Reflectance *versus* interactance reflectance near infrared analysis of homogenised and intact ewe cheese

Nieves Núñez-Sánchez,[a] Ana Garrido-Varo,[b] Juan M. Serradilla-Manrique[b] and José L. Ares-Cea[c]

[a]*C.I.F.A Cordoba Andalusian Department of the Ministry of Agriculture and Fisheries, Spain.*

[b]*Animal Production Department, School of Agriculture and Forestry Engineering, University of Cordoba, PO Box 3048, E-14080, Cordoba. Spain.*

[c]*C.I.F.A, Hinojosa del Duque Andalusian Department of the Ministry of Agriculture and Fisheries, Spain.*

Introduction

Economic and legal constraints require strict control of the composition of dairy products. From the payment of the milk, depending on fat and protein levels, to the control of the production process and the quality control of the final product. The official analytical reference procedures for fat, dry matter, protein and casein, all take too long to be satisfactory for controlling manufacturing processes. Consequently, there is an urgent need of instruments that can rapidly determine quality or important economic parameters.

The aim of this work was to evaluate near infrared (NIR) calibration equations for main constituents of cheese, analysed: (a) under two different sample preparation methods: homogenised and non-homogenised (intact) cheese and (b) under two different analysis modes: reflectance and interactance–reflectance (fibre optic probe).

Materials and methods

Cheese samples

A total of 187 ewe cheese samples were used in this study. The milk used to make the cheeses came from different ewes and different lactations.

After 15 days of ripening, cheeses were barked and split into two halves. A windmill was used for the homogenisation of one of them (Tecator KNIFETEC model 1095 Sample Mill), using these samples for chemical and NIR analysis of homogenised cheese. The other half of each cheese was reserved for the NIR analysis of intact cheese. Chemical analysis of the cheese samples was duplicated, for the determination of fat (Van Gulik), dry matter (oven drying) and protein ($N \times 6.38$) contents.

NIR spectroscopy

Three spectroscopic NIR analysis modes were compared:

- Reflectance, a small ring cup filled with homogenised cheese
- Interactance–Reflectance, placing the fibre optic probe on the homogenised cheese samples.

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■ Interactance–Reflectance, placing the fibre optic probe on a slice of intact cheese.

Near infrared reflectance spectra were obtained on an NIRSystems 6500 monocromator (model I) with a spinning module. Near infrared interactance–reflectance spectra were obtained by an NIRSystems 6500 monocromator (model II) with a fibre optic attachment (NR-6775).

All spectral data were recorded using the ISI-NIR 3 version 3.11 (International Infrasoft, Port Matilda, PA, USA). Optical data recorded as log (1/*R*) were taken at 2 nm intervals. The vis-NIR region (400 nm–2500 nm) was used for reflectance analysis. However, the vis-NIR region was trimmed to 800 nm–2200 nm for interactance-reflectance analysis before mathematical treatment of the data.

Two replicates were performed on each sample for the reflectance measurements and four replicates for the fibre optic probe measurements.

Development of NIR calibrations

Mathematical treatment of all the spectral data was performed using the ISI-NIR 3 version 3.11 software.

Calibrations were developed for each of the three NIR analysis modes with a total of 187 samples for fat and dry matter and 142 samples for protein, since it was not possible to analyse all cheese samples for this last parameter.

The methodology followed for the development and evaluation of NIR calibrations has been described in different publications.[1–4] The statistics used for the selection of the most accurate calibration equations were: the standard error of the residuals for the calibration (*SEC*) and for the cross-validation (*SECV*), the coefficient of determination for the calibration (R^2) and for the cross-validation (r^2), the coefficient of variation (*CV*), calculated as (*SECV*/Mean) × 100 and the RPD, calculated as *SD/SECV*.

