR region. They concluded there was a coherence between water holding capacity, pH, glycogen level and protein conformations which is supported by other research. From the principal components study of de Vries, van der Wal, Long, Eikelenboom, and Merks (1994), it appeared that two components explained 66% of the variance in the meat quality parameters. The two were associated with muscle metabolism and water holding capacity.

Individual pigs will respond in different ways to stressors such as loading, transport, unloading and pre-slaughter handling. The physiological status of the animal at the moment of slaughter (exhausted, stressed or relaxed), is an important factor determining meat quality. Psychological or physical stress immediately before slaughter may lead to excessive glycogenolysis in the muscles which will lower the pH relatively rapidly. As a consequence, meat can become pale and soft, exuding much of tissue's water (Hambrecht et al., 2003; Lamb-ooy et al., 2004) although there can be a discrepancy between colour and water holding capacity (Kauffman et al., 1993). Also, we expected that pigs subjected to transport stress and feed deprivation would be exhausted at the moment of slaughter and that much of the glycogen in the muscles will be depleted yielding a relatively high ultimate pH. This was not confirmed. Probably because the time of feed withdrawal was not big enough to get significant differences between the two treatments. The effect of stress immediately before slaughter was as expected. As no NIRS prediction model could be developed, no groups could be classified for water holding capacity differences.

5. Conclusion

NIRS on the slaughter line has potential as a fast predictor of intra-muscular fat. Predicting drip loss with NIRS early post mortem is not successful. Possibilities for using NIRS to understand about muscle metabolism and post mortem changes are promising. Yorkshire

crossbreds had a better water holding capacity than Piëtrain crossbreds and rough handling before slaughter caused a deterioration in water holding capacity. Time in lairage had no influence. Furthermore, a good correlation for meat quality characteristics was found between different anatomical locations within animals.

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