

Pork fat composition calibration transfer between two near infrared spectrometers using different multivariate standardisation approaches

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Introduction

Spectra obtained by different spectrophotometers are often difficult to compare and this represents a great limitation for NIR spectroscopy as, generally, considerable effort is involved in constructing a robust model since it is meant to be used for an extended period of time.

For many years, calibration transfer has represented the main drawback for a wide application of NIR spectroscopy for laboratory and routine analysis¹.

In this trial, three different multivariate standardisation approaches have been tested aiming to evaluate which of them could provide the best performances for transferring a old, robust Foss NIRSystem 5000 calibration to a Unity Scientific 2500x spectrometer.

Materials and Methods

A Foss NIRsystem 5000 calibration was built over several years of analysis using 344 samples of sub-cutaneous pork fat.

In this trial, 320 other ground samples (Retsch laboratory mill, 4000 rpm x 10 s) were scanned both with the master instrument “Foss NIRSystem 5000” (1100-2498 nm, 2 nm interval) and with a Unity Scientific 2500x (680-2500 nm, 1 nm interval) placing the sample at room temperature in small ring cups. Data acquisition were carried out using the WinISI software (InfraSoft International, Port Matilda, PA, USA) for the FOSS instrument and Scientific Infostar v. 3.80 for the Unity device. Of the 320 samples, 255 were used as a standardisation set, 43 as a validation set and 22 as an updating file for the new calibrations. All calibrations were carried out by modified PLS after spectra pre-treatment with first and second derivatives and standard normal variate and detrend (SNV-D) using WinISI software.

Validation and updating samples were chemically analysed for iodine number (according to the modified Wijs method) and fatty acid profile (gas chromatography analysis performed on an automated apparatus (GC Shimadzu 17A Kyoto Japan) equipped with flame ionisation detector and a Supelco Omegawax 250 type capillary column (30 m and 0.25 mm ID)).

Different standardisation methods were compared using MATLAB as computation software: Direct standardization (DS), Piecewise-DS (PDS), double PLS.

Standardisation performances were evaluated (before and after calibration update with 22 samples) by comparing the responses with those obtained with the original Foss calibration and through a validation test using the external dataset of 43 samples. SEP (standard error of prediction), R^2 (determination coefficient in validation) slope and bias were the statistical parameters used for comparison among standardisation techniques.

Results and Discussion

The information embedded in Foss and Unity spectra is defined by a series of characteristic absorption bands (at 1200, 1400, 1750, 2310 and 234 nm) that are typical for some bonds of the fatty acid molecules² (Figure 1). Table 1 shows the statistics of the original Foss calibration which was developed on ground sub-cutaneous fat over a long period of analysis.

Sample composition revealed a certain level of variability, which is important when searching for calibration equations to be used for prediction. The feeding regime and genetics of animals are the main source of variability.^{3,4} All the coefficients of determination in calibration (RSQ) for the different parameters are very high, reaching values above 0.9. Similar performances were also shown for analysis of Iberian swine fat with a Foss NirSystem 5000 equipped with a fibre-optic probe³.