Results and discussion

The mean, minimum, maximum and standard deviation (*SD*) of the values (% w/w) for each of the three parameters analysed are given in Table 1. A wide variability in the calibration set was observed, ranging from 16.13% to 41.00% for fat content, from 43.34% to 71.92% for dry matter content and from 16.40% to 28.94% for protein content.

Tables 2 and 3 show the calibration statistics for homogenised cheese analysed by reflectance and interactance–reflectance respectively. The coefficient of determination (r^2) were always very high. Shenk and Westerhaus [3] indicate that the calibrations with values of r^2 higher than 0.9 have an excellent capacity for quantitative analysis.

The *SECV* values obtained for the homogenised cheeses and for both analysis modes are comparable for the three parameters studied (*SECV* Fat = 0.51% v. 0.69%, *SECV* Dry Matter = 0.63% v. 0.79% and *SECV* Protein = 0.77% v. 0.63%).

The calibration statistics for the intact cheese analysed by fibre optic probe are presented in Table 4. The *SECV* values obtained for fat (1.11%) and dry matter (1.33%) equations were higher than those

Table 1. Chemical data (% w/w) for cheese samples.

	N	Mean	Minimum	Maximum	*SD*
Fat	187	30.68	16.13	41.00	4.99
Dry matter	187	59.53	43.34	71.92	5.63
Protein	142	21.86	16.40	28.94	2.86

Table 2. Calibration statistics for homogenised cheese NIR equations in the reflectance mode.

	N	Mean	*SECV*	R^2	*CV*	*RPD*
Fat	174	30.5	0.51	0.99	1.67	9.78
Dry matter	165	59.8	0.63	0.98	1.06	8.98
Protein	131	21.8	0.77	0.93	3.54	3.73

Table 3. Calibration statistics for homogenised cheese NIR equations in the interactance–reflectance mode.

	N	Mean	*SECV*	R^2	*CV*	*RPD*
Fat	161	30.7	0.69	0.98	2.24	7.27
Dry matter	163	59.5	0.79	0.98	1.32	6.85
Protein	115	21.3	0.63	0.94	2.96	4.01

Table 4. Calibration statistics for intact cheese NIR equations in the interactance–reflectance mode.

	N	Mean	*SECV*	R^2	*CV*	*RPD*
Fat	167	30.4	1.11	0.95	3.65	4.42
Dry matter	168	59.1	1.33	0.94	2.24	3.96
Protein	115	21.4	0.71	0.91	3.30	3.40

obtained using homogenised cheese (Tables 2 and 3). However, the *SEC* value for protein (0.71%) was similar to the equivalent equations developed using homogenised cheese.

In order to make the results obtained in the present study comparable with other results previously published,[5–7] the *CV* values for all the equations obtained were calculated. The *CV* values for fat (1.67% and 2.24%) and dry matter (1.06% and 1.32%) in the homogenised cheese are similar to those obtained by Pierce and Wehling.[5]

The *CV* values of the intact cheese equations for fat (3.65%) and dry matter (2.24%) are higher than those obtained by Pierce and Wehling [5] and by Rodríguez *et al*.[6] However, part of the differences can be explained by the different cheese surface areas scanned in the three studies. Pierce and Wehling [5] used a fibre optic probe with 5 × 5 cm window to scan the samples and Rodríguez *et al*.[6] used a high fat/high moisture cell, with a 16.5 × 3.5 cm window, approximately. In the present study, a probe with a small window (0.7 cm ϕ) was used.

The value of the RPD statistic (Tables 2, 3 and 4) were always higher than 3 minimum value, recommended by Williams and Sobering[4] to consider a calibration equation suitable to use under real conditions for the control of processes.

Conclusion

This study has shown that NIR calibrations, based on homogenised and intact cheese analysed by fibre optics, can be used for control purposes of the cheese production process, avoiding the need to fill

and clean the cups. Further work is in progress to improve the precision of the equations for the analysis of intact cheese. Factors such as wavelength region, selection and number of subsamples to analyse should be optimised.

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