Developing and monitoring quantitative near infrared spectroscopic models for routine analysis of pork meat

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Introduction

A large number of papers have shown the ability of near infrared (NIR) spectroscopy to predict quality attributes in meat and meat products.[1-4] However, none of the published papers show in detail the complexity of developing and maintaining calibration models applicable in routine analysis. Moreover, most studies are muscle-specific, i.e. only one muscle type is used. In some situations, such as annual breeding programs or in quality control of raw materials used to produce final products composed of a mixture of muscles, it is desirable to analyse multiple muscles and hence development of calibrations requires the inclusion of multiple muscle types. Furthermore, based on the experience of many years by the authors' research group in routine analysis procedures are required to control unexpected results. Although the sample presentation/analysis is standardised or the instrument diagnosis informs that the instrument is working properly, the statistics used to check the certainty of the NIR prediction of unknown samples can be out of the limit control specified in the literature. This paper evaluates a methodology for developing and maintaining routine NIR models for the quantitative prediction of fat, moisture and protein in pork meat. The methodology evaluated is to predict new unknown samples of different times with certainty avoiding the need to send unnecessary samples to wet chemistry laboratories, saving money and time.

Materials and Methods

Sample set and NIR analysis

Three Iberian pork muscles, i.e. *gluteus medius* (3.3% of the total number of samples), *masseter* (11.3% of the total) and *longissimus dorsi* (85.4% of the total)) collected over 1999, 2000, 2001, 2002, 2003, 2004 and 2009 at different slaughterhouses were used for the calibration and validation process. The samples were divided in two sets: 316 samples (set A) collected over 1999-2004 and 514 samples (set B) collected in 2009. Each muscle was ground, homogenised and analysed independently.

A Foss NIRSystems 6500 spectrometer (Foss-NIRSystems Inc., Silver Spring, MD, USA), equipped with a spinning module and the WinISI software package ver 1.50 (Infrasoft International, Port Matilda, PA, USA) were used for spectral acquisition and chemometric analysis, respectively. Two sub-samples were analysed for each sample using standard circular cups (diameter 3.5 cm). A root mean squared (RMS) value of 11 250 μ log(1/R) was used to control the spectra repeatability.

Samples were analysed by wet chemistry in the same year of collection and NIR analysis. Fat content was determined following the Soxhlet procedure (ISO-R-1443). Total protein was determined by the Kjeldahl method (ISO-R-937) and moisture content was measured by oven drying to constant weight at 100ºC in accordance with ISO-R-1442. All values were expressed as percentage (%) of wet weight.

Spectral data treatment and calibration

Calibration models were developed with set A. Principal component analysis (PCA) was performed on set A (n = 316) samples in order to decompose and compress the data matrix. After PCA, the centre of the spectral population was determined in order to detect outlier samples. The global distance (GH) was calculated between each sample and the centre; samples with a GH value greater than 3 were considered outliers.[5] As spectral pretreatments, the standard normal variate (SNV) plus detrending (DT)[6] procedures were used to remove the multiplicative interferences of scatter, and two derivative mathematical treatments were evaluated: first and second derivative.

Modified partial least squares (MPLS) regression[7] was used for the prediction of fat, moisture and protein in pork in the 1100–2500 nm range. Chemical outliers were analysed using Student's *T* statistic.[8] The following statistics were used to select the best equations: standard error of calibration (SEC), standard error of cross-validation (SECV), and determination coefficient of cross-validation (R^2_{CV}), based on four cross-validations groups. The other statistic used was residual predictive deviation (RPD_{CV})[9], calculated as the ratio

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between the standard deviation of the reference data for the training set and the SECV. According to Williams & Sobering (1996),[9] a model is suitable for screening purposes if the RPD is greater than 3.

Prediction and maintenance of calibration models

The fat, protein and water contents of 514 samples of *longissimus dorsi* analysed in 2009 (set B) were predicted with the best calibration models obtained for each parameter. The statistics, global Mahalanobis (GH) distance, which calculates the distance between a sample and the centre of the training set (values higher than 3-4 are deemed far from the training set), and neighborhood Mahalanobis (NH) distance, which indicates how far a sample is from the most similar samples in the training set (values higher than 0.6-1.2 denote samples with few close neighbors in the training set),[7] were calculated to evaluated the certainty of the predictions.

Recalibration samples were selected on the basis of: GH>3.0, NH>1.0 and initial NIRS prediction values, to try to fill gaps in the library concerning each parameter and, therefore, have a more representative reference set for the range of each parameter.

The standard error of differences (SED) calculated as the difference between prediction values of a common sample set before and after recalibration was used to compare the performance of both models.

