Near infrared analysis of Iberian pig fat: repeatability file effect

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Introduction

Previous studies have demonstrated the viability of near infrared (NIR) technology for predicting the fatty acid content of Iberian pig fat.[1,2] However, despite the high degree of precision afforded by the equations obtained, considerable fluctuations were detected when predicting new samples; these fluctuations became more marked as the time interval between obtaining the equation and testing the product increased, thus hindering routine laboratory use of the equation. Moreover, in our hands, the problem was encountered only in products such as fat, which display an absorption pattern with well-defined peaks at given wavelengths; this prompts acute sensitivity to the slight instrumental changes which escape detection during routine instrument monitoring using the standard check cell. To correct this drawback, WinISI 1.04 software offers a mathematical algorithm consisting, briefly, in a "Repeatability File" which, when used for calibration purposes, enables minimisation of the sources of variation that might affect NIR predictions.[3]

The purpose of the present study was to assess the effect of using a repeatability file in the quantitative NIR analysis of subcutaneous Iberian pig fat.

Material and methods

Experimental material

A total of 188 samples of subcutaneous Iberian pig fat were taken from the carcass rump of batches of pigs reared on different feeding systems. Samples were obtained at COVAP, a Spanish pork- producing cooperative taking part in R+D Project IFD-0990, over two production seasons: 1997–98 ($N = 97$) and 1999–2000 ($N = 91$).

After removal of remnant skin, lean tissue and the surface fat layer, fat samples were melted in a microwave oven following De Pedro *et al*.[4]

NIR spectrum collection and reference data

Melted subcutaneous fat samples were analysed in a Foss NIRystems 6500 SY-I scanning monochromator equipped with a spinning cup, working in reflectance mode in the spectral range 400–2500 nm.

Measurements were made using folded-transmission gold reflector cups with a pathlength of 0.1 mm. Two spectra were measured per sample, the mean spectrum being used for subsequent analysis.

The fatty acid composition of each sample was determined by gas chromatography (GC). The methyl esters of fatty acids were extracted with hexane and were determined using a Perkin Elmer Sigma 3 D chromatograph with FID detector.

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Chemometric treatment of data

Spectroscopic and chemical data were subjected to chemometric treatment using WinISI ver. 1.04 software.[5] Initially, the "Center" algorithm was applied to the sample set and six anomalous spectra were detected and eliminated. Calibration equations were then obtained for the 182 remaining samples, using the following: modified partial least squares (MPLS) for regression purposes; wavelength range 1100–2500 nm (at 2 nm intervals); SNV and Detrending treatments to correct for diffuse radiation phenomena. Several mathematical derivation treatments were also tested.

Calibration equations were then obtained using what the WinISI chemometric package termed a "repeatability file", composed of 128 spectra from a single sample, obtained weekly over a nine-month period.

The best equations obtained, with and without the repeatability file, were validated using a validation set composed of 12 fat samples not included in the calibration; sample spectra were collected some time after calibration.

The following statistical parameters were used to evaluate the predictive capacity of the equation: standard error of cross-validation (*SECV*), standard error of prediction corrected for bias [*SEP*(*c*)], coefficient of determination for the cross-validation process (r^2) and for the external validation process (R^2), and bias or mean residual error for the external validation set.

Results and discussion

NIR calibration equations were obtained for predicting the content of six fatty acids in melted Iberian pig fat: myristic acid (C14 : 0), palmitic acid (C16 : 0), palmitoleic acid (C16 : 1), stearic acid (C18 : 0), oleic acid (C18 : 1) and linoleic acid (C18 : 2). Resulting statistics are shown in Table 1.

The calibration statistics obtained (Table 1) suggest that these equations afford a high degree of precision for predicting content of the six fatty acids under study, yielding *SECV* values similar to, and in some cases lower than, those reported by García-Olmo *et al.*[2], using folded transmission aluminium reflector cups.

Table 1. Calibration and validation statistics obtained for predicting fatty acid content in subcutaneous Iberian pig fat with and without repeatability file.

			C14 : 0	C16 : 0	C16 : 1	C18 : 0	C18 : 1	C18 : 2
Without repeatability file	Calibration	*N*	172	176	174	175	166	175
		r^2	0.76	0.97	0.94	0.97	0.99	0.98
		SECV	0.07	0.28	0.08	0.27	0.20	0.16
	Validation	*N*	12	12	12	12	12	12
		R^2	0.52	0.92	0.74	0.94	0.98	0.99
		SEP(*c*)	0.09	0.43	0.13	0.27	0.47	0.13
		Bias	–0.05	-0.42	–0.03	0.47	0.14	0.25
With repeatability file	Calibration	*N*	179	168	175	180	171	163
		r^2	0.65	0.98	0.92	0.96	0.99	0.98
		SECV	0.08	0.24	0.09	0.29	0.20	0.16
	Validation	*N*	12	12	12	12	12	12
		R^2	0.90	0.97	0.79	0.99	0.99	0.94
		SEP(*c*)	0.06	0.26	0.10	0.17	0.32	0.26
		Bias	–0.04	–0.11	0.03	0.28	–0.04	–0.20

The validation statistics obtained suggest that the repeatability file is highly efficient for minimising sources of variation which may undermine the precision of predictions, particularly in products such as fat, which display well-defined peaks in the near infrared range. As Table 1 shows, differences with reference to calibration are negligible and, indeed, better results were sometimes obtained without applying the repeatability file. The positive effect of using the repeatability file is chiefly appreciable when predicting new samples not involved in the calibration process, whose spectra were collected some time after calibration; bias values were much lower than those recorded when not using the repeatability file.

Conclusions

The results obtained here support the use of a repeatability file when obtaining NIR calibration equations; this file ensures the required precision in routine prediction of fatty acid content in new subcutaneous Iberian pig fat samples.

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