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**The use of visible and near-infrared reflectance spectroscopy to predict beef
M. longissimus thoracis et lumborum quality attributes**

**S. Andrés^a, A. Silva^b, A.L. Soares-Pereira^c, C. Martins^b, A.M. Bruno-Soares^{c*}, and
I. Murray^d**

^a – Departamento de Producción Animal I, Universidad de León, E-24071 León,
Spain

^b – Universidade de Trás os Montes e Alto Douro, (CECAV-UTAD), Ap. 1013,
5000-911 Vila Real, Portugal

^c – Instituto Superior de Agronomia, (DPAA- ISA), Tapada da Ajuda, 1399 Lisboa
Codex, Portugal

^d – Scottish Agriculture College, (SAC), Ferguson Building, Craibstone Estate,
Bucksburn, Aberdeen, AB21 9YA, UK

*- Corresponding author (e-mail: abs@isa.utl.pt)

26 **Abstract**

27 Visible and near infrared reflectance spectroscopy was used to predict pH at 24 hours
28 (pH₂₄) *post-mortem*, sarcomere length (SL), cooking loss (CL), Warner-Bratzler shear
29 force (WBSF) and colour parameters (L*, a*, b*) in beef cattle samples. Samples from
30 *M. longissimus thoracis et lumborum* from 30 bulls were aged at 4° C for 1, 3, 7 and 14
31 days and analysed for pH, SL, CL, WBSF and colour. NIRS calibrations for pH₂₄,
32 luminosity at 0 ($L^*_{t_0}$) and 60 minutes ($L^*_{t_{60}}$) showed good predictability ($R^2= 0.97, 0.85$
33 and 0.82 ; SECV = 0.10, 1.16, 1.36, respectively), whereas those related to the rest of the
34 parameters were poorer but the values for WBSF ($R^2=0.65$) could be considered useful.
35 These results indicate that calibration, being a dynamic process, will lead to the
36 improvement of the models, by increasing and diversifying the population, to develop
37 the technical precision of NIRS for meat quality evaluation.

38 Keywords: Near Infrared Reflectance Spectroscopy, beef, meat, quality attributes.

39 **1. Introduction**

40 Visible and Near Infrared Reflectance Spectroscopy (NIRS) is one of the most
41 promising techniques for large-scale meat quality evaluation (Geensink, Schreutelkamp,
42 Frankhuizen, Vedder, Faber, Kranen and Gerritzen, 2003). NIRS has the great potential
43 of predicting quickly and accurately different attributes of meat quality, it allows rapid
44 and frequent measurements, the sample preparation is fast and simple, is suitable for
45 non-contact on-line use and for simultaneous determination of different attributes
46 (Prevolnik, Candek-Potokar and Skorjanc, 2004).

47 The meat industry is an important economic sector in most developed countries as the
48 demand for this product is high, especially as far as beef is concerned. It is well known
49 that all the meat supplied to the markets must undergo quality controls in order to
50 guarantee consumer safety. However, some consumers are willing to pay higher prices
51 for meat products with an additional guarantee of quality. For example, colour is one of
52 the main factors influencing the sale of meat, since it is the only characteristic perceived
53 by the consumer in the market. In addition, the eating quality of this product is highly
54 determined by sensory properties such as tenderness, juiciness and flavour.

55 Regarding the application of reflectance spectroscopy to predict the quality of fresh
56 meat, most attention has been focused on the prediction of colour in CIELAB System
57 (Geensink *et al*, 2003) and there are few available data on other meat quality
58 parameters. Tenderness can be assessed as a measurement of meat mechanical
59 resistance (Warner-Bratzler shear force – WBSF) that is a destructive and time-
60 consuming method (Rødbotten, Nilsen and Hildrum, 2000).

61 Moreover, pH is the most commonly measured parameter in fresh meat, as it affects
62 technological processing ability, keeping ability as well as most sensory traits (Monin,
63 1998). Thus early prediction of ultimate pH would be of interest to resolve problems of

64 DFD (dark, firm and dry) and PSE (pale, soft and exudative) beef carcasses at the end of
65 the slaughter line.

66 Since NIRS is a rapid method, its use by the industry offers the hability to increase
67 control checks during meat processing and retailing (Cozzolino, Barlocco, Vadell,
68 Ballesteros and Gallieta, 2003).