Results and Discussion

The PCA of set A (316 samples) did not show any pattern related to the muscle type. The GH indicated 6 samples with values larger than 3. Examination of the reference data showed that these samples were extreme mainly in terms of percentage fat content (greater than 21% in two, and below 4% in the other four). Once spectral outliers had been removed a set consisting of 310 samples of three different muscles was used to develop calibration models. The calibration models obtained showed R^2_{CV} equal to or above 0.9 for all parameters. Fat displayed a SECV of 0.36%, moisture 0.42% and protein 0.50% (Table 2, set A). The RPD_{CV} indicated adequate values for fat and moisture with values larger than 3. The lower RPD_{CV} for protein compared to fat and moisture was due to the range of this parameter in the training set. This means that the models are suitable, especially for fat prediction which is the most relevant parameter, either in breeding programs or quality control in the meat industry.

New 514 *longissimus dorsi* samples were predicted using the models. The corresponding GH and NH values are shown in Figure 1. It was observed that these new unknown samples were not well represented in the population used for calibration, with average values of 1.60 for GH and 1.21 for NH. Moreover, Figure 1 shows that three samples had GH values above 3, while 78.59% of the 514 unknown samples had NH values above the limit (1.0), indicating little similarity to the training samples. These results were unexpected since there was a sample preparation protocol for the analysis of samples, the type of animals analysed in the different years was similar and the instrument diagnosis informed that the Foss NIRSystem was working properly.

At this stage, a critical step is to choose which, and how many, samples have to be sent to the laboratory for wet chemistry analysis to check the real performance of the models. Accordingly, the GH statistic was used to detect new spectral variability, and the NH statistic in conjunction with the initial NIR prediction for each constituent, to detect new chemically-interesting samples for analysis by wet chemistry, as well as to ensure a uniform range for each parameter in spectral libraries. The range, mean and standard deviation of the training set provide an overview of the structure of the samples used in the model; however, they provide no information on sample distribution or, therefore, on possible gaps in reference values in the training set. Sample distribution (set A) for each parameter in terms of reference data was analysed and the NIRS predictions of the unknown samples were plotted to check for useful samples to fill gaps. Thirty two samples collected in 2009 were selected to update the models, comprising samples deemed spectral outliers (3 samples with high GH) and samples with both a high NH (>1.0) and a prediction value for one or more parameters that was not well represented in the models (29 samples: 5 samples with extreme NH value; 7 samples to fill gaps with large NH for fat, 7 samples for moisture and 7 for protein). Table 1 shows the reference statistics of this set. It is observed, compared to the initial structure of the training library, that real extreme samples were selected for fat and moisture. The standard deviation values indicated large variability of the selected samples for these parameters. Mean values were higher for fat and lower for moisture compared to the training set. The larger difference was observed for the moisture. This can indicate that it is a relevant factor which could be responsible for the unexpected performance of the models during routine analysis. Also, to check if the selected samples were really samples of interest to send to the lab the standard error of prediction (SEP) was calculated with the initial models. SEP was 0.82% for fat, 1.89% for moisture and 0.85% for protein, which were large compared to the SECV of the initial models.

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A new training set of 348 samples (set A+ = 316 samples of set A plus 32 samples from set B) was subjected to the process of developing calibration models. The updated calibration models displayed R^2_{CV} above 0.9 and SECVs values of 0.35%, 0.46% and 0.52% for fat, moisture and protein, respectively (Table 2, set A+), similar to those of the original calibration. There was no improvement in model performance. The standard error of difference (SED) within the predictions for set B (minus the 32 samples included in the training set) obtained using the initial models developed with set A (Table 2; set A) versus the recalibrated models (Table 2; set A+) were: 0.15% for fat, 0.11% for moisture and 0.28% for protein. However, the recalibrated models showed an improvement in GH and NH statistics; the initial models yielded an average GH = 1.60 and NH = 1.21 when set B (minus the 32 samples included in the training set) was evaluated and the recalibrated models yielded an average GH = 0.75 and NH = 0.41. Comparison of GH and NH distribution for the initial models (Figure 2) and for the updated models (Figure 3) showed that only 0.2% of the unknown samples evaluated in routine had NH values above the established limit. Thus, predicted values were more reliable using the recalibrated models, which enabled the detection of real spectral or chemical outliers. This was a very positive finding, given the time and cost involved in analysing meat samples by traditional wet chemistry.