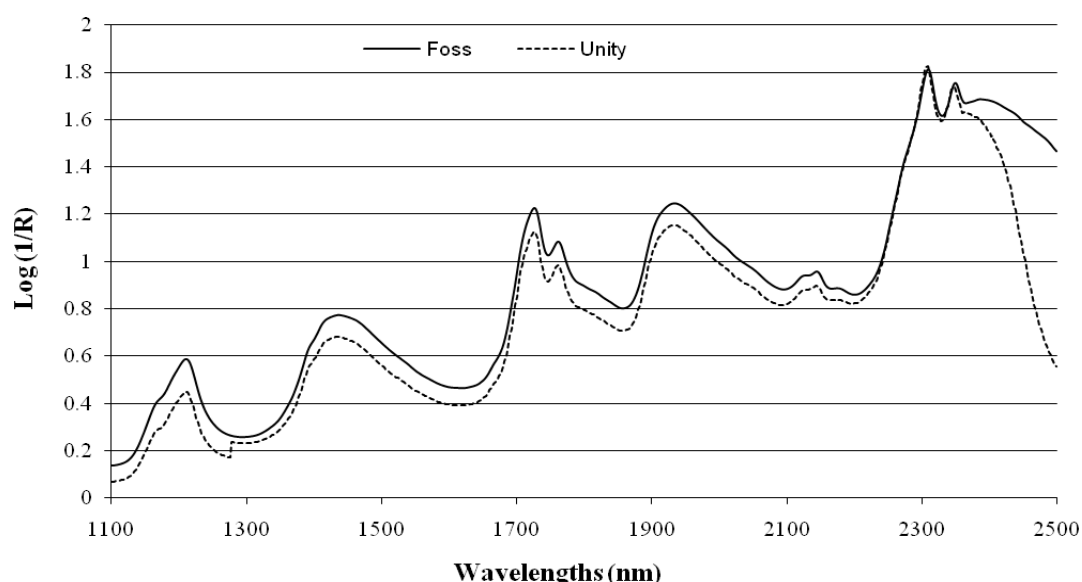


Figure 1. Comparison between Foss and Unity spectra.

Table 1. Foss calibration equations, cross- and external validation.

	Calibration					Cross-validation		External validation		
	N	Mean	SD	SEC	RSQ	SECV	1-VR	SEP	R ²	RPD
Iodine N.	344	71.68	3.16	0.76	0.94	0.82	0.93	0.94	0.92	3.85
C18:0 ¹	332	10.17	1.09	0.35	0.90	0.40	0.86	0.38	0.89	2.73
C18:2n6 ¹	342	13.48	1.84	0.42	0.95	0.50	0.93	0.59	0.90	3.68
PUFA ¹	344	15.58	2.12	0.56	0.93	0.59	0.92	0.56	0.93	3.6
m/p	342	3.19	0.48	0.11	0.94	0.13	0.92	0.16	0.85	3.69

N: samples number, SD: standard deviation, SEC: Standard error of calibration, RSQ: coefficient of determination in calibration, SECV: standard error of cross validation, 1-VR coefficient of determination in cross validation; PUFA = polyunsaturated fatty acids; m/p = ratio of monounsaturated to polyunsaturated fatty acids;¹ Results were expressed as a percentage (w/w) of total fatty acid methyl esters, SEP: standard error of prediction, R²: coefficient of determination in prediction, RPD: ratio performance deviation (SD/SECV)

In the present study, standard errors of calibration (SEC) and cross-validation (SECV) were quite low but higher than those reported previously.⁵

The prediction ability of the FOSS calibrations were also assessed by using the ratio performance deviation (RPD). Values of this ratio are calculated by dividing the standard deviation (SD) of a given parameter by the SECV of that parameter; RPD should be larger than 2.5 to consider the calibration of practical utility.⁶ In our trial, all parameters considered had a RPD higher than 3.5 (Table 1). Stearic acid (C18:0) only produced a RPD value of 2.73 which can still be considered adequate for practical uses of the calibration. Foss predictions are accurate enough to permit the use of this instrument for routine analysis providing important information about the main chemical composition of the pork fat. Therefore, NIR spectroscopy might be a strategic tool to monitor swine thighs and to indicate their most suitable transformation process according to the pork fat composition.

The effort spent in building a robust calibration might be lost once a new instrument is used or when some environmental conditions change during analysis.⁷ Nowadays a wide number of standardisation methods are available to permit the transfer of a calibration developed with a certain spectrometer to other devices. Different multivariate standardisation techniques were tested. Direct Standardisation (DS), Piecewise-DS (PDS) and double PLS are based on spectra using the transfer matrix F . This contains a certain number of coefficients able to transform the spectra to be standardised.

In DS, each wavelength of the two instruments is taken into account to perform the standardisation. This may lead to over-fitting and long computation times. Amongst the multivariate standardisation methods evaluated in this trial, DS obtained the best performances: R² higher than 0.83 for all constituents and SEP values lower than those obtained with the other techniques. The ratio of monounsaturated to polyunsaturated (MUFA/PUFA) fatty acids showed the worst performance but can be explained by the fact that this is a prediction of a ratio and not of a real constituent. Slight improvement of the calibration statistics was obtained updating the dataset with 22 other samples.

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Table 2. Standardisation with Direct Standardisation, Piecewise Direct Standardisation and double PLS.