69 The aim of this study was to examine the accuracy of visible/near infrared spectroscopy
70 for the prediction of beef quality characteristics such as, pH at 24 hours *post mortem*
71 (pH₂₄), colour parameters (L*, a*, b*), sarcomere length (SL), cooking loss (CL) and
72 Warner Bratzler Shear Force (WBSF) using *M. longissimus thoracis et lumbarum* of
73 young Maronesa bulls.

74 **2. Materials and Methods**

75 **2.1. Meat Sampling**

76 Young Maronesa bulls (n=30) aged between 9 and 11 months and with live weights
77 ranging from 90 to 150 kg, were slaughtered and selected according to the ultimate pH
78 (pH measured at 24 h *post-mortem*) in the *M. longissimus thoracis et lumborum* in order
79 to obtain a wide range of pH values. This muscle was excised 24 hours *post-mortem*
80 from between the 8th rib and 2nd lumbar vertebra and divided into four parts. The first
81 part was used for the laboratory procedures performed during the first day *post-mortem*
82 and the three remaining pieces were vacuum packed and aged at 4°C for 3, 7 and 14
83 days. For each aging time (1, 3, 7 and 14 days *post-mortem*) 30 samples were used,
84 being 120 the maximum number of analysed samples per parameter.

85 At the end of each period three slices were taken from each piece: the first one (2.5 cm
86 thick) was vacuum packed and stored at -80°C for NIRS analysis, the second one (3 cm
87 thick) was used for cooking loss and Warner-Bratzler Shear Force (WBSF) and the last
88 one for colour and sarcomere length (SL) measurements.

89 **2.2. Technological analyses**

90 The pH of fresh muscle samples was measured directly at 3 (pH₃; n=30) and 24 hours
91 (pH₂₄; n=30) *post-mortem* using a combined glass electrode with a pH meter WTW PH
92 330.

93 Regarding the colour parameters, two measurements were taken in each of the four
94 times *post-mortem* (n=120); the first one was measured on a fresh meat cut (n=30; 1 day
95 *post-mortem*) or, in the case of the aged meat (n=90; 3, 7 and 14 days *post-mortem*),
96 immediately after opening vacuum package-aged meat (t_0); the second one (n=120; 1, 3,
97 7 and 14 days *post-mortem*) after keeping the meat in a tray covered with a polyethylene
98 film at 4°C during 60 minutes (t_{60}) to allow meat re-oxygenation ("blooming"). L*

99 (luminosity), a^* (coordinate green-red) and b^* (coordinate blue-yellow) were
100 determined with a Minolta Chromometer CR-310 (Minolta, Osaka, Japan).

101 Sarcomere length analyses were made only at day 7 *post-mortem* (n=30). In this case 4
102 g of meat were minced and homogenised at low speed (8000 rpm) in a chilled 0.25 M
103 sucrose solution using an Ultra Turrax T25 mixer (Cross, West and Dutson, 1981). The
104 length of 10 consecutive sarcomeres was measured (15 groups of 10 sarcomeres for
105 each sample) under the phase contrast microscope (40× objective) with a video camera
106 attached and using the image-analysing system Matrox Inspector (Matrox Electronic
107 Systems Ltd, Dorval, Canada).

108 The water holding capacity of meat was evaluated by the cooking loss (CL) method in
109 each of the four time periods *post-mortem* (n=120). Hence, the percentage of lost weight
110 after cooking (70°C in the core the meat) was determined (Silva, Patarata and Martins,
111 1999). After measurement of cooking loss, samples were stored overnight in a
112 refrigerator and after reaching room temperature they were used for Warner-Bratzler
113 shear force (WBSF) determination in the four time periods *post-mortem* (n=120). The
114 WBSF was measured on 10 sub-samples of 1 cm² cross-section and about 4-5 cm
115 length. The sub-samples fibres were positioned perpendicularly to the direction of the
116 blade (driving at 100 mm min⁻¹) attached to a Stevens QTS 25 Texture Analyzer
117 (Stevens Advanced Weighing Systems Ltd, Great Dunmow, England).

118 **2.3. NIRS measurements**

119 **2.3.1. Sample Preparation**

120 The frozen (-80 ° C) meat samples were thawed in a fridge during 24 hours. Thereafter
121 the samples were taken out, stored in a plastic bag to prevent water evaporation and left
122 to reach room temperature. The surface temperature was recorded by an IR 'gun' (IRtec,
123 Miniray 100, Eurotron) and digital colour photos of each sample were collected. Next,

each sample was trimmed to eliminate connective tissue and two pieces of intact meat of 35 mm diameter were cut parallel to the longitudinal orientation of the muscle fibres (Cozzolino and Murray, 2004) and placed inside 35 mm diameter quartz cuvette with aluminium foil backing. The duplicates were photographed inside the cuvette and scanned in order to obtain a mean spectrum for each sample.

