Determining non-destructive quality of fat and low-fat European-style cheese by near infrared spectroscopy

Diana De Santis, Paolo Carlini and Riccardo Massantini

Istituto di Tecnologie Agroalimentari, Facoltà di Agraria, Università degli Studi della Tuscia, Via S.C. De Lellis snc, Viterbo, I-01100, Italy.

Introduction

The use of near infrared (NIR) spectroscopic techniques to collect on-line data about the composition of food products is a very active field of research.

During cheese production it is important that major components are rapidly and directly quantified and, in many instances, NIR methods can be used effectively.[1] However, there have only been a few cases where results for the non-destructive assessment of major constitutents have been presented.[2–4]

The purpose of the present work was to evaluate the use of NIR spectroscopy for the non-destructive quantification of major constituents (i.e. moisture and total nitrogen content) in different kinds of European-style soft cheeses, including those integrated with sodium alginate and carrageenan, characterised by an unusual composition, with high moisture content. Also, a calibration for discrimination between full-fat and no-fat samples was studied.

Materials and methods

Cheese manufacture

Cheeses for our experiments, with two levels of fats (0 g kg^{-1} and about 260 g kg^{-1}) were manufactured in a laboratory-scale plant from pasteurised milk and standard milk with 0 g kg^{-1} and 35 g kg^{-1} in fat content, respectively, according to a previously used process.[5]

Milk for cheese with carrageenan was treated at 85°C for 3–4 mins, to incorporate part of the whey proteins, after the addition of 0.3 g kg^{-1} of carrageenan (type τ and type κ, 60 : 40, Sigma–Aldrich, Italy), then cooled at 32°C and manufactured according to the adopted procedure. Cheeses with alginate were prepared from milk added with 0.25 g kg^{-1} of sodium alginate (Mannuronic type, Sigma).

Chemical determinations

All chemical analyses were conducted in accordance with the AOAC, 1990 methods.

Near infrared spectroscopy

During the first week of ripening, individual forms were picked and conditioned for 30 minutes at 25°C ± 2.

Spectroscopic data were then recorded by a rapid scanning (1.8 scans s^{-1}) vis-NIR spectrophotometer NIRSystems (Silver Spring, MD, USA) model 6500, using a fibre optic

interactance probe and interfaced to a personal computer running NIRS-2 Version 4.00 package with Infrasoft International (Port Matilda, PA, USA).

The probe was hand-placed against the top face of each sample at a random location near the centre for about 15 seconds, corresponding to 20 averaged scans. Light interacted through a ring about 1 cm in diameter. For each sample, a cylindrical piece with a diameter of about 2.5 cm and the full height of the cheese was cut from the same location where the optical scans were conducted, then grated, mixed and analysed by standard AOAC methods.

Results and discussion

Fat content varied from 0 g kg^{-1} to about 5 g kg^{-1} in low fat cheeses and from about 255 g kg^{-1} to 265 g kg^{-1} in the full fat ones, with very small departures from the target values of 0 g kg^{-1} and 260 g kg^{-1}, respectively.

Compositional data showed, as expected, an increase of moisture and total nitrogen content when fat was removed. Due to the formation of a three-dimensional gel network by protein–polysaccharide–water interaction,[6] integrated samples had the highest amount of moisture, with a mean value of about 680–690 g kg^{-1}, followed by standard low fat cheese, 630–635 g kg^{-1}, and then standard full-fat cheese, about 530 g kg^{-1}. A total of 153 samples were considered overall during the calibration development for moisture content: using a randomised procedure 100 samples were assigned to the calibration set, where the remaining 53 samples constituted the validation set (Table 1). For total nitrogen content calibration, 87 and 33 samples were assigned to the calibration and validation set (Table 2).

The same data sets used for moisture calibration were considered in the development of a linear discriminant-based classification technique which were able to tell full-fat samples from no-fat ones.

Calibration development

The combined calibration and validation data sets were extensively studied using the various options available in the "spectra calibration" software module of the NIRS-2 4.00 package.

Table 1. Moisture content (g kg^{-1}).

