# Strategies for calibration transfer between instruments that have similar or different spectral ranges

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## Introduction

The transfer of a calibration between different near infrared (NIR) instruments can be accomplished in different ways.<sup>1</sup> One of the most common methods used involves the development of a transfer function that will account for the difference in the measured absorbance spectra. The application of such a function will permit the generation of spectra from a given instrument that are very similar to the spectra generated on the same samples from another instrument. In the literature, several univariate or multivariate methods are described to develop such functions and these have been applied between similar instruments or between sensors using quite different technologies (e.g. FT-NIR and diode array). None of those methods will be able to transfer between instruments working in different spectral ranges. This study has focused on a set of spectral data of pork fat used to evaluate the suitability of pork thighs to make Parma cured ham. After establishing a robust calibration, prediction of pork fat quality obtained from a reflectance instrument was used to calibrate another reflectance and also a transmission instrument.

## **Materials and Methods**

Three NIR instruments were used in this study; a Foss NIRSystems 5000 (1100-2498 nm, at 2 nm intervals) reflectance instrument that has been used to develop the original pork fat calibration since 2006; a Unity Scientific 2500x (680-2500 nm, at 1 nm intervals) also working in reflectance; and a transmission instrument (Grainit srl) based on a Zeiss MMS1 diode array sensor (400-1100 nm) with 256 pixels and spectra interpolated every 2 nm.

Subcutaneous fat samples were collected after slaughter from the outer layer of back fat of animals, produced under the guidelines of the Designation of Origin "Parma ham" and stored at -20°C. For analysis, frozen fat samples were minced using a Retsch laboratory mill (4000 rpm x 10 s). Minced samples were placed in a round ring cup for measurement on the Foss NIRSystems 5000 and the Unity instruments and placed in small plastic petri dish (30 mm in diameter with a path length of 10 mm) for measurement in transmission on the Zeiss-based system.

The original calibration consisted of 345 samples that were analysed for iodine number (IN) according to the modified Wijs method (AOAC, 2000) and fatty acid content by gas chromatography. A total of 320 samples of pork fat from animals reared during 2009 were used in this study. Composition data were obtained for 65 of these samples, which were divided in a group of 32 for calibration update and 33 for validation. For the remaining 255 samples, NIR predictions were obtained using the original calibration from the Foss system and were used to develop new calibrations for the Zeiss in transmission and for the Unity in reflectance. Also, the original Foss calibration was transferred to the Unity system using the Shenk and Westerhaus standardisation method (Ucal v1.0); the average spectrum of the 255 samples scanned on the common ring cup was used. The software Ucal 1.0 (Unity Scientific, LLC) was used for calibration development and validation. All of the calibrations was evaluated using the external validation set of 33 samples. Calibrations were developed with the UCal software package using the PLS algorithm. Data were pretreated with scatter correction (standard normal variate and detrending), first derivative (gap of 4 data points) and smoothing (4 data points). The full spectral range was used for the Foss NIRSystems and Unity instruments. For the Zeiss instrument, calibration development was limited to the 700-1100 nm range.

# **Results and Discussion**

Figure 1 shows the raw NIR spectra (without mathematical pre-treatment) obtained with the three different instruments. The Foss NIRsystems 5000 and Unity SpectraStar 2400 spectrometers work with similar wavelength ranges while the MMS1 instrument operated in a completely different wavelength range (400-1100 nm) compared to the Foss instrument and had only a small portion in common with the Unity instrument (680-1100 nm). Given the similarity in the spectral range and shape of the spectra between the

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Foss NIRSystems and Unity instrument, it was possible to apply a standardisation algorithm<sup>3</sup> to make the spectra from the two different platforms alike. This was not possible between the Foss NIRSystems and the Zeiss instrument as they did not have any wavelength in common and because one worked in reflectance and the other in transmission.



**Figure 1.** Typical spectra in different spectral ranges of the instruments used in the study.

Considering the calibration statistics (Table 1), it appears that the standardisation process was able to maintain the same level of performance. The standard error of cross-validation (SECV) and root mean square of cross-validation (RSQcv) for the calibration using NIR predictions ("transferred") were surprisingly much better than the original calibration based on wet chemistry values. The value of SECV for the Unity transferred was only 0.37% for both IN and LA compared to 0.88 and 0.58% in the original Foss NIRSystems calibration. Also, for the transfer onto the transmission instrument, the SECVs of IN (0.42%) and LA (0.43%) were much lower than the original calibration. Another peculiarity of these transferred calibrations was that models were optimised with the maximum number of principal components allowed, (fixed at 15) and the models for the data set with laboratory reference data used a maximum of 11 or a lower number of terms. The addition of 23 samples for updates with real laboratory determinations has resulted in an increase in the SECV, particularly for IN on the Unity platform.

The decrease in SECVs and the use of a larger number of principal components must be related to lower error associated with the prediction values, which are affected only by error between replicates. Near infrared is well known for good repeatability of results which would produce a 'laboratory' error much lower than the one associated with the wet chemistry procedures. The fact that the addition of 23 samples with real chemistry analysis had a negative impact on SECV of IN, may confirm that this is an effect due to the error associated with the reference values used in calibration.

To our knowledge, only one other study<sup>2</sup> has reported on the effect of using prediction values from a reflectance instrument to develop a calibration model for forage analysis for a transmission instrument. In their comparison between NIR spectra based and chemistry based models, there was not much of a difference in terms of SEC and SEP of the models based on the different reference values. However, in that case samples were not dried and only manually cut, increasing variability of scan, yielding a repeatability error of triplicates that was similar to the SEC of the calibration. Pork samples are much more homogeneous than coarsely-chopped forage and our samples were also minced to maximise homogeneity of the samples scanned. This must have kept our repeatability error very low.