2.3.2. Spectra collection

The diffuse reflectance spectra were collected at 2 nm intervals from 400 to 2498 nm (1050 data points per sample at 16 bit precision) using a NIRSystems 6500 scanning spectrophotometer (FOSS NIRSystems, Silver Spring, MD, USA) equipped with a spinning module, to increase the scanned sample area and reduce sampling error (Downey and Hildrum, 2004). Absorbance data were stored as $\log(1/R)$, R being the reflectance. The instrument was operated by the software package NIRS2 version 3.01 (Infrasoft International, State College, PA, USA).

2.3.3. Calibration and validation

Calibration development and validation were performed using WinISI II version 1.02 (Infrasoft International, State College, PA, USA). Spectral data pre-treatments such as standard normal variation and detrending -SNV-D- (Barnes, Dhanoa and Lister, 1989), multiplicative scatter correction -MSC- (Dhanoa, Lister, Sanderson and Barnes, 1994) and first or second order derivatives (2, 12, 2, 2) were applied to the spectra to reduce the noise and light scattering effects.

pH₂₄ and SL parameters were predicted by using the spectra corresponding to fresh muscles (n=30). On the other hand, all the spectra (n=120) were used to estimate colour parameters, CL and WBSF. Partial least squares regression (PLSR) was used to predict muscle properties using visible and near infrared spectra as independent variables. Two passes of elimination of outliers were allowed and full cross validation was performed

149 in order to avoid over-fitting the PLSR equations. The accuracy of prediction was given
150 by the standard error of cross validation (SE_{CV}) and the ratio performance deviation-
151 RPD (Edney, Morgan, Williams and Campbell, 1994).

152 3. Results and discussion

153 3.1 Analytical Values

154 Measurements of the technologic parameters (Table 1) showed a wide range of
155 variability, resulting from the aging of meat and the different ultimate pH reached by
156 the samples. Moreover, it must be noticed that in fresh muscle samples (n=30) pH₂₄
157 (5.99) was slightly lower than pH₃ (6.66) as a consequence of the acidification during
158 the *post-mortem* aging process. The ultimate pH of meat greatly affects meat quality. In
159 fact, meat specimens with high pH values are darker, more susceptibility to bacterial
160 spoilage, have reduced flavour but may have better water holding capacity and
161 tenderness (Guignot, Touraille, Ouali and Renerre, 1994; Silva *et al.*, 1999).

162 Cooking loss, measured as the percentage weight loss, showed a mean value of 9.53%,
163 thus much smaller than the 30.70% value measured at day 9 *post-mortem* by Leroy,
164 Lambotte, Dotreppe, Lecocq, Istasse, and Clinquart, (2003). This difference can be due
165 to the high values of pH₂₄ observed in the present study, which could have been
166 partially responsible for the superior water holding capacity measured.

167 The colour parameters showed substantial variation, maybe as a result of the range
168 observed in the ultimate pH, which exerts a marked effect on meat colour. Meat re-
169 oxygenation and oxymyoglobin formation after 60 minutes was the reason why the a^*
170 and b^* parameters at t_{60} showed higher values than at t_0 .

171 SL is a measurement of muscle shortening during *rigor mortis* development and affects
172 meat quality, mainly tenderness (Tomberg, 1996). Considering 2.00 μm as the length of
173 the relaxed sarcomere (Quali, Lepetit, Touraille and Kopp, 1994), the values observed
174 in this study (1.51-1.84 μm), correspond to about 25% shortening.

175 WBSF showed a mean value of 10.37 kg cm^{-2} with a higher coefficient of variation
176 (37.01%). This variation could be mainly due to the aging time, ultimate pH and
177 possible effect of sarcomere length.

178 **3.2. Observations on near infrared spectra**

179 **3.2.1. Absorbance spectra ($\log(1/R)$)**

180 Figure 1 shows the mean spectra corresponding to each group of samples (meat
181 samples aged during 1, 3, 7 and 14 days *post-mortem*). No significant differences could
182 be observed between these mean spectra. In addition, when principal components
183 analysis (PCA) was performed on all the spectra ($n=120$), the scores corresponding to
184 each sample showed no differences between different groups of samples (Figure 2). In
185 other words, no differences due to the aging process could be observed in meat samples
186 by NIRS.