	Statistics	DS N _{tot} =344	Updated DS N _{tot} =366	PDS with window size=1 N _{tot} =344	Updated PDS N _{tot} =366	Double PLS 16 PCs N _{tot} =344	Updated Double PLS N _{tot} =366
Iodine number	SEC	0.77	0.76	0.78	0.77	1.02	1.01
	RSQ	0.94	0.94	0.94	0.94	0.90	0.90
	SECV	0.88	0.86	0.84	0.83	1.06	1.06
	1-VR	0.92	0.93	0.93	0.93	0.89	0.89
	SEP	0.97	0.96	1.35	1.33	1.43	1.56
	R ²	0.92	0.92	0.85	0.86	0.85	0.79
	Bias	-0.23	-0.27	-0.42	-0.43	-0.55	-0.30
	SEP C	0.95	0.93	1.30	1.28	1.34	1.55
	Slope	1.02	1.02	0.98	0.97	0.91	0.90
C18:0¹	SEC	0.41	0.37	0.33	0.34	0.47	0.47
	RSQ	0.86	0.88	0.90	0.90	0.81	0.81
	SECV	0.45	0.43	0.39	0.40	0.49	0.50
	1-VR	0.83	0.85	0.87	0.86	0.79	0.79
	SEP	0.48	0.43	0.61	0.66	0.73	0.67
	R ²	0.83	0.86	0.74	0.69	0.67	0.71
	Bias	-0.10	0.07	0.19	0.03	0.23	0.20
	SEP C	0.47	0.43	0.59	0.67	0.70	0.64
	Slope	0.93	0.94	0.89	0.81	0.78	0.81
C18:2n6¹	SEC	0.42	0.41	0.50	0.50	0.74	0.73
	RSQ	0.95	0.95	0.92	0.93	0.84	0.85
	SECV	0.49	0.47	0.53	0.54	0.76	0.75
	1-VR	0.93	0.94	0.91	0.92	0.83	0.84
	SEP	0.51	0.52	0.91	0.90	0.63	0.59
	R ²	0.92	0.92	0.81	0.84	0.88	0.90
	Bias	0.04	-0.05	-0.25	-0.32	-0.10	-0.09
	SEP C	0.52	0.53	0.88	0.85	0.63	0.59
	Slope	1.04	1.01	0.82	0.81	1.01	1.00
PUFA¹	SEC	0.46	0.44	0.56	0.54	0.85	0.80
	RSQ	0.95	0.96	0.93	0.93	0.83	0.85
	SECV	0.55	0.53	0.61	0.60	0.88	0.85
	1-VR	0.93	0.94	0.91	0.92	0.82	0.84
	SEP	0.59	0.56	0.90	0.77	0.66	0.69
	R ²	0.93	0.93	0.84	0.87	0.90	0.90
	Bias	-0.25	-0.18	-0.16	-0.10	-0.11	-0.22
	SEP C	0.54	0.54	0.89	0.78	0.65	0.66
	Slope	0.96	0.95	0.85	0.89	0.97	0.94
MUFA/ PUFA	SEC	0.11	0.11	0.11	0.11	0.21	0.20
	RSQ	0.94	0.95	0.94	0.94	0.80	0.81
	SECV	0.14	0.14	0.13	0.13	0.22	0.21
	1-VR	0.91	0.92	0.92	0.93	0.79	0.80
	SEP	0.18	0.18	0.26	0.18	0.19	0.19
	R ²	0.83	0.83	0.71	0.84	0.80	0.82
	Bias	-0.06	0.01	-0.03	0.00	-0.01	0.05
	SEP C	0.18	0.18	0.26	0.18	0.19	0.18
	Slope	0.85	0.84	0.69	0.83	0.86	0.83

SEC: Standard error of calibration, RSQ: coefficient of determination in calibration, SECV: standard error of cross-validation, 1-VR coefficient of determination in cross-validation, SEP: standard error of prediction, R²: coefficient of determination in prediction, SEP C = standard error of prediction corrected for bias. ¹ Results were expressed as a percentage (w/w) of total FA