	Overall	Standard	St. low fat	Carrag.	Alginate
Calibration					
Samples	100	25	26	24	25
Range	488.5–716.8	488.5–566.7	573.3–666.0	665.3–712.0	643.2–716.8
Mean	635.4	532.5	636.0	692.7	683.0
Std dev.	66.2	19.1	21.5	13.9	20.9
Validation					
Samples	53	13	13	14	13
Range	500.7–716.1	500.7–564.0	592.2–667.9	635.9–714.5	633.2–716.1
Mean	634.9	531.8	632	684.8	684.8
Std dev.	66.1	17.0	24.1	19.7	20.9

Table 2. Nitrogen content (g kg^{-1}).

	Overall	Standard	St. low fat	Carrag.	Alginate
Calibration					
Samples	87	24	23	24	16
Range	23.748.1	26.4–31.0	35.8–48.1	28.3–37.2	217–36.0
Mean	33.1	28.9	39.9	32.0	31.2
Std dev.	4.9	1.1	2.6	2.8	2.8
Validation					
Samples	33	7	8	10	8
Range	210–49.2	26.3–31.5	34.3–49.2	27.1–35.1	22.0–35.1
Mean	32.7	28.6	39.4	31.8	31.0
Std dev.	5.2	1.6	4.3	2.8	4.5

During preliminary experiments, the wavelength range processed was restricted to 600–1090 nm and 1110–2000 nm for vis and NIR regions, respectively, since the initial and final part of the spectra were affected by noise.

We concentrated on the well-established stepwise regression technique over a number of "full-spectrum" regression methods available in the software. A second-derivative mathematical

Table 3. Calibrations for moisture content (*SEC*, *SEP* and Bias in g kg^{-1}).

		Wavelength (nm)	Coefficient
5 wavelengths			
18 points segment 16 points gap	B(0) B(1) B(2) B(3) B(4) B(5)	 1386 1238 1336 1342 1346	708.39 –5500.45 –17285.07 –88981.99 124684.04 –43643.46
SEC = 13.76 R_C^2 = 0.96 *SEP* – 14.22 Bias = 0.76			
6 wavelengths			
18 points segment 18 points gap	B(0) B(1) B(2) B(3) B(4) B(5) B(6)	 1398 1392 1336 1410 1238 1340	694.12 51886.31 – 47584.35 –126135.33 –16233.99 –19310.30 116156.95
SEC 13.64 R_C^2 = 0.96 *SEP* 13.98 Bias = 1.30			

Table 4. Calibrations for nitrogen content (*SEC*, *SEP* and Bias in g kg^{-1}).

		Wavelength (nm)	Coefficient
5 wavelengths			
18 points segment 4 points gap	B(0) B(1) B(2) B(3) B(4) B(5)	 1576 1272 1164 902 1520	1.39 1981.02 –1786.86 _2099.53 –5178.95 1533.06
SEC 1.59 R_C^2 = 0.89 *SEP* 1.67 Bias = 0. 14			
6 wavelengths			
18 points segment 3 points gap	B(0) B(1) B(2) B(3) B(4) B(5) B(6)	 1518 1526 1272 1164 902 1578	3.51 724.50 836.38 –1685.29 –1951.00 –5288.94 2119.06
SEC = 1.5 R_C^2 = 0.90 *SEP* = 1.61 Bias 0. 12			

treatment consistently led to better calibrations and validation results. We tried various choices for the important *segment* and *gap* options, related to the numerical computation of the derivative: in order to reduce the impact of noise, segments of data points are averaged by the software and then differences are taken between neighbouring sums, spaced by a prescribed gap.[7] A *detrending* preprocessing step was beneficial, accounting for the variation in baseline shifts and curvilinearity, which are generally found in the reflectance spectra of powder and densely packed samples.[8]

In Tables 3 and 4, two different calibrations for moisture and total nitrogen content are shown, using five and six wavelengths, respectively. They resulted in the lowest combined standard error of calibration (*SEC*) and standard error of prediction (*SEP*) values among those obtained varying the preprocessing options. The optimal number of wavelengths, for each set of preprocessing treatments, was automatically determined by the software, in a stepwise process using *F*-statistics tests.

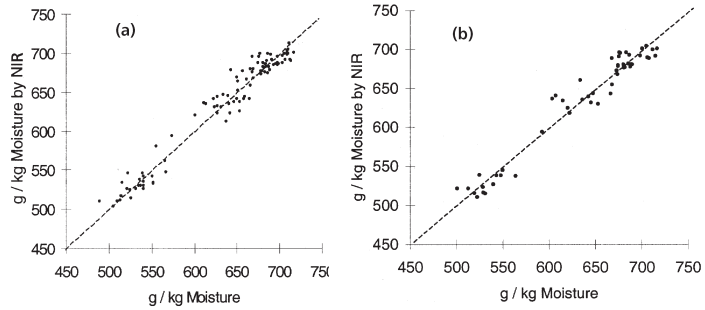

Figure 1. Comparison between moisture values for calibration (a) and validation (b) samples as predicted by NIR calibration (five wavelengths) and as determined by oven drying.