187 Regarding the mean spectra (Fig. 1) two main broad bands can be identified in the
188 visible region (400 – 750 nm) at 435, 575 nm, and five more bands in the near infrared
189 area (750 – 2500 nm) around 760, 980, 1200, 1450, and 1950 nm. The absorption band
190 at 435 nm is the Soret band attributed to an intense $\pi \rightarrow \pi^*$ transition observed in all
191 conjugated porphyrin (macrocyclic tetrapyrrole) rings in which electron delocalisation
192 extends throughout the macrocyclic ring (e.g. the haem prosthetic group in myoglobin
193 Mb). The spectral features in the visible region are very similar to those reported by
194 Swatland (1995), Cozzolino *et al.* (2003) and Barlocco, Vadell, Ballesteros, Gallieta
195 and Cozzolino (2006) in pork meat samples and by Cozzolino and Murray (2004) in
196 beef, pork and chicken meat samples, since all these species contain the same primary
197 pigment responsible for meat colour, myoglobin.

198 A weak near infrared band at 760 nm can be due to the OH third overtone or an
199 absorption band produced by myoglobin oxidation (Cozzolino and Murray, 2004 and

200 Liu, Lyon, Windham, Realini, Pringle and Duckett, 2003). Moreover , characteristic
201 bands of water were identified at 980 nm (OH second overtone), 1450 nm (OH first
202 overtone), and 1950 nm (OH combination band) (Cozzolino and Murray, 2004;
203 Cozzolino *et al.*, 2003; Leroy *et al.*, 2003). Water is the main component of meat
204 samples, typically 74% in lean beef, thus explaining the water bands previously
205 mentioned (Cozzolino and Murray, 2004). A band around 1200 nm (CH second
206 overtone) can be attributed to fat content (Cozzolino and Murray, 2004, Leroy *et al.*,
207 2003, Rødbotten *et al.* 2000).

208 **3.2.2. Prediction of the technological characteristics of the meat samples**

209 Currently there are few published reports on the ability of NIRS to predict meat quality
210 parameters. According to Prevolnik *et al.* (2004) there are no successful attempts to
211 estimate pH values. However in the present study absorbance data of meat samples
212 aged for 24 hours (n=30; 1 day *post-mortem*) showed good correlations with pH₂₄
213 parameter (Fig. 3). We found that this parameter (Table 2) could be accurately
214 predicted by NIRS ($R^2=0.97$, RPD=3.17). Light scattering properties of muscle tissue is
215 well known to be affected by tissue pH (Swatland, 1995), so this may well explain how
216 the ultimate pH could have been accurately predicted by visible and near infrared
217 reflectance spectroscopy (Fig. 4). The good repeatability of the reference method in the
218 present study (SEL pH₂₄ = 0.10) could also have contributed partially to the successful
219 prediction of pH₂₄ obtained by NIRS. In addition, it does not seem probable that this
220 equation has been over-fitted (number of PLS factors in the equation, p=3) as a
221 consequence of the small population (n=30), since there was a high correlation
222 observed between the absorbance data and pH₂₄ (Fig. 3).

223 The prediction of pH₂₄ using NIRS technology was also possible when all the samples
224 having different aging times (n=120) were included in the calibration set (data not

225 shown). Success arises probably as a result of the stability of pH₂₄ (also known as
226 ultimate pH) during the aging process, value that did not change significantly on longer
227 maturation.

228 In agreement with different authors (Meulemans, Dotreppe, Leroy, Istase and
229 Clinquart, 2003; Leroy *et al.*, 2003) CL parameter could not be accurately predicted by
230 NIRS ($R^2=0.20$, RPD=1.01). However, important correlations between the absorbance
231 in some regions and the CL parameter could be observed (Fig. 5). For example,
232 absorbance data related to meat respiratory pigments at 530 nm (myoglobin), and 780
233 nm (deoxymyoglobin) seemed to explain somehow part of the variability related to the
234 CL parameter. In addition, those areas related to the absorption of C-H bonds in the fat
235 fraction and O-H bonds of water showed coefficients of correlation of near 0.60 with
236 CL parameter. This is reasonable since water is the main fraction lost after cooking and
237 water and fat fraction are negatively correlated in meat.