Validation of calibrations with an independent set has highlighted a few problems (Table 2). The standardised calibration had a slight increase in the SEP for IN over the original SEP but after the updates, there was an unexpected marked increase in the prediction error; similar behaviour was observed also for the LA predictions.

As expected, performance of the transferred calibration did not hold up in comparison with real reference values. Nevertheless, the SEP for the Unity transferred calibration was very similar to the original one for LA and slightly better for IN. This confirmed what was already observed by Dardenne and coworkers<sup>2</sup>, i.e. that the use of prediction values to develop calibrations on other platforms leads to a fairly good transfer of the information in a data set to another one; even if the instrument is quite different.

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Table 1. Calibration statistics.								
	Ν	Mean	SD	Terms	SEC	RSQ	SECV	RSQcv
NIRSystems (original data)								
lodine number	331	71.54	3.11	8	0.81	0.93	0.88	0.91
Linoleic acid (%)	328	13.43	1.82	10	0.53	0.92	0.58	0.89
NIRSystems (updated)								
lodine number	364	71.65	3.16	8	0.84	0.93	0.91	0.91
Linoleic acid (%)	359	13.54	1.84	11	0.51	0.93	0.58	0.90
Unity (standardised data)								
lodine number	331	71.54	3.11	8	0.80	0.93	0.87	0.91
Linoleic acid (%)	328	13.43	1.82	10	0.51	0.92	0.57	0.89
Unity (standardised and updated)								
lodine number	360	71.62	3.17	10	0.78	0.94	0.89	0.91
Linoleic acid (%)	360	13.54	1.84	10	0.56	0.91	0.64	0.87
Unity (transferred)								
lodine number	255	72.09	1.27	15	0.22	0.99	0.37	0.97
Linoleic acid (%)	255	14.55	1.27	15	0.21	0.97	0.37	0.90
Unity (transferred and updated)								
lodine number	284	72.11	2.48	15	0.31	0.98	0.52	0.95
Linoleic acid (%)	285	14.56	1.96	15	0.23	0.97	0.38	0.92
Zeiss (transferred)								
lodine number	252	72.09	2.25	14	0.36	0.97	0.42	0.96
Linoleic acid (%)	253	14.55	1.62	14	0.37	0.91	0.43	0.88
Zeiss (transferred and updated)								
lodine number	279	72.17	2.47	13	0.42	0.97	0.47	0.96
Linoleic, %	282	14.57	1.96	14	0.38	0.93	0.44	0.90

#### Table 2. Validation statistics.

	Mean	bias	SEP	SEP(C)	Slope	RSQ
lodine number						
NIRSystems original	72.52	0.01	0.82	0.83	1.04	0.88
NIRSystems updated	72.64	-0.10	0.82	0.82	1.04	0.88
Unity standardised	72.55	-0.02	0.93	0.94	0.94	0.84
Unity standardised and updated	72.33	0.21	1.29	1.29	0.78	0.75
Unity transferred	72.70	-0.16	0.75	0.74	1.01	0.90
Unity transferred and updated	72.67	-0.13	0.79	0.78	1.00	0.89
Zeiss transferred	72.41	0.12	1.00	1.00	1.05	0.82
Zeiss transferred and updated	72.37	0.17	0.97	0.97	1.04	0.83
Linoleic acid (%)						
NIRSystems original	14.83	0.18	0.56	0.54	1.00	0.85
NIRSystems updated	15.05	-0.05	0.49	0.50	1.00	0.87
Unity standardised	14.87	0.13	0.79	0.79	0.71	0.81
Unity standardised and updated	14.79	0.22	0.88	0.86	0.72	0.72
Unity transferred	15.07	-0.07	0.48	0.48	0.97	0.88
Unity transferred and updated	15.11	-0.10	0.54	0.53	0.92	0.86
Zeiss transferred	14.63	0.37	0.98	0.92	0.92	0.57
Zeiss transferred and updated	14.66	0.35	0.94	0.88	0.91	0.61

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In our study, the custom-made transmission instrument had SEP values slightly greater for IN than the original calibration (1.0 vs 0.82). On the other hand the SEP for LA was almost double that of the NIRSystems instrument. The transmission system had a halogen lamp that generated a lot of heat; the system did not control sample temperature that was routinely scanned at room temperature and the variation in temperature may have affected the prediction of LA.

## Conclusion

This study showed that the use of NIR prediction values instead of spectral data, is a valid alternative to transfer calibration data sets and models. The fact that this method does not limit the transferability between instruments with similar spectral ranges and sample presentations increases the flexibility of possible instruments that can be used thereby using the information and value invested in a well-established calibration.

## Acknowledgements

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## References

- 1. T. Fearn, J. Near Infrared Spectrosc. 9, 229-244 (2001).
- 2. P. Dardenne, G. Sinnaeve, R. Biston, and P. Leconte, in *Making Light Work: Advances in Near Infrared Spectroscopy. Proceedings of the 4th International Conference on Near Infrared spectroscopy.* Ed by I. Murray and I.A. Cowe, J. Wiley and Sons, UK, pp. 277-283 (1991).
- 3. J.S. Shenk and M.O. Westerhaus, Crop Sci. 31, 1694-1696 (1991).