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The number of constants to estimate is reduced when PDS is used. In fact smaller spectral regions are considered rather than the whole spectrum. A practical drawback of the PDS method is that different parameters have to be carefully defined such as the “window size” and the “optimal rank” of each local multivariate regression model. In our case, different window sizes were tested (1, 3, 5, 7; data not shown) to evaluate which of them provided the best results. The window size represents the number of wavelengths which are considered by the model for standardisation computation. The software will select a certain number of wavelengths and, in the case of a window size of 3, the software will standardise the spectra considering the selected wavelengths and the ones prior and subsequent simultaneously. In this trial, higher performances were obtained with a window size of 1 and 3 (only the performance obtained with the window size of 1 has been reported on Table 2). R^2 values were quite satisfactory for all the parameters but performances were much lower than those obtained with DS standardisation. A decrease in R^2 in comparison to DS was observed for all constituents which never exceeded 0.9 and especially for C18:0 which reached a value of 0.74 (while DS obtained an R^2 of 0.86). A decrease of prediction performances was also observed considering the SEP which was higher in PDS than in DS for all the constituents. The update of the equations with the 22 samples showed contrasting consequences: a general improvement of R^2 for all the constituents considered but a slight decrease noticed for stearic acid (R^2 of 0.74 before and 0.69 after update) and concomitant increase of SEP (from 0.61 to 0.66).

Double PLS was proposed⁸ to decrease the number of coefficients to consider in the transfer matrix (F) using PCs rather than all variables. The procedure uses partial least squares (PLS) regression twice: to compute the relationship between the spectra of the two instruments and to compute the regression equation (relationship between chemical variables and spectral variables) of the first instrument. In our trial, we used different numbers of PCs - 8, 10, 12, 14, 16 (data not shown); the best performances were obtained with 16 PCs (Table 2). This is a threshold quantity of PCs to consider as, after that, there is a risk of over-fitting which should be avoided. Low numbers of PCs (<10) did not provide any interesting performances. R^2 was quite satisfactory for all the parameters but SEP was much higher in Double PLS than in DS for all the constituents. Good performances were observed for PUFA ($R^2 > 0.9$) while a decrease in prediction ability was shown for the rest of the constituents in comparison to the other standardisation techniques. In particular, iodine number produced the lowest R^2 (0.79) and the highest SEP (1.56). The update of the standardised equations with the 22 samples did not provide improvements of the calibration or prediction performances.

Conclusion

Standardisation performances vary according to the tested techniques but satisfactory results were obtained with all the methods evaluated. DS seems to be the best one for pork fat calibration transfer. Different window sizes were tested with PDS and narrow windows size (1-3) provided better results. Double PLS was tested using different numbers of principal components. Sixteen (16) PCs seemed to be the threshold amount as this number guaranteed a certain level of accuracy but avoided over-fitting. Really low numbers of PCs did not provide any useful results.

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References

1. E. Bouveresse and D.L. Massart, *Vib. Spectrosc.* **11**, 3-15 (1996).
2. P.C. Williams and K.H. Norris (Eds), *Near Infrared technology in the agricultural and food industries*, American Association of Cereal Chemists International, St Paul, MN (1987).
3. I. Gonz  les-Mart  n, I. Gonz  les-P  rez, J. Hern  ndez-M  ndez, and N. Alvarez-Garc  a, *Meat Sci.* **65**, 713-719 (2003).
4. E. Sturaro, C. Romani, M. Noventa, L. Gallo and P. Carnier, *Ital. J. Anim. Sci.* **2**, 373-375 (2003).
5. J. Garc  a-Olmo, A. Garrido-Varo and E. De Pedro, *J. Near Infrared Spectrosc.* **9**, 49-62 (2001).
6. N. Prieto, S. Andr  s, F.J. Gir  ldez, A.R. Mantec  n and P. Lav  n, *Meat Sci.* **74**, 487-496 (2006).
7. R.N. Feudale, N.A. Woody, H. Tan, A.J. Myles, S.D. Brown and J. Ferr  , *Chemom. Intell. Lab. Syst.* **64**, 181-192 (2002).

Reference paper as:

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8. M. Forina, G. Drava, C. Armanino, R. Boggia, S. Lanteri, R. Leardi, P. Corti, P. Conti, R. Giangiaco, C. Galliena, R. Bigoni, I. Quartari, C. Serra, D. Ferri, O. Leoni and L. Lazzeri, *Chemom. Intell. Lab. Syst.* 27: 189-203. (1995).

Reference paper as:

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