238 Regarding colour parameters, luminosity L^*t_0 (Fig. 6) and L^*t_{60} (Fig.7) showed the best
239 predictions by NIRS ($R^2 > 0.80$; RPD > 2.00). This is in agreement with the results
240 reported by Leroy *et al.* (2003) in beef samples ($R^2=0.83$). Liu *et al.* (2003) found
241 poorer predictions of luminosity in beef samples because the visible region (400-1080
242 nm) was used to measure the spectra, whereas meat components responsible for L^* are
243 water and fat, which can be detected in the near infrared region. Indeed, the successful
244 prediction of L^*t_0 and L^*t_{60} achieved in the present study could be due to the good
245 correlation between them and the intramuscular fat. In the present study intramuscular
246 fat was not determined, but it is well known that this is one of the best parameters
247 predicted using near infrared spectra of meat samples (Prieto, Andrés, Giráldez,
248 Mantecón and Lavín, 2006).

249 On the other hand, a^* (red-green) and b^* (yellow-blue) parameters measured after 0
 250 and 60 minutes *post-mortem* could not be predicted by visible and near infrared
 251 spectroscopy ($R^2 < 0.60$, $RPD > 0.90$), almost certainly as a result of the time elapsed
 252 between the reference method (performed on fresh meat) and the spectra measurement
 253 (performed later on frozen and thawed meat). During this period of time the proportions
 254 of respiratory pigments determining a^* and b^* values (myoglobin, oxymyoglobin and
 255 metmyoglobin) might well have been modified, thus giving rise to a change in the
 256 colour of meat samples and hence decreasing the accuracy of prediction by visible and
 257 near infrared spectroscopy (Leroy *et al.*, 2003). This statement is supported by the
 258 results obtained by Liu *et al.* (2003) in beef samples, who could predict a^* and b^*
 259 parameters with a high degree of accuracy ($R^2 = 0.90$, 0.78, respectively) due to
 260 absorbance of different forms of myoglobin in the visible region. Successful prediction
 261 of a^* and b^* probably requires simultaneous measurement of these parameters and the
 262 spectra, since colour changes can happen between them.

263 Mathematical models performed to estimate SL ($R^2 = 0.16$, $RPD = 0.84$) were poorer,
 264 probably because SL in fresh muscle is still in a state of dynamic change, so the spectra
 265 corresponding to these samples was not useful to estimate this parameter.

266 The prediction results for WBSF ($R^2 = 0.65$, $RPD = 1.46$) could be considered as useful.
 267 These statistics are in agreement with the ones described by Rødbotten *et al.* (2000),
 268 Park, Chen, Hruschka, Shackelford, and Koohmaraie (1998) and Byrne, Troy, Downey
 269 and Buckley (1997) showing R^2 values of 0.68, 0.63 and 0.67, respectively. The
 270 different aging times considered in our study could have improved the variability of this
 271 parameter, an essential requirement for NIRS performance. Nevertheless, the low
 272 precision of the reference procedure might constrain visible and near infrared
 273 spectroscopy predictability of WBSF.

274 It should be stressed that direct comparison of prediction results with other studies is
275 difficult for several reasons. For instance, meat samples were fresh, or aged for
276 different periods of time, sometimes frozen and thawed. Furthermore, the instruments
277 used to perform the technological analysis were different, and the spectra were obtained
278 in reflectance/transmittance modes often having quite different presentation geometry
279 to the instrument, the radiation source and the detectors. Such operational constraints
280 can profoundly affect performance (Swatland, 1995).

281 However, it must be pointed out that intact muscle samples with the complete
282 organization of tissue unaffected were used in the present study so, possibly this fact is
283 the reason why the obtained results are more accurately predicted than those previously
284 described by other authors (Venel, Mullen, Downey and Troy, 2001; Liu *et al.* 2003).

285 **Conclusions**

286 Although the beef samples studied in this work arose from a small number of animals
287 (n=30) and muscle samples (n=120), some conclusions can be drawn. The results
288 obtained in this study suggest that visible and near infrared spectroscopy instruments
289 (400 – 2500 nm) can accurately predict pH_{24} and L^* parameters, and have a good
290 potential to provide useful prediction of WBSF on intact beef muscle samples.
291 The lower prediction performance observed in some parameters could have been
292 partially due to the low repeatability of the reference methods.
293 Further development work is needed over a wider animal population to improve the
294 accuracy of the NIRS predictions, leading to a higher acceptance of NIRS technology
295 in meat quality.

