Calibration transfer of fatty acids equations between NIR instruments located in different geographical sites

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Introduction

To obtain robust calibrations for agro-food products need to have the scan and reference data of a high number of samples produced during different years and covering the complete range of variability of the constituent to analyse. The equations produced in such way have not only a high scientific value but also an important economic value. Thus, it is desirable that robust calibrations could be of use on instruments placed in different laboratories. However, even if instruments meet manufacturer's specifications, their spectra may not be alike enough for consistent prediction without adjustment to the spectra or equation. Shenk and Westerhaus[1,2] developed a standardisation procedure to correct for these differences between instruments.

Previous work[3] showed that two near infrared (NIR) instruments located on the same laboratory (side by side) were successfully standardised to transfer calibrations equations to predict main fatty acids (palmitic, stearic, oleic and linoleic acids) of Iberian pig liquid fat samples.

The objective of this work is to examine the possibility of transferring fatty acids calibration equations developed in one instrument to other NIR instruments located on different geographical sites.

Material and methods

NIR instruments

Three NIR monochromator instruments were optically matched:

- Master: a Foss-NIRSystems 6500 monochromator located in Cordoba and equipped with a spinning module.
- Satellite A: a Foss-NIRSystems 6500 monochromator attached with a transport module and located in Zaragoza.

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- Satellite B: a Foss-NIRSystems 6500 monochromator equipped with a transport module located in Cabrils (Barcelona).

Instrument diagnostic were measured on each instrument before attempting to measure the NIR spectra of the samples.[2]

Equations and standardisation samples

Robust calibrations equations used to transfer between the different instrument were developed on the master instrument to predict main fatty acids composition on Iberian pig fat (palmitic acid or C16:0, steaic acid or C18:0, oleic acid or C18:1 and linoleic acid or C18:2).[3] These equations are shown on Table 1 and they were discussed in details by Garcia-Olmo *et al*. (2001).[3]

Table 1. Statistics of NIR equations to predict fatty acids composition on Iberian pig fat (*N* = 352).

Constituent	Mean	*SD*	*SECV*	r^2
C16:0 (%)	21.00	1.39	0.26	0.97
C18:0 (%)	10.68	1.34	0.28	0.96
C18:1 (%)	52.24	2.37	0.26	0.99
C18:2 (%)	9.40	1.33	0.15	0.99

SD: standard deviation

To evaluate the possibility of successfully transferring to both satellites the fatty acids equations developed on the master, it was used a unsealed sample of Iberian pig fat. This sample of liquid pig fat had a fatty acids composition similar to the mean values of the NIR equations described on Table 1 (Table 2).

Table 2. Fatty acids composition (%) of the standardisation and validation sets.

Constituent	Standardisation set (n = 1)	Validation set (n = 10)	
		Mean	*SD*
C16:0	20.40	21.12	2.18
C18:0	10.00	10.51	1.43
C18:1	52.60	52.53	3.36
C18:2	10.40	9.32	1.19

A validation set of ten unsealed samples of Iberian pig liquid fat were used to evaluate the success of the instrument standardisation and fatty acids equations transfer. These samples had fatty acids values which were well represented in the calibration set (Table 2).

As NIR instruments were located in different geographical sites, it was necessary to transport standardisation and validation samples to the participant laboratories. Frozen fat samples were placed inside of an insulated food container and they were carried out by train from Cordoba (master) to Zaragoza (satellite A) and Barcelona (satellite B). Samples were melted at 40°C before its NIR spectra were taken.[3]

Standardisation and validation samples were analysed by duplicate in the master and satellite instruments using folded transmission and aluminium reflector surface cam-locks cup (reference IH-03459). A diffuse reflecting surface placed at the bottom of the cup reflects the radiation back trough the sample to the reflectance detectors.[4] Each sample of the standardisation and validation sets was analysed by the same analyst by repacking of two subsamples using the same sample cell

as for the development of the fatty acids calibrations. Spectra were collected with ISI NIRS 3 software ver. 3.11 (Infrasoft International, Port Matilda, PA, USA).

Data treatment

All the spectra scanned on the master were compared with the corresponding spectra scanned on the satellite using the Clone 1 programme in the ISI software according to Shenk and Westerhaus methodology.[1,2,4,5] It was produced a standardisation file for each satellite instrument that was applied to the validation set scanned on both satellite instruments in order to make satellite spectra to look like spectra from the validation set scanned on the master instrument. The statistics used to evaluate the performance of the standardisation and calibration transfer were the standard error of the differences (*SED*) between the predicted values of validation set by the master and the satellite before and after standardisation and the mean *H* Mahalanobis distance.[4,5]

Results and discussion

Figure 1 and 2 show similarities and differences between liquid fat spectra scanned on the master, satellite A and satellite B instruments before and after standardisation. As can be seen, the standardisation completed with a liquid pig fat sample produced spectra on both satellite instruments which look like spectra scanned on the master instrument.