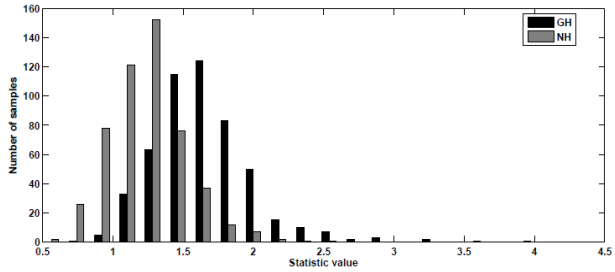

Figure 1. Frequency histogram of GH and NH values of set B (514 samples) predicted with initial models (Table 2; set A).

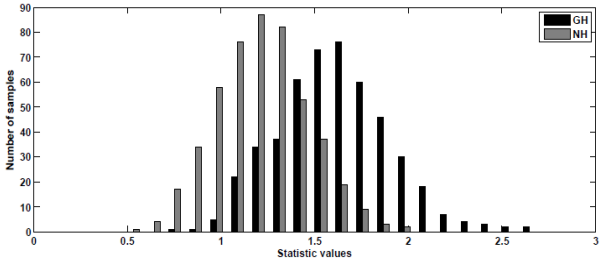

Figure 2. Frequency histogram of GH and NH values of set B minus 32 recalibration samples (482 samples) predicted with initial models (Table 2; set A).

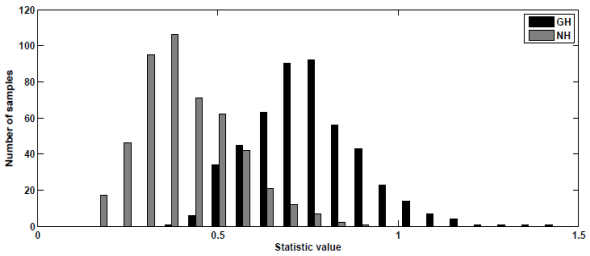

Figure 3. Frequency histogram of GH and NH values of set B minus 32 recalibration samples (482 samples) predicted with recalibrated models (Table 2; set A+).

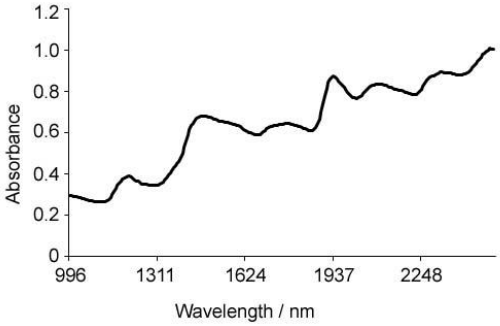

Table 1. Descriptive statistics for training and recalibration sets (values expressed as % of wet weight).

Parameter	Training set (310 samples)				Recalibration set (32 samples)			
	Min	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*
Fat (%)	2.10	21.90	6.87	3.23	2.05	16.20	8.40	4.90
Moisture (%)	56.00	77.00	70.48	2.97	51.40	72.70	67.33	4.50
Protein (%)	16.80	26.20	21.45	1.65	18.90	24.10	22.10	1.47

Min: minimum; Max: maximum, SD: standard deviation

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Table 2. Statistics for chemometric models developed for fat, moisture and protein with the set A (310 samples) and set A+ (342 samples = set A + recalibrated samples).

Parameter	Set	Data preprocessing	No. samples	No. factors	SEC (%)	SECV (%)	R^2_{CV}	RPD_{CV}	GH*	NH*
Fat	A	SNV + DT (1,10,5,1)	279	7	0.33	0.36	0.98	9.19	1.60	1.21
	A+	SNV + DT (1,10,5,1)	308	6	0.34	0.35	0.99	10.02	0.75	0.41
Moisture	A	SNV + DT (2,5,5,1)	279	7	0.37	0.42	0.97	7.33	1.60	1.21
	A+	SNV + DT (1,10,5,1)	308	7	0.44	0.46	0.97	7.28	0.75	0.41
Protein	A	SNV + DT (1,10,5,1)	296	6	0.47	0.50	0.91	3.36	1.60	1.21
	A+	SNV + DT (1,10,5,1)	322	7	0.49	0.52	0.90	3.23	0.75	0.41

*Average value calculated with the 514 samples of set B for the models developed with set A and with 482 samples of set B for models developed with set A+.

Conclusion

The use and understanding of spectral and chemical distance statistics to ensure the reliability/suitability of the NIR spectroscopy predicted values is critical in meat routine analysis. The methodology proposed ensures reliable predictions, saving time and money for recalibration, although the accuracy of the models does not change in terms of cross-validation errors. NIR spectroscopy is suitable for routine analysis of chemical composition of a large number of Iberian pig meat samples, by using well structured spectral libraries and regular testing of equation performance.

Acknowledgements

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