Comparison of near infrared measuring techniques for cheese analyses

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Introduction

The contents of fat and total solids (TS) in cheese and the ratio between these parameters are important factors in process control of semi-hard cheese production because of their influence on quality and because of regulatory requirements regarding the content. These parameters are typically controlled from a knowledge of casein transfer factors, by standardisation of the process milk and by registration weight balances. Supplementary chemical analyses are performed on the cheese by simple analytical methods, for example, rapid drying methods for moisture determination and the Gerber van Gulik method for fat determination. However, infrared spectroscopic methods have proved to be a useful alternative to these methods.

Different accuracies of NIR spectroscopy have been reported for determination of fat and TS.[1–3] The variation in reported results indicates a dependence of accuracy on the technique used for sample presentation and measurement. To elucidate this point in more detail, a study was carried out in which measurements were performed on intact and ground cheese by dispersive and interferometric reflectance and transmittance near infrared (NIR) spectroscopic techniques.

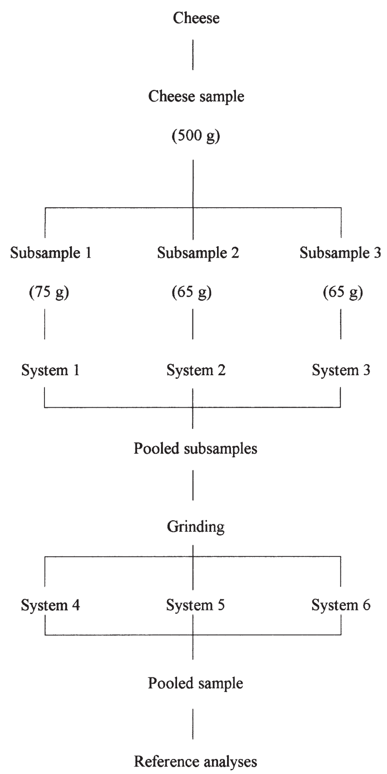

Figure 1. Flow chart for cheese analyses performed in the present study. NIR measurement systems are described in Table 1.

Materials and methods

Samples (*c*. 500 g) of semi-hard cheese containing *c*. 24% fat were collected from a dairy during a period of two months. A total of 52 samples of Danbo 45+ ripened for three weeks, 45 samples of Danbo 45+ ripened for five weeks and 15 samples of Danbo 45+ spiced with caraway and ripened for five weeks were collected. The samples were measured in duplicate by spectroscopic techniques in ground and unground form (Figure 1, Table 1). A 1 cm layer of

Table 1. NIR measuring techniques.

System	Sample presentation	Spectroscopy
1	Five cylindrical cheese blocks (diam. 25 mm, height 15 mm) in an open sample cup with 5 windows.	NIR transmission, 850–1050 nm (Infratec 1255).
2	Cheese slice (l × w × h, 70 × 50 × 9 mm^3) packed in polyethylene bag. Two spots (diam. *c*. 8 mm) were measured.	NIR transmission, 850–1250 nm (Bomem MB 160 with Bag Sampler unit).
3	Cylindrical cheese block (diam. 50 mm, height 15 mm) placed directly on window of reflectance unit	NIR reflection, 925–1725 nm (Bomern MB 160 with diffuse reflectance unit).
4	An open sample cup with glass bottom (diam. 90 mm, height 25 mm) was filled with ground sample.	NIR transmission, 850–1050 nm (Infratec 1255).
5	An open sample cup with glass bottom (l × w × h, 170 × 40 ×15 mm^3) was filled with ground sample.	NIR reflection, 410–2490 nm (NIRSystems 6500 with horizontal operating transport module).
6	An open sample cup made of glass (diam. 50 mm, height 15 mm) was filled with ground sample.	NIR reflection, 925–1725 nm (Bomem MB 160 with diffuse reflectance unit).

the sample surface was discarded before measurement on unground subsamples. Grinding of samples was performed in a mini food processor (Krups, Germany). All subsamples and ground samples were stored in airtight polypropylene containers at 12 ± 0.5°C. The sample temperature, when measured, was 12 to 18°C. The instruments used were an Infratec 1255 Food and Feed Analyser (Foss Tecator, Hillerod, Denmark), an NIRSystems 6500 with sample transport module (Foss NIRSystems, Hillerod, Denmark) and a Bomem MB 160 FT-NIR with bag sampler, diffuse reflectance module and InAs detector (Bomem, Quebec, Canada). All instruments were operated in accordance with manufacturers' instructions. The measuring time was adjusted to *c*. 60 s on all systems. Reference analyses were performed by International Dairy Federation (IDF) standard methods. The TS content was determined by IDF Standard 4A[4] while fat content was determined by IDF Provisional Standard 5B.[5] Fat content in 70 samples was also analysed by the more rapid Gerber van Gulik method. The percentage of fat in total solids (fat/TS%) was calculated from TS and fat. Two double determinations were carried out on different days. Calibration was performed on mean spectra by partial least square (PLS) regression using six cross-validation segments. Data from NIRSystems 6500 and Infratec 1255 were treated with ISI version 3.00 software (Infrasoft International, Silver Spring, MD, USA). Data from Bomem MB 160 were treated with PLSPlus version 2.1G (Galactic Industries Corporation, Salem, NH, USA).