In every case a segment length of 18 points performed best, with varying results for the optimal gap lengths, shown in the tables together with the wavelengths selected by the software during the stepwise selection procedure.

Calibrations developed for moisture have almost the same accuracy in terms of *SEC*, *SEP* and (R_C^2), with the more complex model characterised, unfortunately, by a larger bias. Everything considered, in our opinion the simpler one should be preferred, even if the size of the data sets suggests the use of richer regression models. Figure 1 presents scatter plots for moisture values predicted by the regression law vs. reference laboratory values. Total nitrogen content calibrations shows a lower accuracy $(R_C^2 \cong 0.9)$ with the six wavelengths calibration favoured, in this case not penalised by an higher bias, always close to 0.1 g kg^{-1} and far smaller than the *SEC*s and *SEP*s, in the 1.5–1.6 g kg^{-1} range.

We also studied a calibration able to distinguish, automatically, fat from no-fat samples (integrated and not). The "discriminate groups" module, based on a "full spectrum" technique, called *PLS2*[9] could be usefully employed for this kind of "two-values" problem. Spectra were processed by II-derivative and detrend steps, with a 15 point segment and a 15 point gap. A regression onto a binary dummy variable representing cheese type was developed, with fat coded as 1 and no-fat coded as 0.

In fact, this problem proved quite simple to deal with, with just one PLS factor selected by the software in a 6-fold cross-validation procedure, sufficient to discriminate with a 100% accuracy our 75 (26 + 24 + 25) samples without fat and 25 samples of full-fat. This performance was not strongly affected by the pretreatment choices: a 20 point segment with 20 point gap gave a similar, very satisfying, result. The same classification accuracy was achieved on the validation set, comprising 40 (13 + 14 + 13) and 13 samples, respectively.

Conclusions

Cheese types, ranges of moisture and total nitrogen, which, to the best of our knowledge have not been previously studied, can be reliably determined by NIR spectroscopy, which is a fast (approx. 20 sec/sample) and non-destructive method.

Admittedly, calibration accuracy should be improved, beyond the 14–15 g kg^{-1} level presented, but, on the other hand, we should rely on more accurate laboratory reference measurements (see also Reference 10). Perhaps a different measuring probe, with a broader measuring area, could be effectively tried.

Moreover, in our experimentation, a simple linear discriminant-based calibration could easily tell full-fat samples from no-fat ones, either integrated or not, a task otherwise not easily carried out by untrained personnel by visual inspection.

References

1. J.L. Rodriguez-Otero, M. Hermida, J. Centeno, *J. Agric. Food Chem*. **45,** 2815 (1997).
2. M. Klenke and T. Schneemann, *Dtsch. Milchwirtsch*. **44,** 944 (1993).
3. M.M. Pierce and R.L. Wehling, *J. Agric. Food Chem*. **42,** 2830 (1994).
4. J.L. Rodriguez-Otero, M. Hermida and A. Cepeda, *J. AOAC Int.* **78,** 802 (1995).
5. D. De Santis, M.T. Frangipane, P. Carlini and R. Forniti, in *V Convegno Nazionale di Reologia Applicata.* Viterbo, Italia, 4–7 Giugno, p. 64 (1997).
6. S.K. Samant, R.S. Singhal, P.R. Kulkarni and D.V. Rege, *Int. J. Food Science Tech*. **28,** 547 (1993).
7. B.G. Osborne, T. Fearn and P.H. Hindle, *Practical NIR Spectroscopy: with applications in food and beverage analysis.* Longman Scientific and Technical, Harlow, UK (1993).
8. R.J. Barnes, M.S. Dhanoa, S.J. Lister, *Appl. Spectrosc*. **43,** 772 (1989).
9. H. Martens and T. Næs, *Multivariate Calibration*. John Wiley and Sons, New York (1991).
10. R. DiFoggio, *Appl. Spectrosc*. **49,** 67 (1995).