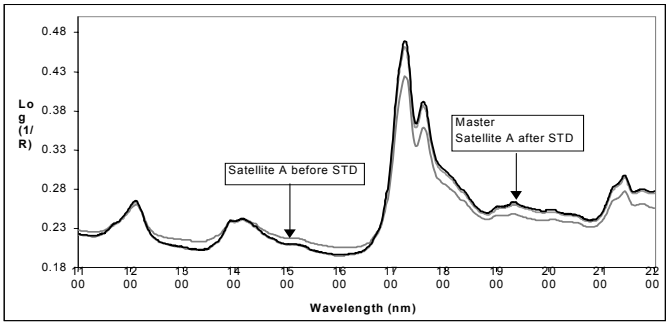

Figure 1. Comparison of liquid fat spectra scanned on the master and satellite A (Zaragoza) before and after standardisation.

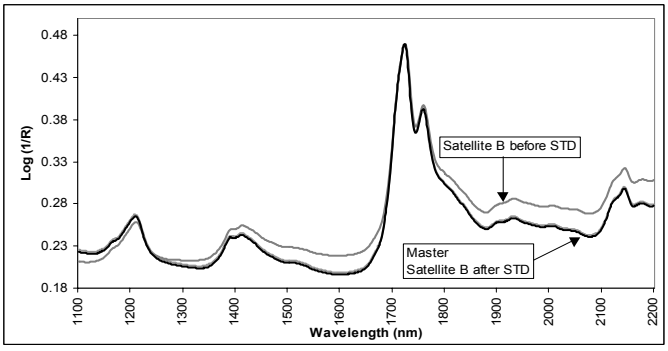

Figure 2. Comparison of liquid fat spectra scanned on the master and satellite B (Barcelona) before and after standardisation.

Validation samples scanned in both satellite instruments were standardised by the standardisation file. Then, the standardised spectra were predicted with the fatty acids equations developed in the master (Table 1). Table 3 and 4 include the standard error of the differences (*SED*) between the predicted values of validation set by the master and the satellite instruments before and after standardisation and the standard error of prediction (*SEP*) values for the validation samples scanned on the master instrument. Table 3 and 4 also include the mean *H* Mahalanobis distance reached by the validation set scanned on the master and satellite A and B before and after standardisation.

Table 3. Standardisation statistics for the validation set (n = 10) scanned on the master and satellite A (Zaragoza) before and after standardisation.

Statistic	Master	Satellite A before STD	Satellite A after STD
SED C16:0	0.30[(1)]	12.28	0.42
SED C18:0	0.29[(1)]	2.63	0.48
SED C18:1	0.35[(1)]	1.98	0.58
SED C18:2	0.11[(1)]	20.35	0.75
H Mahalanobis	1.38	46.11	1.91

[a]*SEP* values

Table 4. Standardisation statistics for the validation set (*n* = 10) scanned on the master and satellite B (Barcelona) before and after standardisation.

Statistic	Master[a]	Satellite B before STD	Satellite B after STD
SED C16:0	0.30[a]	11.51	0.46
SED C18:0	0.29[a]	0.24	0.21
SED C18:1	0.35[a]	1.73	0.37
SED C18:2	0.11[a]	0.50	0.29
H Mahalanobis	1.38	185.38	2.69

[a]*SEP* values

As can be seen on Table 3 and 4, *SED* values of validation set before the standardisation of satellite A (C16:0 = 12.28; C18:0 = 2.63; C18:1 = 1.98 and C18:2 = 20.35) and satellite B (C16:0 = 11.51; C18:0 = 0.24; C18:1 = 1.73 and C18:2 = 0.50) were much higher than *SEP* values obtained on the master (C16:0 = 0.30; C18:0 = 0.29; C18:1 = 0.35 and C18:2 = 0.11). SED values for samples scanned on satellite A before standardisation were always higher than those statistics obtained on the satellite B, particularly for the prediction of C18:2. However, the average *H* Mahalanobis distance of validation samples scanned on satellite B (*H* = 185.38) were higher than the obtained on satellite A (*H* = 46.11). This indicates that latent variables obtained after PCA and PLS analysis extract different information from a sample or spectrum.

After standardisation, *SED* values of validation set on satellite A (C16:0 = 0.42; C18:0 = 0.48; C18:1 = 0.58 and C18:2 = 0.75) and satellite B (C16:0 = 0.46; C18:0 = 0.21; C18:1 = 0.37 and C18:2 = 0.29) decreased reaching similar values to the *SEP* obtained on the master instrument. The average *H* Mahalanobis distance of validation set on satellite A (H = 1.91), satellite B (2.69) and the master (*H* = 1.38) were also similar. To ensure these low *SED* and *H* Mahalanobis values obtained after standardisation, standardisation and validation samples must be well conserved while travelling from one laboratory to another.

Conclusions

The results show that the Shenk and Westerhaus' standardisation algorithm allows a successful optical matching and equation transfer for the prediction of fatty acids on NIR instruments located on different geographical sites. The optical matching can be obtained by using only one sample of liquid fat.

Acknowledgements

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