Results and discussion

The chemical composition of the investigated cheeses determined by reference methods is shown in Table 2.

Near infrared spectra were scatter corrected by different techniques before PLS regression, including multiplicative scatter correction (MSC), Detrend and SNV, first and second derivative treatment. Although the difference in effect of the different procedures was relatively small, Detrend and SNV was found to be optimal for treatment of data from the Infratec 1255, while Detrend and SNV combined with first derivative treatment was found to be optimal for data from the NIRsystems 6500. Data

Table 2. Chemical composition of cheese samples determined by reference analyses.

	Concentration %			
Parameter	Mean	Min	Max	*SD*
TS	52.3	50,7	53.7	0.57
Fat	23.6	22.5	24.5	0.37
Fat/TS	45.1	44.3	46.2	0.40

from the Bomem MB 160 were pre-treated with MSC. The optimal pre-treatment was assessed from standard error of prediction values obtained by cross-validation on the calibration set [*SEP(CV)*]. The optimal number of PLS terms ranged from 3 to 5 for TS, fat and fat/TS.

Best overall accuracy was obtained with systems 1 and 4, applying transmission spectroscopy on a relatively large sample volume (Table 3). No significant difference in accuracy was observed between measurement on cheese blocks and ground cheese by these systems. Transmittance measurement was also applied in system 2, but with less success. Reflectance measurement by system 5 on ground samples showed comparable results to systems 1 and 4. Reflectance measurements on unground cheese (system 3) showed *SEP(CV)* values for TS and fat which were twice the corresponding values for the optimal systems and 85% and 25% higher than corresponding techniques on ground samples (system 6). This relatively low accuracy obtained by reflectance measurements on unground cheese is in agreement with results reported using another instrument type.[3]

Direct calibration against fat/TS resulted in better accuracy compared with calculations from independent accuracies obtained on fat and TS (Table 3). This observation may be explained by the fact that fat/TS can be predicted by a linear combination of fat and TS.

The method repeatability standard deviation (SD) of single NIR measurements was calculated from duplicate analyses on the calibration set. Measuring systems 2 and 3 showed considerably higher SD_r values in comparison with other systems (Table 4).

Table 3. Results obtained by NIR spectroscopy.

Sample	Mode	System pretreatment	*SEP(CV)*			
			TS	Fat	Fat/TS[a]	Fat/TS[b]
Unground	Trans.	1	0.17	0.14	0.27	0.31
Unground	Trans.	2	0.33	0.26	0.44	0.57
Unground	Refl.	3	0.37	0,24	0.37	0.56
Ground	Trans.	4	0.17	0.15	0.25	0.32
Ground	Refl.	5	0.20	0.16	0.24	0.35
Ground	Refl.	6	0.20	0.19	0.31	0.40

[a]Results obtained by direct calibration
[b]Results obtained by calculation from fat and TS standard deviations

Table 4. Method repeatability standard deviation (SQ) for NIR spectroscopic determination of cheese constituents.

Sample pretreatment	Mode	System	SQ		
			TS	Fat	Fat/TS
Unground	Trans.	1	0.09	0.07	0.09
Unground	Trans.	2	0.27	0.15	0.16
Unground	Refl.	3	0.18	0,09	0.14
Ground	Trans.	4	0.11	0.04	0.11
Ground	Refl.	5	0.06	0.04	0.08
Ground	Refl.	6	0.11	0.05	0.06

Table 5. Accuracy of the Gerber van Gulik method for fat determination when compared with the IDF standard method 5B.

Parameter	*SEP*[a]
Fat % (Gerber van Gulik)	0.28
Fat/TS % (Gerber van Gulik/IDF 4A)	0.54

[a]Results were corrected for bias

Table 6. Repeatability standard deviation (SD,) and intra-laboratory reproducibility standard deviation ($SD_{R,\ intra}$) for reference methods. Values are valid for single determinations and were calculated from results obtained by routine analyses on the samples investigated.

Parameter	SD_r	$SD_{R,\ intra}$
TS % (IDF 4A)	0.08	0.16
Fat % (IDF 5B)	0.13	0.18
Fat/TS % (IDF 5B/IDF 4A)	0.24	0.37

In process control, fat is traditionally determined by the Gerber van Gulik method, which is less time consuming than the reference method. In the present study, NIR measuring systems 1, 4, 5 and 6 were significantly more accurate than the Gerber van Gulik method (Table 5). The accuracy of these NIR measuring systems was also significantly better in determination of percent fat/TS when compared with values calculated from Gerber van Gulik and reference total solids results.

The accuracy of NIR spectroscopy was compared with the precision of the reference methods on the samples studied. The *SEP*(*CV*) values for the best NIR measuring systems were on the same level as the intra-laboratory reproducibility standard deviation of the reference methods obtained in routine analyses, or even better (Table 6).

Conclusions

The results have shown the possibility of improving the accuracy of fat and fat in total solids determination by substituting NIR spectroscopy for the traditional Gerber van Gulik method, thereby improving process control in cheese-making. However, the success rate can be highly dependent on the spectroscopic concept applied. Generally, the size of the interacting sample volume appears to be a significant system factor influencing accuracy in cheese analysis when unground samples are measured.

References

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