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 364

Figure 1. Four mean spectra corresponding to the four meat sample groups (1, 3, 7 and 14 days *post-mortem*)

Figure



Figure 2. PCA scores corresponding to the meat samples (n=120)

Figure

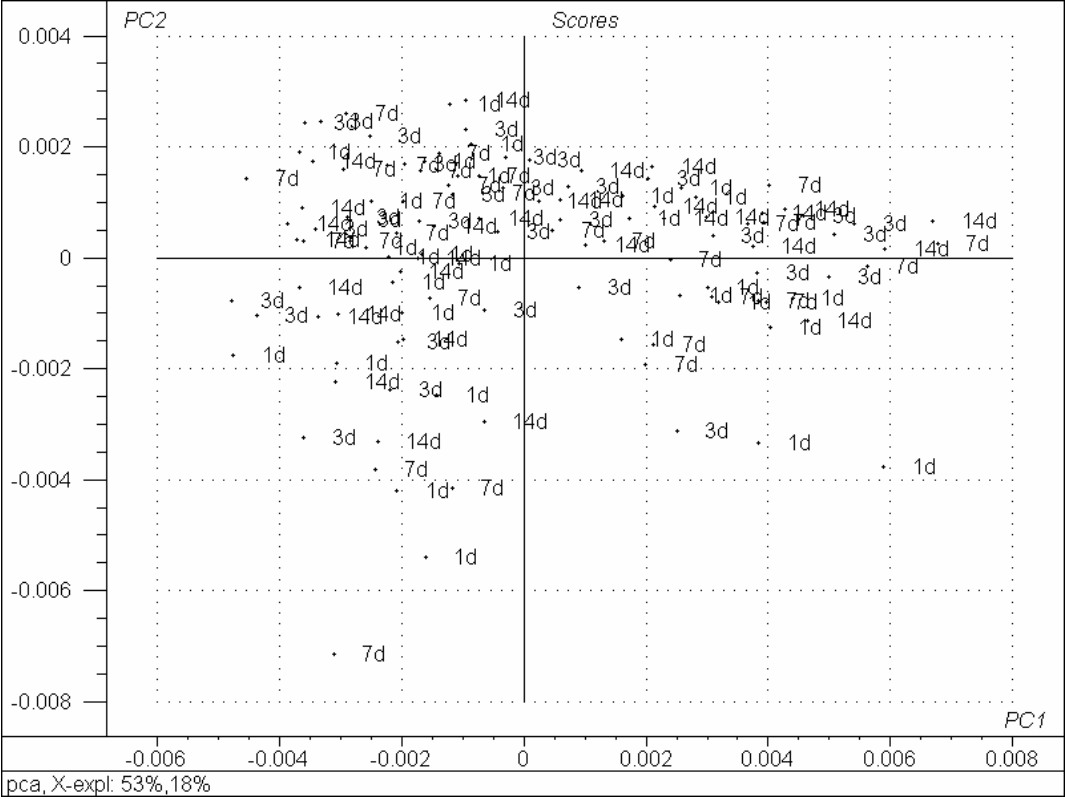


Figure 3. Correlation coefficients (R) between pH₃ or pH₂₄ and the absorbance data of the average second-order derivative spectra corresponding to the beef samples aged during 24 hours (n = 30)

Figure

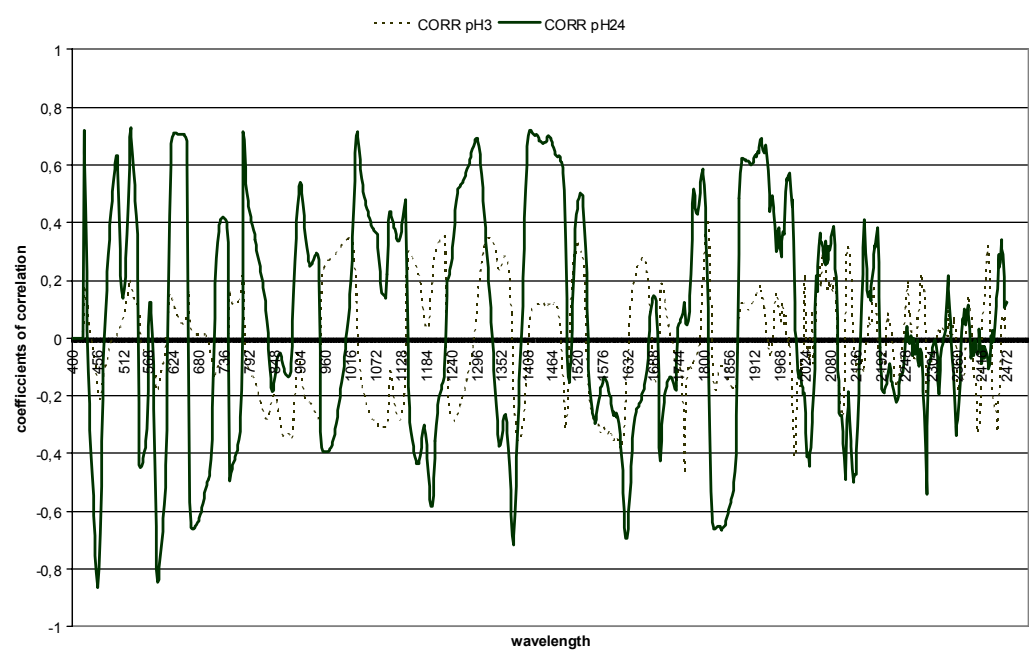


Figure 4. Relationship between the pH_{24} reference data and those predicted by NIRS (n=30).

Figure

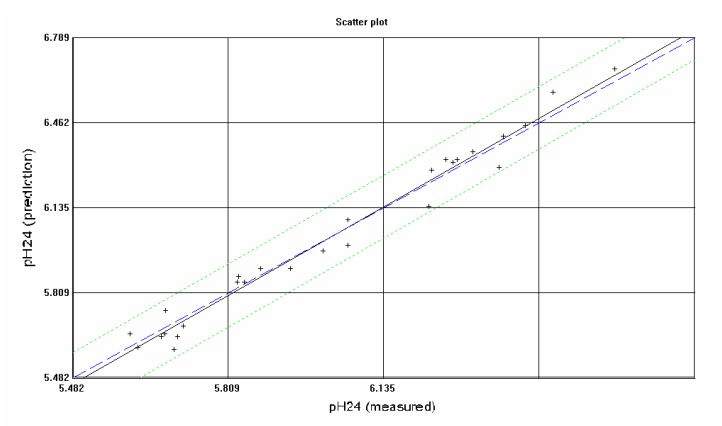


Figure 5. Correlation coefficients (R) between CL and the absorbance data of the average second-order derivative spectra corresponding to the beef samples ($n = 120$)

Figure

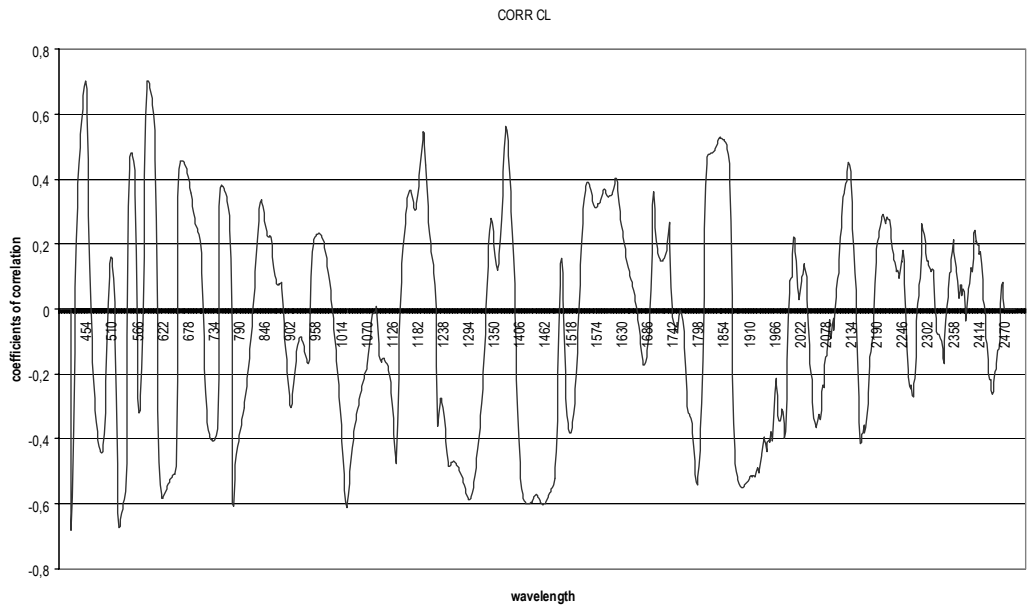


Figure 6. Relationship between L^* reference data on fresh meat cut ($t=0$) and those predicted by NIRS ($n=120$).

Figure

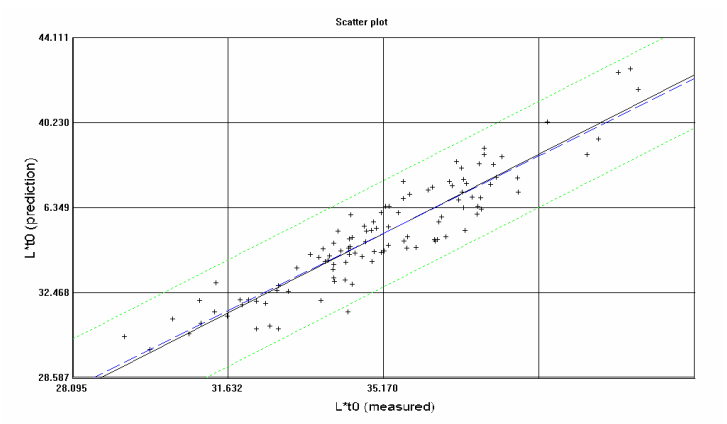


Figure 7. Relationship between L^* reference data after 60 minutes *post-mortem* ($t=60$) and those predicted by NIRS ($n=120$).

Figure

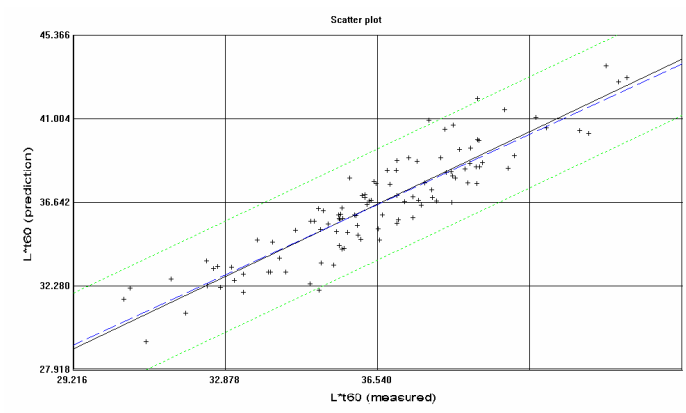


Table 1

Range, mean, standard deviation (SD) and coefficient of variation (CV) of the technological parameters of beef samples

Parameter	n	Mean	Range	SD	CV(%)
pH ₃	30	6.66	6.17 – 6.95	0.20	2.93
pH ₂₄	30	5.99	5.50 – 6.67	0.33	5.53
CL (%)	120	9.53	2.91 – 16.81	3.07	32.18
L*t ₀	120	35.03	27.62 – 42.70	2.76	7.87
a*t ₀	120	17.85	12.88 – 21.90	1.36	7.60
b*t ₀	120	2.94	0.88 – 5.59	0.95	32.33
L*t ₆₀	120	36.14	10.38 – 43.78	3.76	10.40
a*t ₆₀	120	20.23	15.23 – 26.40	2.10	10.39
b*t ₆₀	120	6.56	2.96 – 11.37	1.77	26.92
SL (µm)	30	1.70	1.51 – 1.84	0.09	5.06
WBSF (kg cm ⁻²)	120	10.37	3.85 – 19.88	3.838	37.01

n: number of samples; SD: standard deviation; CV: coefficient of variation.

1 **Table 2**
2 Prediction of technological characteristics of beef samples by near infrared reflectance
3 spectroscopy

Parameter	n	p	SEC	R ²	SE _{CV}	1-VR	RPD
pH ₂₄	27	3	0.06	0.97	0.10	0.91	3.17
CL	99	1	0.07	0.20	0.08	0.02	1.01
L*t ₀	108	5	1.00	0.85	1.16	0.80	2.22
a*t ₀	104	2	1.04	0.29	1.09	0.23	1.14
b*t ₀	109	3	0.63	0.49	0.75	0.27	1.17
L*t ₆₀	109	4	1.19	0.82	1.36	0.75	2.07
a*t ₆₀	100	2	1.22	0.35	1.28	0.29	0.90
b*t ₆₀	99	4	0.95	0.51	0.99	0.46	1.37
SL	30	1	0.08	0.16	0.10	0.02	0.84
WBSF	112	5	2.30	0.65	2.67	0.53	1.46

4 n: number of samples; p: number of PLUS factors in the equation; SEC: standard error of calibration; R²: coefficient
5 of determination for calibration; SECV: standard error of cross validation; 1-VR: coefficient of determination for
6 validation; RPD; ratio performance deviation calculated as SD/SECV.

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